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Gary S. Hoffman, MD, AND John H. Stone, MD, MPH

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SPECIAL ISSUE

Foreword

Welcome to the 10th International Vasculitis and ANCA Workshop. This supplement to the *Cleveland Clinic Journal of Medicine* contains the proceedings of the Workshop.

Over the years, the Workshop has enjoyed extraordinary growth in numbers of attendees, abstracts, and knowledge. The first International Workshop on ANCA in Copenhagen (1988) attracted, by invitation, a hardy band of 34 individuals interested primarily in antineutrophil cytoplasmic antibodies (ANCA) and a small number of vasculitides, particularly Wegener's granulomatosis and microscopic polyangiitis. (Many of these 34 have attended every meeting since.) Recent meetings have brought together more than 400 investigators and students of vasculitis. We have discovered substantial commonalities among many vasculitides, be they diseases associated with ANCA or not. Our understanding of disease pathogenesis has grown, and new treatments are being explored that promise to change the quality of health for our patients.

Unquestionably, one of the most enjoyable aspects of the Workshops has been the cultural, geographic, and scientific diversity of our members. The meeting has benefited from participation of practitioners and scientists from many fields, including immunology, rheumatology, nephrology, pulmonology, pathology, cardiology, infectious diseases, and even a variety of surgical subspecialties. We hope that you will share our pleasure in learning about new areas of study and discovery in inflammatory vascular diseases.

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Host and viral genes that control herpesvirus vasculitis

HERBERT W. VIRGIN IV, MD, PhD

The human vasculitides are idiopathic syndromes for which both autoimmune and infectious etiologies have been proposed. Depending on the syndrome, arteries and veins of all sizes can be affected. Signs of immune activation (inflammation, upregulated MHC Class II, expanded T-cell subsets, increased antibody titers) have been observed in several diseases, but antigens responsible for this activation have not been defined. While many postulate that these inflammatory diseases are autoreactive, infectious etiologies have also been proposed. Both bacterial and viral infections have been implicated (reviewed in references 1-3). The strongest viral candidates for human vascular disease are ones that establish long-term persistent infections, such as hepatitis B and hepatitis C viruses, human cytomegalovirus, Epstein-Barr virus, herpes simplex virus, and HIV (reviewed in references 3-5). However, it is difficult to prove causality because the presence of bacteria or virus may merely be bystander infection. In some cases (eg, CMV), latent infection of unaffected vessels further complicates evaluation of the etiology of human vasculitis and atherosclerosis.[6,7]

Evidence for infectious etiologies of atherosclerosis, arteritis, and coronary artery and transplant restenosis has also been reported[8-9] (reviewed in references 1-4 and 10-15). A number of studies have associated herpesvirus infection with human atherosclerosis (reviewed in references 10, 14, 16, and 17), and restenosis after angioplasty,[18,19] but proof of causality is lacking. Human cytomegalovirus (HCMV) infection has been more convincingly associated with rapidly progressive atherosclerotic-like lesions in cardiac transplant recipients.[9,20,21] In addition, active HCMV infection has been documented in inflammatory arteritis involving the human aorta.[22-24] Seroepidemiologic studies have suggested a modest association between HCMV infection and atherosclerosis,[25-27] although this is far from generally accepted.[28,29] HCMV and/or HSV nucleic acid or proteins have been detected in the aorta or in cells cultured from the aorta.[5,6,14,23,30-33] However, herpesvirus nucleic acid is found in normal as well as abnormal regions of the aorta,[6,34] and no specific viral transcripts or proteins have been identified in classical atheromatous lesions.[5,6,30,33] Thus, studies of human disease remain inconclusive with respect to a direct role of herpesviruses in vascular pathology.

While a direct relationship between infection and atherosclerosis or vasculitides such as Takayasu arteritis remains unproven, it is nevertheless an attractive possibility that infection may provide the initial injury, or chronic antigenic stimulation, required for chronic vascular disease. Thus, it is possible that vasculitis and atherosclerosis are pathogenetically related in that each may be caused by or maintained by infection. Whether this is correct or not, it is true that immune activation in the wall of the great vessels occurs in both human vasculitis and atherosclerosis. Therefore, it is essential that we understand the fundamental immune mechanisms that are operative in the vascular wall.

To study infection-mediated vascular disease, different animal models have been developed. Infections with the bacteria *Chlamydia pneumoniae*,[35,36] the porcine RNA virus PRRSV,[37-40] a retrovirus,[41] and herpesviruses all cause vascular pathology. Many aspects of the human diseases are recapitulated in these models, so further animal studies may help elucidate mechanisms of infection-mediated vasculitis. Such results may improve management, and potentially prevention, of these important human diseases. In particular all three classes of herpesviruses (α-, β-, and γ-herpesviruses) can cause vasculitis.

α-HERPESVIRUS-ASSOCIATED ARTERITIS

The initial observation that herpesviruses can cause vasculitis was reported by Paterson and Cottral who demonstrated cosegregation of neurolymphomatosis (NL), or Marek's disease, with a vasculitic process, with some similarities to atherosclerosis, in chickens. Chickens with a low incidence of NL developed vasculitis after inoculation with tracheal washings from chickens with a high NL incidence. Churchill and Biggs later reported that a herpesvirus was the etiologic agent of Marek's disease. These reports were followed by an extensive set of experiments by Fabricant et al analyzing the vascular disease induced by Marek's disease virus (MDV) in chickens (reviewed in reference 42). Certain strains of quail also develop large vessel arteritis which may be linked to infection with an MDV-related herpesvirus.[43,44]

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These studies demonstrated that infection of genetically susceptible newborn chickens resulted in inflammatory lesions in coronary arteries, aortas, and major arterial branches. Additionally, the fatty proliferative lesions contained intimal and medial foam cells, cholesterol clefts, extracellular lipid, and calcium deposits. Adventitial inflammatory infiltrates as well as mononuclear cells in the intima were evident. High-cholesterol feeding synergized with viral infection resulting in increased fatty proliferative lesions. Uninfected controls did not have lesions, regardless of diet. Gross arterial lesions were visible after 3 months postinfection, and persisted for at least 4 months. Microscopic lesions were discernible one month postinfection. There was no change in incidence of diseases related to cholesterol levels in the diet. Antibody-mediated injury seems unlikely as IgG and C3 deposits in the lesions were not detectable by immunohistochemistry.

Immunofluorescence revealed viral antigen in a few smooth muscle cells (SMCs) of the arterial media, providing evidence of direct MDV infection within arterial lesions. Infection was demonstrated in SMCs within plaques by in situ DNA hybridization. The authors argue that latent infection may result in SMC proliferation, thereby resulting in atherosclerotic-like proliferative plaques. It should be noted that latent and lytic infections were not formally distinguished in these studies.

■ β-HERPESVIRUS-ASSOCIATED ARTERITIS

Murine CMV (MCMV) or rat CMV (RCMV) infection are widely used as model systems for studying aspects of HCMV infection. Given the interest in HCMV as a possible agent in human vascular disease, several groups have used the murine system to test whether CMV infection can result in vasculitis. Persoons et al[45] demonstrated small-vessel pathology following local (subcutaneous in the foot pad) and generalized (intraperitoneal) infection of irradiated rats with RCMV. This work builds on a large body of work with RCMV in a transplant model[46,47] of large-vessel arteritis. MCMV infection can cause vascular disease, as seen by infection of mice at one to two weeks of age, as well as of wild-type and immunocompromised adult mice. Dangler et al[48] infected suckling BALB/c and C57BL/6 mice at 7–15 days of age with near-lethal doses of MCMV intraperitoneally (i.p.), and demonstrated very significant arteritic lesions at the base of the aorta. We infected adult (6–8 weeks old) wild-type 129 mice as well as IFNγR-/- mice and found significant arteritis in the same locale **(Figure 1)**.[49] Berencsi et al[50] found arteritis after MCMV infection of irradiated adult BALB/c mice. For each group, aortic inflammation developed and was characterized by mononuclear cells in the intima and adventitia, although the adventitial inflammation was often most severe, with few infiltrates in the media. Dangler et al[48] demonstrated such pathology at 8 and 15–16 weeks postinfection, with increased penetrance when younger mice were infected. We found that, although lesions were present in wild-type mice at 28 and 56 days postinfection, they resolved by 84 days postinfection. In contrast, IFNγR-/- mice had significant arteritis as late as 154 days postinfection.[49]

Immunohistochemistry for CD3, CD8, and CD4 resulted in staining predominantly in the adventitial infiltrate, with only scattered positive cells in the medial and intimal infiltrates.[48] Although the media contained the least infiltration, we demonstrated medial infection by the presence of cytomegalic nuclear inclusion bodies and MCMV antigen in SMC of the elastic media.[49] It is therefore likely that the continued inflammatory response is directed against viral antigens. The prevalence and severity of aortic lesions in MCMV induced vasculitis were shown to be independent of diet.[48,50] However, an increase in serum LDL-cholesterol levels with MCMV infection was reported,[50] as well as increased aortic lipid deposition in infected versus uninfected mice fed a high fat diet.[48]

These studies demonstrate that CMV infection can cause aortic inflammation and that disease can persist for many weeks after infection. The presence of nuclear inclusions and detection of viral antigen suggest continued viral gene expression and replication, thereby providing stimulation for chronic disease. As with MDV, dietary factors can influence lipid deposition in the arterial wall, but do not affect the vascular inflammation per se.

■ γ-HERPESVIRUS-ASSOCIATED ARTERITIS

Recognizing the importance of understanding mechanisms of immunity to γ-herpesvirus infection, we and others have been studying murine γ-herpesvirus 68 (γHV68 or MHV-68), which is closely related at the genomic level to EBV and KSHV.[51-53] While γHV68 is clearly distinct from EBV and KSHV in important ways, this model has many advantages, including the availability of mutant mouse strains and the ease of generating viral mutants. Conservation of multiple nonessential genes (eg, *v-cyclin*, *v-bcl-2*, *K3* [MHC regulation], *regulator of complement activation*) between γHV68 and EBV and/or KSHV, together with the fact that these viruses are associated with induction of lymphomas, makes γHV68 a relevant model for understanding aspects of EBV and KSHV pathogenesis and immunity.

γHV68, a natural pathogen of wild rodents,[54,55] is capable of infecting both outbred and inbred mice. Following inoculation with γHV68, acute productive replication occurs in multiple organs, and is cleared 9 to 15 days postinfection.[56-58] γHV68 establishes latent infection in macrophages (Møs) and B cells in the peritoneum,[59] and in B cells and dendritic cells (DCs) in the spleen.[60,61] Chronic γHV68 infection is associated with three diseases: (i) lymphomas and lymphoproliferative disease (reference 62 and unpublished data), (ii) severe large-vessel arteritis,[63-65] and (iii) splenic fibrosis.[64,66]

We found that γHV68 caused death of infected IFNγR-/- mice over weeks to months. Based on our experience with MCMV, and the tropism of the human γ-herpesvirus KSHV for vascular tissues, we investigated the possibility that γHV68 also caused severe arteritic lesions. We found that IFNγR-/- mice were profoundly susceptible to induction of arteritis by infection with either MCMV or γHV68 **(Figure 1)**.[64]

Several things were striking about the lesions we observed. First, they were restricted to the great elastic arteries and manifested as skip lesions **(Figure 2A)** with a

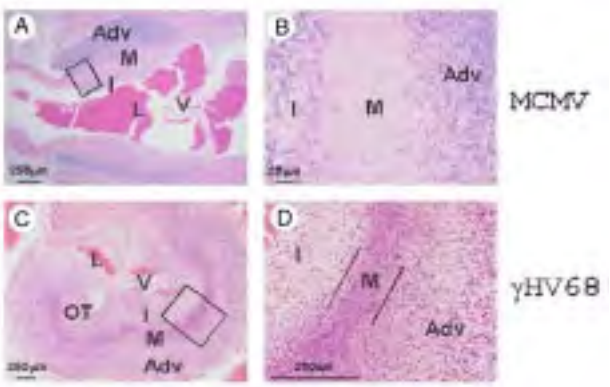

Figure 1. Arteritis induced by both MCMV and γHV68. (A) Aorta from an IFNγR-/- mouse sacrificed 154 days after infection with 10^4 PFU MCMV. (B) High-power view of the boxed region shown in A. (C) Base of the aorta from a γHV68-infected IFNγR-/- mouse that died 5.6 weeks after infection. (D) Higher-power view of the boxed region shown in C. Reprinted from *International Journal of Cardiology*; 75(suppl 1). Dal Canto AJ, Virgin HW IV. Animal models of infection-mediated vasculitis: implications for human disease, pp. S37-S45. Copyright 2000, with permission from Elsevier Science.

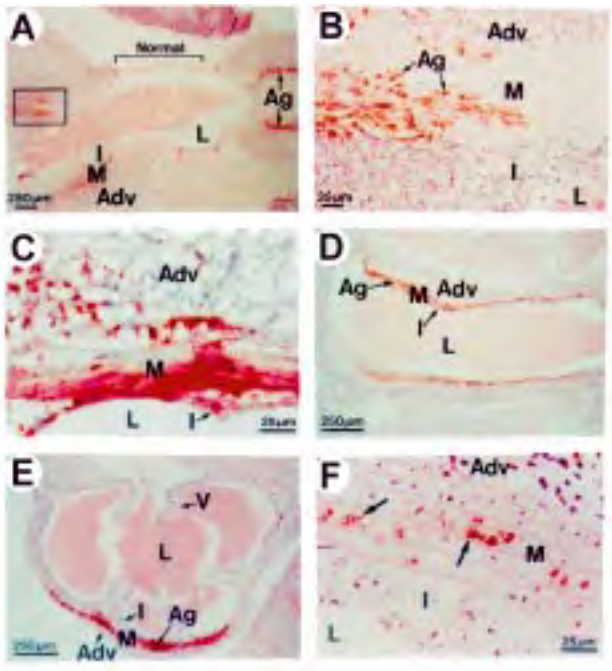

Figure 2. Immunohistochemical localization of γHV68 antigen and lipid deposition in γHV68-induced arteritic lesions. For all panels, L, lumen; I, intima; M, media; Adv, adventitia; and Ag, γHV68 antigen. (A) Longitudinal section of the aorta from a γHV68-infected IFNγR-/- mouse that died 4 weeks after infection stained with anti-γHV68 antibody. Note skip lesions in the aorta, and the correlation between inflammatory arteritis and regions containing viral antigen (brown color). The bracket indicates a region of normal aortic wall between two arteritic lesions. (B) High-power view of the boxed region in (A) demonstrating the nature of the inflammatory process occurring in IFNγR-/- mice infected with γHV68. Note staining for viral antigen (brown color) in elongated cells within the media of the vessel. (C) Oil Red O–stained frozen section from the base of the aorta of a γHV68-infected IFNγR-/- mouse fed a high-fat diet that died 8 weeks after infection. Lipid deposition, demonstrated by red staining, is evident in subendothelial and medial regions. (D) Longitudinal section of pulmonary artery from a γHV68-infected B-cell–deficient mouse that was sacrificed 13 weeks after infection. Note the inflammation in both the intima and adventitia as well as viral antigen in the media (brown color). (E) Aorta from a normal pup that died 9 days after infection (day 16 of life). Viral antigen was detected within the media (brown color), and intimal inflammation is present. (F) Aorta from a pup that died 19 weeks after infection with γHV68. Note adventitial inflammation, intimal thickening, and the presence of viral antigen (brown color, examples indicated by arrows) in the media. From *Nature Medicine* 1997; 3:1346–1353.

high frequency of involvement of the base of the aorta. Second, the pathology had similarities to both atherosclerotic lesions and to lesions of Takayasu's arteritis, including extensive deposition of lipid in the lesions. Third, γHV68 antigen was found in the SMC of the elastic media for months after infection **(Figure 2B, D, E, and F)**.[64] Remarkably, this was the same distribution seen for MCMV.[49] Fourth, in addition to deficiency of IFNγ, B cell deficiency and MHC Class II and therefore CD4 T cell deficiency **(Figure 2D)**[64] predisposed to lesion development. However, mice lacking most CD8 T cells (β2microglobulin-/-, β2m-/-) mice did not.[64] This latter result was surprising since it is generally considered that CD8 T cells are important components of the host defense against herpesvirus infection. However, it was consistent with the finding that CD8 T cells may play an immunopathologic role during γHV68 infection.[66] Lastly, we found that young mice without immunodeficiency were susceptible to arteritic lesions **(Figure 2E and F)**.[64] Since infection with herpesviruses often occurs in young persons, this raised the possibility that infection of young hosts sets up a situation in which secondary genetic factors might predispose to atherosclerosis or vasculitis. This was similar to findings by Dangler et al for MCMV showing that younger mice are more susceptible to MCMV induced arteritis than adult mice.[48]

These initial observations begged several basic questions. Were arteritic lesions secondary to persistent lytic replication, or latent infection? What was the basis for the tropism of the virus to the media of the great elastic arteries? Was tropism due to a specific interaction between a viral protein and cells of the elastic media or due to some intrinsic property of the arteries themselves? This was particularly of interest since MCMV localized to the same site generating similar lesions,[49] suggesting that viral tropism for the elastic arteries might be due to properties of the elastic media rather than specific tropism determinants of a given virus. Did normal adult mice also develop arteritis, or was this a phenomenon only observed in the artificial situation of immunodeficiency? Where and how did IFNγ act to protect against chronic arteritis? What were the specific roles of CD4 T

cells, CD8 T cells, and B cells in protection against or induction of arteritis? What components of the virus contributed to the capacity to induce arteritis?

Over the past 3 years we have addressed a number of these questions. Our major findings have been that: (i) persistent viral replication in the SMC of the media of the great elastic arteries is responsible for arteritic lesions,[65] (ii) the tropism of virus for the media of the great elastic arteries is explained by the finding that the media of the great elastic arteritis is an immunoprivileged site,[63] (iii) the M3 protein of γHV68 is a high affinity chemokine binding protein of novel structure that regulates virus induced inflammation but not arteritis (article submitted and references 67 and 68), and (iv) two genes in γHV68, one encoding a homolog of host bcl-2 proteins and the other encoding a homolog of host D-type cyclins, are important for both persistent replication and induction of arteritis (submitted).

ROLE OF PERSISTENT REPLICATION

We have shown using immunofluorescence, in situ hybridization, and electron microscopy that the virus infects, replicates, and kills vascular SMC of the arterial media during chronic infection.[63,65] Studies using the antiviral drug cidofovir show that replication is required for maintenance of arteritic lesions.[65] Interestingly, we found that persistence of virus was specific for the media of the great elastic arteries. These studies led to the identification of the media of the great elastic arteries as a novel immunoprivileged site.[63] The mechanism(s) responsible for the immunoprivilege are not defined. We found that lymphocytes and macrophages, but not neutrophils, are excluded from the vascular media, consistent with an anatomic blockade to entry and/or a viral protein that modulates inflammation at this site.[63,68] The possibility that a viral protein might prevent lymphocyte entry into the media of the great vessels led to studies of the γHV68 M3 chemokine binding protein. This protein is a high affinity chemokine scavenger of novel structure (unpublished and references 67 and 68). We found that it is not involved in vasculitis induction by γHV68, but is involved in encephalitis induced by the virus (submitted).

ROLE OF IFNγ

We showed that normal adult mice develop arteritis. However, transient depletion of IFNγ increased the incidence of arteritic lesions, and chronic depletion of IFNγ increased the severity of arteritic lesions.[63] Using bone marrow transplantation we found that IFNγ responsiveness of somatic cells is critical for controlling arteritis, but that hematopoietic cell IFNγ responses regulate the nature of the pathology. This latter finding suggests that IFNγ may limit immunopathologic injury to the wall of the great vessels.[63] We have also found that IFNγ protects cultured primary aortic SMC from infection, making it likely that IFNγ acts both by direct control of viral replication in SMC and by immunoregulatory mechanisms.[63]

ROLE OF VIRAL GENES

Since persistent replication in SMC of the media is required for vasculitis,[65] and the v-bcl-2 and v-cyclin are important for persistent replication in IFNγ-/- mice (submitted), we evaluated the role of the v-bcl-2 and v-cyclin in arteritis. We isolated the appropriate mutants and found that the v-bcl-2 and v-cyclin are critical for both persistent replication and virulence in IFNγ-/- mice which develop arteritis. The incidence of arteritis is significantly decreased in IFNγR-/- mice infected with v-bcl-2 mutant or v-cyclin mutant virus as compared to wild-type virus (submitted). This is the first work identifying viral genes important for induction of chronic vascular pathology.

CONCLUSION

Studies in the MCMV and γHV68 systems are beginning to unravel the immunologic mechanisms responsible for protection of the great elastic arteritis from viral infection. During the course of this work the vulnerability of the elastic media to chronic infection has become clear. This has implications for human disease, raising the possibility that a number of pathogens may persistently replicate in this immunoprivileged site. Identification of specific host (IFNγ) and virus (v-bcl-2 and v-cyclin) genes that are involved in vasculitis provides a basis for further studies on mechanisms responsible for chronic arterial injury.

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1-090

INDUCTION OF PAUCI-IMMUNE NECROTIZING AND CRESCENTIC GLOMERULONEPHRITIS (NCGN) BY INTRAVENOUS ADMINISTRATION OF ANTI-MYELOPEROXIDASE (ANTI-MPO) ANTIBODIES TO RECOMBINASE ACTIVATING GENE-2 DEFICIENT (RAG-2 -/-) MICE

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Background: Association of antineutrophil cytoplasmic autoantibodies (ANCA) with pauci-immune NCGN raises the possibility that they are pathogenic. We report an animal model that should facilitate understanding the pathogenesis of ANCA disease.

Methods: MPO knockout mice were immunized with murine MPO purified from WEHI cells, or with bovine serum albumin (BSA). Development of anti-MPO and anti-BSA was monitored by ELISA and by indirect immunofluorescence microscopy assay (IFA) using mouse neutrophils as substrate. IgG was isolated from serum of mice immunized with MPO or BSA by 50% ammonium sulfate precipitation followed by protein G affinity chromatography. 50 µg/g mouse weight IgG in PBS was injected i.v. into RAG2-/- mice, which lack B and T lymphocytes. Induction of circulating anti-MPO and anti-BSA was monitored by anti-MPO and anti-BSA ELISA and by ANCA IFA. Serum creatinine, BUN, proteinuria and hematuria were monitored. At the termination of the experiments on day 6 or day 14, tissue was obtained for pathologic examination by light and immunofluorescence microscopy.

Results: Passive transfer of anti-MPO and anti-BSA resulted in an immediate peak in circulating antibodies that declined progressively during observation. At the time of the first urine analysis at day 3, mice that received anti-MPO IgG (n=7) already had developed hematuria and proteinuria, but not mice that received anti-BSA IgG (n=3). Mice sacrificed at day 6 that had received anti-MPO (n=5) all had focal necrotizing glomerulonephritis (mean 18% glomeruli with necrosis) and crescents (mean 11% crescents), whereas mice that received anti-BSA (n=3) had no histologic lesions. Anti-MPO mice had a mean BUN of 47.4 compared to a mean of 22.7 in anti-BSA mice. Mice sacrificed at day 14 that had received anti-MPO had an average of 1.5% glomerular necrosis, 11% crescents, and 34% glomerular sclerosis. Immunofluorescence microscopy demonstrated only a paucity of glomerular staining for immunoglobulins and complement, most pronounced at sites of injury.

Conclusions: In mice with no T lymphocytes, circulating anti-MPO (MPO-ANCA) causes pauci-immune necrotizing and crescentic glomerulonephritis. Necrotizing lesions progress to sclerotic lesions in less than a week.

2-091

INDUCTION OF NECROTIZING AND CRESCENTIC GLOMERULONEPHRITIS (NCGN) AND SMALL-VESSEL VASCULITIS (SVV) BY ADOPTIVE TRANSFER OF ANTI-MYELOPEROXIDASE (ANTI-MPO) LYMPHOCYTES INTO RECOMBINASE ACTIVATING GENE-2 DEFICIENT (RAG-2 -/-) MICE

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Background: Association of antineutrophil cytoplasmic autoantibodies (ANCA) with NCGN and SVV raises the possibility that they are pathogenic. We report an animal model that should facilitate understanding the pathogenesis of ANCA disease.

Methods: MPO-knockout mice were immunized with murine MPO from WEHI cells or bovine serum albumin (BSA), or received no immunization. 1×10^8, 5×10^7, or 1×10^7 splenocytes from these mice were injected into RAG2-/- mice, which lack functional lymphocytes. Anti-MPO, anti-BSA, creatinine, BUN, proteinuria and hematuria were monitored. At termination, tissue was obtained for pathologic examination.

Results: Mice that received anti-MPO splenocytes developed circulating anti-MPO within 3 days, and the titer rose until sacrifice at 13 days. Mice that received anti-BSA splenocytes developed circulating anti-BSA. Mice that received MPO-ANCA splenocytes developed renal failure, hematuria and proteinuria, whereas mice that received anti-BSA or control splenocytes did not. All mice that received 1×10^8 (n=12) or 5×10^7 (n=4) anti-MPO splenocytes developed severe NCGN, 3 developed pulmonary capillaritis, 1 had necrotizing vasculitis in spleen and lymph nodes, and 1 had necrotizing granulomatous inflammation in spleen. Mice that received 1×10^7 anti-MPO (n=4), or 1×10^8 anti-BSA (n=14) or control (n=9) splenocytes developed no crescents or renal failure. They did have mild glomerular hypercellularity and rare segmental necrosis. All mice that received anti-MPO, anti-BSA or control splenocytes developed moderate glomerular localization of mouse immunoglobulin and complement.

Conclusions: All RAG2-/- mice that receive splenocytes from immune-competent mice develop low-level, functionally insignificant, glomerular immune complex localization. Only mice that receive splenocytes that produce MPO-ANCA developed NCGN and SVV. Thus, circulating MPO-ANCA cause NCGN and SVV, possibly by synergistic interactions with another minor inflammatory stimulus.

3-049

CONTRIBUTION OF ACTIVATED NEUTROPHILS AND MPO-ANCA TO THE DEVELOPMENT OF CRESCENTIC GLOMERULONEPHRITIS IN SCG/Kj MICE

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A role of activated neutrophils in the development of nephritis was investigated in the correlation between neutrophil functions and renal failure of SCG/Kj mice that spontaneously develop crescentic glomerulonephritis from early phase of life. MPO release, superoxide generation of peripheral neutrophils, MPO-ANCA titer in serum and histology of glomeruli were examined in young and aged mice. Neutrophil number in peripheral and glomeruli increased depend on the development of nephritis showing proteinuria. Particularly, in the early phase of nephritis, spontaneous release of MPO from peripheral neutrophils of SCG/Kj mice increased, but superoxide generation stimulated with fMet-Leu-Phe and cytochalasin B decreased. MPO-ANCA titer, a marker for vasculitis, in serum of SCG/Kj mice was higher than that of normal mice. While the comparison of neutrophil functions with histological findings, spontaneous release of MPO was correlated to activity index, crescent formation score and chronicity index. On the contrary, superoxide generation was negatively correlated to crescent formation score. Moreover, the number of neutrophils infiltrated in glomeruli was well correlated with MPO-ANCA titer in sera, activity index, crescent formation score and chronicity index. These findings suggest that spontaneously activated neutrophils of SCG/Kj mice may contribute to nephritis development. Aseanostatin (ai-15:0), an inhibitor of human MPO release from neutrophils, was inhibited spontaneous MPO release from peripheral neutrophils in SCG/Kj mice, but not neutrophils of C57BL/6 mice. From these findings, it is strongly suggested that activated neutrophil could trigger the development of crescentic glomerulonephritis in SCG/Kj mice. This mouse is believed to be a good model of crescentic glomerulonephritis.

Known infectious causes of vasculitis in man

STANLEY J. NAIDES, MD

An array of pathogens is known to cause vasculitis in man.[1,2] For several of these agents, vasculitis is the major manifestation of disease. The majority, however, typically present as infectious processes in which vasculitis is an occasional manifestation of disease. For many, vasculitis may be a component of disease pathogenesis but is not a prominent feature of the clinical presentation. The various agents—viruses, bacteria, and fungi—share a common target, blood vessels. The involvement of vessels may be direct, with vascular structures serving as targets. Many infectious pathogens have tissue tropism that includes endothelium. Other agents may bind to the vessel wall because the vascular endothelium expresses specific receptors for the pathogen or another moiety with which the pathogen travels. Even when the agent does not enter the endothelial cell, the immune response to the agent may be focused at the vessel wall because the pathogen is adherent to the endothelial cell surface, thereby promoting innocent by-stander injury to the vessel. Processes that target the endothelium directly are usually acute in nature. Innocent bystander injury is often chronic and may be insidious in onset.

Demonstration of infectious agents as the cause of some cases of vasculitis fuels interest in searching for infectious etiologies of idiopathic vasculitis. The advent of highly sensitive molecular techniques has encouraged searches for various known pathogens in idiopathic vasculitis. Recognition that infectious agents are dynamic populations of organisms prompts us to search for emerging pathogens as previously unknown causes of vasculitis. Such pathogens "emerge" as new species or strains develop from older species in their traditional host population. Others may emerge due to spread into a new host population. The new, previously non-susceptible population may become infected because the pathogen adapts to the new host species. Alternatively, the agent may spread to a new susceptible host population as a consequence of changes in the physical environment or human or vector behavior that promotes geographical spread. In mirror fashion, changes in the behavior of pathogens, vectors, and hosts, and our ability to intervene in disease processes, have rendered some causes of vasculitis far less common.

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VIRAL CAUSES OF VASCULITIS

Our knowledge of viral pathogenesis has exploded in the last quarter of the twentieth century, accelerated in large part by epidemics of "emerging" viral diseases. Hepatitis C virus, discovered in 1989, has worldwide prevalence.[3] The 10- to 20-year latent period before hepatic or rheumatic manifestations of disease explains the increasing number of cases of hepatitis C virus–mediated vasculitis currently being seen in the United States following the epidemic of new infections in the 1980s.[4] Prior to the discovery and characterization of hepatitis C virus in the late 1980s, the triad of arthritis, palpable purpura, and type II cryoglobulinemia was given the sobriquet "essential mixed cryoglobulinemia" and considered an idiopathic vasculitis. Availability of diagnostic testing for hepatitis C virus demonstrated that almost all of these cases were associated with hepatitis C virus infection. Immune response to the virus elicits a response to the Fc portion of immunoglobulin with the majority of elicited antibody having the Wa idiotype.[5,6] Immune complexes of anti–Fc Wa idiotypic antibody and pre-existing antibody, and virus have the peculiar physical property of precipitating out of solution in the cold ("cryoglobulins"). Presumably, Wa idiotype recognizes a cross-reactive epitope found on hepatitis C virus and immunoglobulin. Extremities and skin are sufficiently cold so as to explain a predilection for small-vessel leukocytoclastic vasculitis of the skin; gravity enhances vascular injury in dependent distal vessels, giving rise to palpable purpura predominantly in the lower extremities. More severe cases may manifest visceral organ involvement including membranoproliferative glomerulonephritis and bowel involvement. Small- and medium-sized arteries may be involved as well, especially in the kidneys.

Hepatitis B virus (HBV) infection provides the classic example of virally mediated immune complex disease. A lymphocytic venulitis or neutrophilic vasculitis of small vessels with leukocytoclastic or fibrinoid changes presents typically as an "urticaria-arthritis syndrome."[7] Immune complexes of hepatitis B virus surface antigen (HBsAg) and antibodies to hepatitis B virus surface antigen (HBsAb) circulate in the blood and are found deposited in vessels in association with complement.[8,9] The long latency period of HBV allows time for an immune response to occur. Viral replication increases HBsAg load, and is tem-

porally associated with jaundice.[10] The immune complexes eventually no longer form in antigen excess, and the serum sickness-like illness resolves. HBV has also been associated with large-vessel polyarteritis nodosa-like illness.[11] Onset is early in the course of chronic HBV hepatitis. Immune complexes containing HBsAg, HBsAb, and complement are found in the vessel wall.[12] The determinants of small vessel versus larger vessel disease in the two syndromes of HBV infection are unknown.

Human immunodeficiency virus (HIV) patients may present with a variety of vasculitides. However, it is difficult to specifically attribute the various vasculitides seen to HIV because of frequent co-infections with other agents that may cause vasculitis in the absence of HIV infection. Human T lymphotropic virus 1 infection may cause retinal, cutaneous, or central nervous system vasculitis.[13-16]

The herpesviruses (cytomegalovirus, varicella-zoster, herpes simplex viruses 1 and 2, and herpes hominis) may be associated with retinal vasculitis in immunocompromised patients.[17-23] Varicella-zoster may also cause a diffuse central nervous system small arterial granulomatous vasculitis, or a small- and/or large-artery vasculopathy.[24-27] Herpes simplex viruses 1 and 2 have been associated with cutaneous vasculitis and necrotizing arteritis of small and medium vessels.[28-30] Epstein-Barr virus has been suggested as a cause of both small- and large-vessel disease in a number of cases and short series.[31-36] However, the ability to demonstrate causality in many instances is made all the more difficult by the latency of herpesvirus infection.

Parvovirus B19 has been suggested as the causative agent of Wegener's granulomatosis and polyarteritis nodosa in a number of cases and short series.[37-42] However, the issue of latency and the failure to eliminate B19 from pooled blood products provides a cautionary note when considering causality.[43-46] Rare cases of vasculitis have similarly been reported following rubella virus, adenovirus, echovirus, coxsackievirus, parainfluenza virus, herpes simplex viruses, and hepatitis A virus infections.[1,47-57]

■ BACTERIAL CAUSES OF VASCULITIS

Bacterial seeding of vessels may lead to necrosis through direct bacterial action. Vessels may be seeded intraluminally at sites of endothelial injury or flow turbulence. Seeding of vasa vasora may cause destruction of vessels from the outside in. An injury of a large vessel by this mechanism is classically termed a "mycotic aneurysm." Contiguous spread from an infected site to a vessel may occur. Vessels may also be seeded from within the lumen, as in subacute bacterial endocarditis in which septic emboli embed within the wall of smaller vessels, causing a "mycotic" process via a luminal route. Immune response to bacteria or to bacterial components may also lead to vasculitis, usually by immune-complex–mediated mechanisms.[2]

In subacute bacterial endocarditis, direct spread via septic emboli and immune complex injury may occur. Patients may present with evidence of elevated acute-phase reactants, fever, malaise, myalgia, arthralgia, Osler's nodes, Janeway lesions, and septic infarcts.[58,59] Staphylococcus and streptococcus infections are common causes. Gram-negative organisms, other gram-positive cocci, fungi, and parasites may be causative as well, and their occurrence depends on the clinical setting.[60-66] Mycotic aneurysms resulting from septic emboli are common with staphylococcus, streptococcus, and *Salmonella* species.[67-69] Patients with subacute infections may develop cryoglobulins.[70-72] Bacteremia may present as leukocytoclastic vasculitis.[73,74] Small-vessel vasculitis may be associated with post-streptococcal infection, distinct from endocarditis.[75,76] The *Rickettsiae* are a group of obligate intracellular bacteria with tropism for vascular endothelium.[77] Infection results in widespread microvascular leak, local thrombosis, and ultimately multisystem failure if untreated.[78,79]

In the lung, necrosis of vessels may occur from septic emboli or from contiguous spread in primary pneumonias. In the latter setting, *Pseudomonas aeruginosa* and *Legionella pneumophila* often cause direct necrosis via contiguous spread.[80] The presentation, however, is that of pneumonia. Mycobacterial or fungal pulmonary infections may mimic Wegener's granulomatosis or Churg-Strauss vasculitis in eliciting a granulomatous reaction in vessels.[81] Spread of *Mycobacterium tuberculosis* to the aorta may be seen as a cause of tuberculous aortitis, coronary arteritis, and mycotic aneurysm.[82-84] *Aspergillus aeruginosa*, *Aspergillus fumigatus*, and *Mucor* may be characterized by direct vessel invasion and necrosis.[68,85,86]

Coccidioides immitis meningitis may be associated with vasculitis that can be confused with central nervous system angiitis.[87,88] *Coccidioides immitis* may also present as an immune-complex–mediated disease with erythema nodosum, periarthritis predominantly of the ankles, and bihilar lymphadenopathy.[89,90] This presentation is often confused with Löfgren's syndrome of sarcoidosis. While sarcoidosis as a cause of Löfgren's syndrome is more prevalent in eastern United States populations, *Coccidioides immitis* is a more likely cause of a Löfgren's-like presentation in the western United States.

Neisseria species may be associated with small-vessel vasculitis. In *Neisseria gonorrhea* infection, cutaneous papules vesiculate, then becomes necrotic.[91] In *N meningitides* infections, vasculitis may manifest in the skin and gastrointestinal tract with the endothelium showing necrosis and thrombosis.[92-94] In immunocompromised hosts, *Pseudomonas aeruginosa* and other gram-negative organisms can present as a large 1- to 5-cm macular erythema that develops central necrosis and peripheral edema and induration—a condition termed "ecthyma gangrenosum." Vessel thrombosis results from direct bacterial invasion of the vessels. Similar lesions may be seen in immunocompromised patients with disseminated *Pseudomonas*, *Nocardia*, *Aspergillus*, *Mucor*, *Curvularia*, *Pseudallescheria*, *Fusarium*, *Morganella*, *Metarrhizium*, *Xanthomonas*, *Klebsiella*, *E coli*, and *Aeromonas* infections.[95-107]

Before AIDS, syphilis was the infectious agent known as the "great imposter," presenting as large- or medium-size vessel disease (aortitis or coronary arteries) or as the small-vessel rash of secondary lues. Aortic aneurysms

were insidious in clinical presentation. *Treponema pallidum* spirochetes were rarely detected in fibrosed and scarred vessels.[108-110] At least briefly, *Borrelia burgdorferi*, the causative agent of Lyme disease, was known as an "imposter." Vasculitic changes may be seen in the central nervous system, retina, and temporal arteries.[111-124]

Parasites are a rare cause of vasculitis. The local response to a parasite may include vessel changes typical of vasculitis, but these are localized to the offending pathogen. In a few instances, however, more distant effects have been reported. *Toxocara canis* presented in an adolescent as palpable purpura with additional features suggesting Henoch-Schönlein purpura.[125] *Cysticercus* has caused vasculitis and arachnoiditis as it infects the central nervous system.[126] *Angiostrongylus* nematodes apparently caused a Wegener's granulomatosis-like pulmonary angiitis.[127] *Loa loa*, a filarial parasite, presented with cutaneous leukocytoclastic vasculitis.[128]

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HCV and cryoglobulinemic vasculitis

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CRYOGLOBULINEMIA

The presence in the serum of one (monoclonal cryoimmunoglobulinemia) or more immunoglobulins (mixed cryoglobulinemia), which precipitate at temperatures below 37 °C and redissolve on re-warming[1-3] is termed "cryoglobulinemia." This is an in vitro phenomenon; the actual mechanism(s) of cryoprecipitation remains obscure. It could be secondary to intrinsic characteristics of both mono- and polyclonal immunoglobulin (Ig) components; it can be caused as well by the interaction among single components of the cryoprecipitate.

Monoclonal cryoimmunoglobulinemia is almost invariably associated with a well-known hematological disorder, and it is frequently asymptomatic per se. Similarly, circulating mixed cryoglobulins are commonly detected in a great number of infectious or systemic disorders.[1-3] On the contrary, "essential" mixed cryoglobulinemia (MC) represents a distinct disorder[1] characterized by leukocytoclastic vasculitis of small- and medium-sized vessels and frequent multiple organ involvement **(Table 1)**.

Cryoglobulinemia is usually classified into three subgroups: type I, composed by a single monoclonal immunoglobulin, usually a paraprotein; type II and III, characterized by polyclonal IgG and mono- or polyclonal IgM RF, respectively.[2] Cryoglobulinemia type I is mainly found in patients with overt lymphoid tumors (ie, immunocytoma/Waldenström's macro-globulinemia, multiple myeloma, etc.). MC type II and III can be associated with different infectious, immunological or neoplastic diseases.[1-3] The analysis of cryoprecipitates is generally carried out by means of immunoelectrophoresis or immunofixation. Using more sensitive methodologies (immunoblotting or two-dimensional polyacrylamide gel electrophoresis), type II MC shows a microheterogeneous composition; in particular, oligoclonal IgM or a mixture of polyclonal and monoclonal IgM can be detected. This particular serological subset, termed type II-III MC, could represent an intermediate, evolutive state from type III to type II. In any case, this serological condition agrees with the most recent molecular studies showing the presence of oligoclonal B-lymphocyte proliferation in liver and bone marrow biopsies in the majority of type II MC patients.[4]

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CRYOGLOBULINEMIC VASCULITIS

The so-called "essential" MC was first described by Meltzer et al in 1966.[7] Originally, this term was referred to as autonomous disease when other well-known systemic, infectious, or neoplastic disorders had been ruled out by means of a wide clinico-serological work-up. MC syndrome is characterized clinically by a triad—purpura, weakness, arthralgias—and by a series of pathological conditions,[1-4] including chronic hepatitis, membranoproliferative glomerulonephritis, peripheral neuropathy, skin ulcers, diffuse vasculitis, and less frequently by lymphatic and hepatic malignancies.[1-4] The prevalence of MC manifestations reported in **Table 1** regards an Italian patient population referred to a rheumatology-immunology division. A variable patient recruitment at different specialist centers together with racial differences among patient series is often responsible for some contrasting data present in the literature.[1-5] The prevalence of MC presents great geographic heterogeneity; the disease is more common in Southern Europe than in Northern Europe or Northern America. The disease is considered to be a relatively rare disorder; however, as yet there are no adequate epidemiological studies regarding its overall prevalence. Given its clinical polymorphism, a single manifestation (skin vasculitis, hepatitis, nephritis, peripheral neuropathy, etc.) is often the only apparent or clinically predominant feature, so that MC patients are often referred to different specialties. A correct diagnosis might thus be delayed or overlooked entirely. Consequently, the actual prevalence of MC is probably underestimated.

There are no available diagnostic criteria for MC. In 1989, the Italian Group for the Study of Cryoglobulinemias proposed preliminary criteria for MC classification. A revised version of these criteria, including pathological and virological findings, has been recently proposed.[4] Circulating mixed cryoglobulins, low C4, orthostatic skin purpura, and leukocytoclastic vasculitis are the hallmarks of the disease. Leukocytoclastic vasculitis, involving medium- and, more often, small-sized blood vessels (arterioles, capillaries, and venules) is responsible for MC tissue injury.[3,4] Cryoglobulinemic vasculitis (CV) is secondary to vascular deposition of circulating immune complexes (CIC), mainly cryoglobulins, and complement,

with the possible contribution of both hemorheological and local factors.[1-5] Due to its clinical and histological features, CV is classified among systemic vasculitides, in the subgroup of small-vessel vasculitides, which also includes cutaneous leukocytoclastic vasculitis and Schönlein-Henoch purpura.[1-5]

HCV AND CRYOGLOBULINEMIC VASCULITIS

Following the discovery of hepatitis C virus (HCV) as the major etiologic agent of non-A non-B chronic hepatitis,[6] the role of this virus in the pathogenesis of MC has been definitely established on the basis of numerous clinicoepidemiological and virological studies.[7-12] A direct involvement of HCV antigens in CIC responsible for cryoglobulinemic vasculitis has also been demonstrated.[9] Therefore, the term "essential" no longer seems to be appropriate for the majority of MC patients.[4,9-12] On the other hand, numerous epidemiological studies demonstrated low levels of circulating mixed cryoglobulins in over 50% of HCV-infected individuals, while overt cryoglobulinemic syndrome develops in only a minority of patients.[4] The large diffusion of HCV infection worldwide contrasts with the geographical heterogeneity observed in the prevalence of HCV-related MC.[4] Thus, a role for particular HCV genotypes, unknown environmental and/or genetic factors may contribute to the pathogenesis of MC.[11] However, few and often contrasting studies are available, and the actual role of the above co-factors remains to be demonstrated.[4]

An increasing number of autoimmune disorders have been observed in individuals with chronic HCV infection,[4,5,10-12] suggesting that the same virus might be responsible for different hepatic and extrahepatic immune-mediated disorders. **Table 2** summarizes the main organ or systemic diseases variably associated with HCV infection. Interestingly, different HCV-related diseases show a clinico-serological overlap.[4,10] In particular, MC can represent a crossroads between some classic autoimmune disorders (autoimmune hepatitis, sicca syndrome, glomerulonephritis, thyroiditis, etc.) and malignancies (B-cell lymphomas, hepatocellular carcinoma, thyroid cancer).[4,8,10,12,13]

TABLE 1
DEMOGRAPHIC, CLINICO-SEROLOGICAL, AND VIROLOGICAL FEATURES OF 190 MC PATIENTS

Age, mean ± SD yrs (range)*	50 ± 10 (29-74)
Female/male ratio	3
Disease duration, mean ± SD yrs (range)	12 ± 6.5 (1-34)
Purpura	91%
Weakness	90%
Arthralgias	80%
Arthritis (nonerosive)	9%
Raynaud's phenomenon	35%
Sicca syndrome	33%
Peripheral neuropathy	39%
Renal involvement**	34%
Liver involvement	71%
B-cell non-Hodgkin's lymphoma	7%
Hepatocellular carcinoma	2%
Cryocrit, mean ± SD %	3.2 ± 8
Type II/type III mixed cryoglobulins	2/1
CH50, mean ± SD units (normal 160-220)	85 ± 60
C3, mean ± SD mg/dl (normal, 60-130)	80 ± 29
C4, mean ± SD mg/dl (normal, 20-55)	9 ± 14
Antinuclear antibodies	25%
Antimitochondrial antibodies	12%
Anti-smooth muscle antibodies	25%
Anti-extractable nuclear antigen antibodies	8%
Anti-HCV antibodies	92%
HCV RNA	88%
Anti-HBV antibodies	42%
HBsAg	4%

* at presumed disease onset; ** invariably, membranoproliferative glomerulonephritis.

HCV, CV, AND LYMPHOPROLIFERATION

HCV has been recognized to be both an hepato- and lymphotropic virus, as suggested by the presence of active or latent viral replication in the peripheral lymphocytes of patients with type C hepatitis or MC.[8,14] The infection of lymphoid tissues could represent an HCV reservoir contributing significantly to viral persistence; moreover, the quasispecies nature of HCV permits it to escape immune surveillance and favors the persistence of infection in the host.[8] These biological characteristics may explain the appearance of a constellation of both autoimmune and lymphoproliferative disorders in HCV-infected individuals.[4,5,10-12] It has been proposed that HCV infection exerts a chronic stimulus on the immune system, which facilitates the development and selection of abnormal clones.[4,8] Patients with type II MC may develop a B-cell lymphoma, usually after a long-term follow-up.[4,8,10] This complication may be related to peripheral B-cell expansion and to lymphoid infiltrates observed in the liver and bone marrow of MC patients.[4,8,10] In particular, these infiltrates have been regarded by some authors as "early lymphomas," since they are sustained by lymphoid components indistinguishable from those of B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL) and immunocytoma (Ic).[8]

The HCV-related lymphoproliferation, varying from the benign polyoligoclonal B-cell expansion frequently observed in MC to overt malignant lymphoma, is a multifactorial and multistep process for which multiple genetic aberrations are probably necessary.[4,8] The recent identification of HCV envelope protein E2 able to bind CD81 molecule expressed on both hepatocytes and B lymphocytes[15] could help to clarify the pathogenesis of HCV-related autoimmune and lymphoproliferative diseases. In fact, CD81 is a cell-surface protein, which, on B cells, is part of a complex along with CD21, CD19, and Leu 13.

This complex reduces the threshold for B-cell activa-

TABLE 2
ASSOCIATION BETWEEN HEPATITIS C VIRUS INFECTION AND DISEASES

1—Proven association
Cryoglobulinemic vasculitis
Porphyria cutanea tarda
2—Significant association
Autoimmune hepatitis
B-cell NHL
Monoclonal gammopathies
3—Possible association
Sicca syndrome
Polyarteritis nodosa
Poly/dermatomyositis
Chronic polyarthritis
Lung fibrosis
Diabetes mellitus
Thyroiditis
Thyroid cancer
Lichen planus
Mooren corneal ulcer

1 – HCV infection in the majority of patients.
2 – Found in a significant percentage of patients if compared to general population.
3 – Suggested but unproven association.

tion by bridging antigen-specific recognition and CD21-mediated complement recognition. It can be hypothesized that the interaction between HCV-E2 and CD81 may increase the frequency of VDJ rearrangement in antigen-reactive B cell. One possible consequence could be the bcl-2 recombination observed in a significant number of HCV-infected individuals and particularly in MC patients.[16] This proto-oncogene is able to inhibit the apoptosis, leading to extended cell survival. The aberration of bcl-2 may explain, at least in part, the B-lymphocyte expansion and the wide autoantibody production observed in HCV-infected individuals.[4,8] Other mechanisms such as molecular mimicry may be involved in B-lymphocyte activation responsible for different autoimmune disorders. On the other hand, prolonged B-cell survival can expose these cells to other genetic aberrations[4,8] leading to overt malignant lymphoma.

■ TREATMENT OF CV

Due to its complex etiopathogenesis, the treatment of CV is particularly challenging. For a correct therapeutic approach we must deal with three important factors: HCV infection, autoimmune disorders, and neoplastic complications. Following the cascade of events leading from HCV infection to cryoglobulinemic vasculitis we can treat the disease at different levels by means of different—etiologic, pathogenetic, symptomatic—therapies.[4,8] Since HCV represents the triggering factor of the disease and probably exerts a chronic stimulus on the immune system, an attempt at HCV eradication should be done in all cases of HCV-associated MC. Unfortunately, the beneficial effect observed with this drug is often transient and not rarely associated with important immune-mediated complications—in particular, peripheral sensory-motor neuropathy.[4,8] There are no parameters available for predicting this harmful complication; thus, alpha-interferon therapy should be avoided at least in those patients with clinically evident peripheral neuropathy. On the whole, the usefulness of alpha-interferon treatment in MC patients is limited by the low rate of responders and frequent side effects. The association of alpha-interferon and ribavirin might achieve the eradication of HCV infection in a rather significant number of treated subjects, as recently demonstrated in patients with type C chronic hepatitis.[4]

Hopefully, with the rapid growth of molecular biology, a vaccine against HCV may be available in the near future. The recent identification of the interaction between HCV envelope protein E2 and CD81 on both hepatocytes and lymphocytes[4,15] suggests the possibility of interfering with HCV binding to target cells.

Immunosuppressive treatment is still the first-line intervention in cases of non-HCV–associated MC. For HCV-associated MC, immunosuppressive treatment should be considered, especially in patients who have failed to respond to alpha-interferon. This includes steroids, low-antigen-content (LAC) diet, plasma exchange, and immunosuppressors, mainly cyclophosphamide.[4,8,10] A reduction in circulating immune-complex levels can be achieved by means of plasmapheretic therapy including both traditional plasma exchange and double-filtration plasma exchange.[4] The use of oral cyclophosphamide (50-100 mg/day for 2-6 weeks) during the tapering of apheretic sessions can reinforce the beneficial effect of plasma exchange; moreover, it can prevent the rebound phenomenon that may be observed after the discontinuation of apheresis. Plasma exchange is useful in severe MC complications, and particularly in active cryoglobulinemic nephropathy. LAC diet has been employed in some immune-complex–mediated disorders such as MC and IgA-nephropathy.[4,10] In MC patients, this particular dietetic treatment can improve the CIC clearance by restoring the activity of the reticulo-endothelial system, overloaded by large amounts of circulating cryoglobulins.[4] LAC diet and/or low dosage of steroids may be sufficient to improve mild to moderate manifestations of MC. As commonly observed, MC patients with mild to moderate symptoms, such as palpable purpura, are particularly sensitive to the smallest variations of daily steroid dosage (1-2 mg). On the whole, MC treatment should be tailored for the individual patient according to the severity of clinical symptoms.[4] Therefore, patients with severe vasculitic manifestations must be promptly treated with high doses of steroids and/or plasma exchange and/or cyclophosphamide, whereas clinically asymptomatic patients usually do not need any treatment, even in the presence of high levels of cryocrit. Careful clinical monitoring of the disease is mandatory in all cases, with particular attention to neoplastic complications.[4,8,13]

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Infections in primary vasculitides

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Atherosclerosis and vasculitis are both inflammatory vascular disorders. In lesions of both disorders T lymphocytes are found that are clonally expanded, suggesting an antigen-driven immune response.[1,2] The antigens involved are, however, not well characterized in most cases. Auto-antigens and/or microbial antigens are likely candidates. In atherosclerosis, several infectious agents have been proposed such as cytomegalovirus, *Chlamydia pneumoniae*, and *Helicobacter pylori*.[3] Also, vasculitis is linked to a wide variety of microbes.[4,5] Classic examples are syphilitic aortitis, vasculitis of the skin related to subacute bacterial endocarditis, and hepatitis-B–associated polyarteritis nodosa (PAN). More recently, evidence was found that also in the so-called primary or idiopathic forms of vasculitis, microbes could play a role in the pathophysiology of these diseases. The mechanisms that could be operative in these latter will be discussed in this review.

LARGE-VESSEL VASCULITIDES

Several bacteria and fungi cause large-vessel vasculitis. So-called mycotic aneurysms are nowadays more frequently caused by *Salmonella*, *Campylobacter*, *Yersinia*, *Pseudomonas*, and other gram-negative bacteriae and anaerobes than the classic examples such as syphilis, mycobacteria, and/or aspergillus.[4] Primary large-vessel vasculitides, ie, giant-cell arteritis and Takayasu's arteritis, have no clearly established association with an infection, although previous exposure to mycobacterial, streptococcal, or spirochetal infection has often been mentioned in Takayasu's arteritis.

VASCULITIS INVOLVING MEDIUM- AND SMALL-SIZED ARTERIES

Kawasaki disease, also known as mucocutaneous lymph node syndrome, is a form of systemic vasculitis of unknown cause that primarily affects infants and young children. Clinical features include acute fever; cervical lymphadenopathy; conjunctival injection; redness of the lips, tongue, or oral mucosa; erythema of the palms and soles; edema of the hands and feet; a polymorphous cutaneous rash; desquamation of the skin; and cardiac abnormalities in about 20–30% of the patients. Arteritis of the coronary, iliac, or other systemic arteries can be found on histologic examination. The syndrome can occur sporadically, but sometimes clear outbreaks are observed. Several infectious agents have been suspected to be responsible for the syndrome such as streptococci, staphylococci, Epstein-Barr virus (EBV), retrovirus, or parvovirus B19.[4] In a few cases a clear pathophysiological role for toxic shock syndrome toxin-1 (TSST-1)–positive *Staphylococcus aureus* has been documented.[6]

In PAN, immune-complex and complement deposits are often found in involved tissues.[4] An infectious agent has to be searched for and is often found. In children, β-hemolytic group A streptococci are especially associated with PAN. In adults, hepatitis B virus (HBV) infection has a firm link with PAN. During recent years, however, a decline in HBV-related PAN has been documented in France, and at present less than 10% of PAN cases are HBV positive.[7] PAN-like disease has been found increasingly often, however, in patients that are infected with HIV.[5] Whether PAN in these cases is a result of a direct effect of HIV infection of blood vessels, a result of the immune activation that accompanies HIV infection, or a result of accompanying drug hypersensitivity or complicating infections with viruses such as CMV, HBV, or hepatitis C virus, is at present unknown.[5] Finally, many other incidental microbial associations with PAN-like vasculitis has been described (reviewed in 4).

VASCULITIS INVOLVING SMALL-SIZED VESSELS

In a few children with Wegener's granulomatosis (WG), a disease characterized by chronic inflammation of the respiratory tract, glomerulonephritis, and vasculitis, chronic parvovirus B19 has been suspected.[5] In adults, however, this virus is not found. A more important infectious association in WG is chonic nasal carriage of *S aureus*.[8] Previously, we found that 60–70% of our patients were chronic nasal carriers of *S aureus* and that those patients that were chronically carrying *S aureus* relapsed nearly eight times more frequently than noncarriers.[9] Recently, we found that 40–50% of the *S aureus* strains that are found in these nasal cultures are positive for staphylococcal superantigens.[6,8,10] Superantigens that were most frequently found were TSST-1, staphylococcal enterotoxin A and C, and exfoliative toxin A. Importantly, in a long-term observational study, we found that TSST-1–positive *S aureus* strains but not strains that

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were positive for other superantigens increased the risk for a relapse of WG.[10] Another observation that links WG with bacterial infection and/or colonization is the finding that patients with WG in which the disease is restricted to the respiratory tract can be successfully treated with co-trimoxazole as monotherapy.[11] Furthermore, we previously demonstrated in a placebo-controlled trial that co-trimoxazole maintenance therapy in patients with WG not only reduced the infection rate but also reduced the risk to develop a relapse by more than 60%.[12] It has been postulated that co-trimoxazole may exert this effect by eradication of chronic nasal *S aureus* carriage. In a recent study, however, we were unable to demonstrate such an effect since nasal *S aureus* carriage was terminated in only 5 of 21 WG patients during co-trimoxazole maintenance therapy. This finding suggests that co-trimoxazole exerts its beneficial effect in WG through a different mechanism, possibly an anti-inflammatory action. Importantly, it has been demonstrated in in vitro studies that co-trimoxazole inhibits myeloperoxidase-mediated halogenation of proteins.

In the other two forms of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides, ie, microscopic polyangiitis and the Churg-Strauss syndrome (CSS), the role of chronic nasal *S aureus* carriage is less clear. Cases have been described in which CSS was linked to infection with *Ascaris, Aspergillus fumigatus,* and/or HIV.[4] In addition, in several CSS cases a link with immunostimulation due to vaccination and/or desensitization is suspected (but never proven).

In Henoch-Schönlein purpura and isolated leukocytoclastic vasculitis of the skin, a precipitating microbial agent is often found. Bacteria, viruses, and sometimes parasites, such as *Ascaris,* have been associated with these forms of small-vessel vasculitis.[4] Microorganisms that are most frequently involved are streptococci, staphylococci, neisseriae, cytomegalovirus, parvovirus B19, HIV, HBV, and hepatitis C virus. Vasculitic lesions in these patients are assumed to be the result of immune-complex deposition initiated by microbial antigens. In some cases, however, other mechanisms may be operative such as in rickettsiae infections, in which infections primarily infect and damage endothelial cells resulting in vasculitis.

During the past decade, it became clear that there is a firm link between hepatitis C virus infection (HCV) and mixed essential cryoglobulinemia (MEC).[13] Patients with MEC due to HCV often suffer from palpable purpura and arthralgias; furthermore, many patients have hepatic, renal, and neurologic involvement. In the majority of MEC patients, hypocomplementemia and circulating rheumatoid factors can be detected. Whereas in Mediterranean countries more than 80% of the patients are positive for HCV RNA as detected by polymerase chain reaction, it is assumed that MEC in Northern European countries is less often associated with HCV. Cryoprecipitates in patients with HCV-associated MEC may contain both HCV and specific antibodies to HCV. Furthermore, HCV has been demonstrated in vasculitic skin lesions.[13]

■ MECHANISMS BY WHICH INFECTIOUS AGENTS TRIGGER VASCULITIS

Different mechanisms may be operative in the induction of vasculitis by infectious agents.[6] Three mechanisms are most likely to be involved: a) direct microbial invasion of endothelial cells; b) participation in immune-complex mediated damage of vessel walls; and c) stimulation of (auto-reactive) B and/or T lymphocytes.

A. Direct microbial invasion

Rickettsiae are responsible for Rocky Mountain spotted fever, a disease which is characterized by a vasculitic rash. Rickettsiae primarily infect the endothelium of the microvasculature and later on also endothelial cells of small arteries and veins. Another example of microbial invasion of endothelial cells is *S aureus*. It has been demonstrated that *S aureus* binds more readily to endothelial cells than most other bacteria. Following binding, bacteria are internalized and can persist in phagosome-like vacuoles as small colony variants. The interaction between *S aureus* and endothelial cells may result in activation of the endothelial cells resulting in enhanced expression of adhesion molecules such as P-selectin and ICAM-1 and in the production of cytokines and chemoattractants such as IL-8 and MCP-1. Furthermore, endothelial cells may be damaged following internalization of alpha-toxin producing strains.

B. Immune-complex–mediated damage of vessel walls

In biopsies of patients with vasculitis, deposits of immunoglobulins and complement components are often found. The nature of the antigen is, however, in most cases unknown. In skin biopsies with vasculitis due to MEC, HCV has been identified.[13] Since electrical charge is an important factor for antigen deposition, we studied the possibility that cationic staphylococcal antigens may be involved in immune-complex formation in WG. We found that one of these proteins, staphylococcal acid phosphatase (SAcP), had in vitro high affinity for endothelial cells and that renal perfusion of SAcP in SAcP-immunized rats resulted in a severe crescentic glomerulonephritis. Furthermore, antibodies to SAcP were frequently detected in patients with WG, and SAcP was present in 3 of 19 renal biopsies from patients with WG.[14] From these studies, we hypothesized that in WG immune complexes play a role in the initiation of the disease and that staphylococcal antigens are likely candidate antigens.

C. Stimulation of (autoreactive) B and/or T lymphocytes

Infections may stimulate autoimmune responses by different mechanisms.[6] These include shared epitopes between pathogens and host, upregulation of heat shock proteins, and stimulation of lymphocytes by factors such as peptidoglycan, protein A, CpG motifs in bacterial DNA, and superantigens. Superantigens are extremely potent activators of lymphocytes. Stimulation of T cells is dependent on the presence of MHC class II molecules on antigen-presenting cells. In contrast to classical T-cell

antigens, processing of the superantigens is not needed. Superantigens bind to MHC class II molecules on antigen-presenting cells and to conserved regions of T-cell receptor V-beta chains. Virtually all T cells expressing a superantigen-binding V-beta chain proliferate. After proliferation, activated T cells undergo apoptosis. Furthermore, repetitive stimulation may induce anergy, a process that is possibly dependent on stimulation of CD4+ regulatory T cells. Superantigens may induce autoimmunity by stimulation of autoreactive cytotoxic T cells and/or by T-cell–dependent activation of antigen-specific B cells. In Kawasaki disease vasculitic disease activity, TSST-1 producing *S aureus*, and the presence of corresponding V-beta 2+ T cells have been simultaneously documented.[6] In patients with WG, a condition in which T-cell expansions and staphylococcal superantigens are frequently found, we failed to show superantigen-related T-cell expansions.

CONCLUSION

Infectious agents have been clearly demonstrated in various vasculitides. Direct evidence of a pathophysiological role of specific microbial agents is, however, scarce. Recently developed molecular approaches such as DNA microarrays may be helpful for studying this issue in the near future.

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4-093

STAPHYLOCOCCAL TOXIC-SHOCK-SYNDROME-TOXIN-1 (TSST-1) IS A RISK FACTOR FOR DISEASE RELAPSE IN WEGENER'S GRANULOMATOSIS

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Nasal carriage of *Staphylococcus aureus* has been identified as a risk factor for disease relapse in Wegener's granulomatosis (WG). We hypothesized that local immunostimulation by secreted staphylococcal superantigens (SAg) may be responsible for this association.

We investigated the presence of *S aureus* and staphylococcal SAg in relation to the occurrence of relapses in a cohort of 63 patients with WG followed at our clinic (follow-up 53 ± 25.2 months). Patients were seen every 6 weeks and evaluated for signs and symptoms of active WG. A nasal swab culture was performed at every visit to detect *S aureus*. *S aureus* was identified by standard techniques. *S aureus* DNA was extracted and analyzed by multiplex PCR for the presence of genes encoding for staphylococcal exotoxin A to E (sea-see), exfoliative toxin-A (eta), and toxic-shock-syndrome-toxin 1 (tsst-1). Cox proportional hazards analysis of time to first relapse as dependent variable and presence or absence of *S aureus* and SAg as time-dependent covariates was used to analyse the association between *S aureus* and SAg and disease activity of WG. Results are expressed as relative risk (RR) with 95% confidence interval.

Of 1711 nasal cultures taken (mean 14, range 4 to 51 per patient), 709 were positive for *S aureus* (41%). Of these 709 *S aureus* isolates, 326 (46%) were positive for ≥1 SAg gene, most frequently sea (48%), followed by tsst-1 (37%), and sec (19%). Relapse of WG occurred in 35 patients (renal involvement in 19). In 27 of the 35 first relapse episodes, *S aureus* had been cultured in the 3 months preceding the relapse. Compared to the absence of *S aureus*, presence of a SAg-negative and SAg-positive *S aureus* were associated with a RR of 2.26 (0.99-5.14; p=.054) and 2.88 (1.17-7.07; p=.021) for relapse within 3 months, respectively. Analysis of individual SAg genes showed that only tsst-1 was associated with a significant risk for relapse (RR 13.36, 4.19-42.62; p<.001). The results were not different when the analysis was restricted to the 52 patients with at least 1 nasal culture positive for *S aureus*.

Conclusion: The association of nasal carriage of *S aureus* and relapse of WG was confirmed. Furthermore, the risk for relapse is modulated by the presence or absence of the staphylococcal tsst-1 gene, suggesting a possible pathogenic role for this superantigen in disease activation of WG.

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CHLAMYDIA PNEUMONIAE AND GIANT-CELL ARTERITIS: FAILURE TO DETECT *CHLAMYDIA PNEUMONIAE* IN TEMPORAL ARTERY BIOPSIES BY POLYMERASE CHAIN REACTION IN 90 CASES AND 90 CONTROLS

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Purpose: To examine the reported correlation between the presence of *Chlamydia pneumoniae* in temporal artery biopsy specimens and the diagnosis of temporal arteritis (TA).

Methods: We reviewed reports of all the temporal artery biopsies performed at our institution between 1968 and 2000, identifying 90 possible cases of TA. Seventy-nine of the biopsy specimens (88%) demonstrated giant cells. The other 11 cases (12%) had other histopathological features compatible with TA. Through a rigorous chart review, we confirmed that all 90 patients with positive biopsies met the 1990 American College of Rheumatology classification criteria for TA. We chose controls from the group of individuals who had negative temporal artery biopsies during the same time 32-year period. We reviewed the charts of potential controls to ensure that their post-biopsy courses were not compatible with TA, and matched one control to each case on 3 variables: gender, year of biopsy, and age within 10 years. The biopsies of all cases and controls were re-evaluated in a masked fashion by an experienced eye pathologist; all of the original readings were confirmed. Following de-paraffinization of the samples and DNA extraction, PCR analyses were performed for C *pneumoniae* on the 180 samples. We used 2 CDC-recommended sets of PCR primers (which target 2 different genes) for C *pneumoniae*. A primer set targeting the *ompA* gene (CP1-CP2/CPC-CPD) was used to perform a nested PCR, followed by confirmation of the findings with primers targeting the 16S rRNA gene in a touchdown enzyme, time-released PCR (CPN90/CPN91). We used positive and negative controls as well as controls made from infected and non-infected Hep-2 cells, suspended in a formalin-fixed, paraffin-embedded matrix.

Results: The results of PCR analyses are shown in the table below.

PCR primer set	Cases (positive TA bx) N = 90	Controls (negative TA bx) N = 90
ompA gene	1 (1.1%)	1 (1.1%)
16S rRNA gene	0	0

Conclusions: The results of this comprehensive study, which involved a large number of biopsy-proven cases of TA and matched controls and employed sensitive and specific PCR analyses, do not support an association of C *pneumoniae* in the pathogenesis of TA.

Pathogenic mechanisms in giant cell arteritis

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Giant cell arteritis (GCA) is a systemic vasculitis affecting medium-size and large arteries. In contrast to vasculitides of capillaries and small arteries, the inflammation does not accumulate in the perivascular space but directly attacks structures that are part of the arterial wall.[1] Besides the vascular lesions, an intense systemic inflammatory syndrome characterizes GCA. This systemic inflammatory syndrome also exists in the absence of fully developed vascular inflammation in patients diagnosed with polymyalgia rheumatica (PMR). Patients with PMR can have a forme fruste of vessel wall inflammation that escapes detection by histomorphology and requires molecular methods to document activation of immunopathways in the artery.[2]

Despite the systemic character of GCA, inflammatory injury of blood vessel walls is not a random process. To the contrary, this arteritis displays a stringent tissue tropism with clear preference for specific arterial trees. Intriguingly, the extracranial branches of the carotid arteries (superficial temporal, occipital, ophthalmic and posterior ciliary arteries), the vertebral arteries, and the subclavian arteries are most frequently affected. Also, the aortic wall, itself, is a well-recognized site of GCA. Coronary arteritis has been described and femoral arteries can be involved, but the likelihood that these vascular territories are attacked by the inflammatory disease is low. Most impressively, GCA essentially spares the intracranial arteries and GCA in mesenteric arteries is extremely unlikely. The mechanisms underlying the selective targeting of vascular beds are not understood, but molecular and structural characteristics of the arteries must play a role in defining susceptibility towards intrawall inflammation. A selection of structural features displayed by GCA-susceptible arteries is listed in **Table 1**. Molecular approaches will be necessary to identify the underlying principles of how molecular composition can translate into tissue tropism.

Vascular manifestations in GCA are a reflection of the affected vascular beds. Reduction in blood flow in the extracranial arteries causes jaw claudication, tongue claudication, scalp tenderness, or transient ischemic attacks. If the compromise of flow is severe enough, the patient will present with ischemic optic neuropathy, stroke, or, infrequently, with myocardial infarction or gangrene. Vascular complications of aortitis are generally related to aneurysm formation and aortic insufficiency.[3] Because of the irreversibility of ischemic tissue damage and the risk to vision and the central nervous system, GCA qualifies as a medical emergency. However, it is important to note that most of the clinical manifestations of GCA are not a direct reflection of arteritis but rather are connected to the syndrome of systemic inflammation, manifesting with a profound acute phase response, fever, anorexia, malaise, and myalgias.

UNCONTROLLED INTIMAL HYPERPLASIA – THE PATHOGENIC LESION OF GCA

Focal arteritic lesions in extracranial arteries do not cause rupture and hemorrhage but instead cause vasoocclusion with subsequent ischemia. Luminal thrombosis is uncommon. Stenosis and occlusion are generated by excessive intimal hyperplasia, a process in which myofibroblasts migrate towards the lumen, settle in the subendothelial layer, begin to proliferate, and deposit extracellular matrix. Intimal hyperplasia is not unique to the vascular injury pattern in GCA. To the contrary, it is a shared mechanism in a diverse set of vasculopathies, including atherosclerotic disease and vascular-occlusive syndrome in transplantation. Of all the different mechanisms leading to vascular injury **(Table 2)**, uncontrolled hyperproliferation of the intimal layer produces clinically the most significant consequences of GCA, particularly blindness and stroke. As such, this mechanism has a key position in the pathogenic events of this arteritis.

As a rule, hyperplasia of the intima never precedes the accumulation of inflammatory cells in the arterial wall. From that timely relationship, it follows that T cells and macrophages infiltrating into the layers of the artery's wall directly or indirectly regulate the process of myofibroblast proliferation and matrix deposition. Studies in temporal arteries from patients with GCA have established that platelet-derived growth factor (PDGF) is a critical growth factor in arteries with lumenal occlusion.[4] PDGF is sup-

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TABLE 1
CHARACTERISTICS OF ARTERIES TARGETED BY GCA

- Topography—extracranial carotid branches, vertebral arteries, and upper extremity arteries
- Well-developed elastic membranes
- Multilayered wall
- Vasa vasorum network

plied from several different cellular sources, the most significant being multinucleated giant cells and macrophages located at the media-intima border. These cells not only produce PDGF, they also provide vascular endothelial growth factor (VEGF).[5] This angiogenesis factor appears to be required in supporting structural adaptations necessitated by the hyperplastic intima, specifically the growth of neocapillaries. The release of PDGF and VEGF from giant cells and macrophages emphasizes the central position of macrophages in mechanisms of vascular injury. The question then arises how macrophages are triggered to secrete potent growth and angiogenesis factors.

VEGF production in the arterial wall has been closely correlated with the concentration of interferon (IFN)-γ in the tissue.[5] Not all patients develop lumenal stenosis; in some patients, the intima is only minimally thickened and blood flow is not compromised. The subset of patients with clinically relevant loss of blood flow, identified by ischemic manifestations, is characterized by production of large amounts of PDGF and VEGF in the tissue. Other tissue cytokines associated with clinical evidence of compromised blood flow are IFN-γ and interleukin (IL)-1β.[6] If jaw claudication and visual symptoms occur in patients who generate higher tissue concentrations of IL-1β, IFN-γ, VEGF, and PDGF, it is likely that these mediators are mechanistically linked. One possible link derives from the fact that PDGF and VEGF are found in multinucleated giant cells. Such specialized macrophages are most frequent in IFN-γ–containing tissue samples. This finding suggests that it is not macrophages but IFN-γ–producing cells that are the primary decision makers in injury-induced intimal hyperplasia.

IFN-γ has been traced back to a relatively small population of T lymphocytes that lie in the adventitia of the inflamed artery.[7] IFN-γ–producing T cells undergo clonal expansion in the artery, are highly selected, and boost the disease process when adoptively transferred.[8,9] Their eradication eliminates the disease, yet IFN-γ production has been shown to be relatively resistant to standard corticosteroid treatment.[10] Recent data have indicated that IFN-γ production in the artery is susceptible to aspirin, providing this non-steroidal anti-inflammatory drug a possible role as a steroid-sparing agent in GCA.[11] IFN-γ–producing T cells are far removed from the growth factor–producing macrophages at the media-intima junction. Possibly, cells or matrix molecules serving as intermediaries are involved in transporting the information from the adventitia to the proximal layers of the arterial wall. Alternatively, it has been proposed that fibroblasts positioned in the adventitia are the precursors of the cells that migrate towards the intima to become hyperplastic myofibroblasts. These myofibroblasts, which compose the expanding intima, may receive their initial instructions directly from activated T cells.

TABLE 2
CELLULAR AND MOLECULAR TARGETS IN GCA

Cellular target	Effect	Pathogenic consequence
Endothelial cells	Activation	Increased adhesiveness, increased cell recruitment
Smooth muscle cells	Smooth muscle cell death	Media degeneration
Myofibroblasts	Dedifferentiation (secretory → migratory)	Intimal hyperplasia
Elastic membranes	Loss of contractility, fragmentation, loss of tissue compartments	Myofibroblast migration

Based on these data, we have proposed that the formation of multinucleated giant cells is under the control of the adaptive immune system through the release of IFN-γ.[12,13] Once formed, these multinucleated giant cells provide PDGF, thus fueling the process of intimal proliferation. Adaptive immune responses in inflamed temporal arteries do not necessarily lead to giant cell formation, growth factor secretion, and lumenal occlusion. In patients whose clinical presentation is dominated by PMR, temporal artery specimens predominantly contained the T-cell cytokine IL-2 and not IFN-γ.[6] Differences in cytokine production are not a reflection of the stage of disease. Patients who presented with primarily subclavian GCA and who had disease for several months before the biopsy was performed and the diagnosis was established, had non-occlusive disease in the temporal arteries and preferential production of IL-2.[14] The different cytokine response pattern regulating the extent of intimal hyperplasia may reflect genetic host factors. As an additional host factor, the vascular response to the inflammatory injury may be genetically diverse and may or may not induce the proliferation of myofibroblasts and the formation of intimal hyperplasia.

■ ARTERIAL MEDIAL WALL DESTRUCTION – A PREREQUISITE FOR INTIMAL HYPERPLASIA

Gene expression profiling of temporal arteries of patients with GCA has been used to identify novel pathways of tissue injury. This approach demonstrated a gene cluster related to oxidative damage that was upregulated in inflamed arterial specimens when compared with normal ar-

terial specimens. One of the first signs for oxidative stress derived from the observation that mitochondrial genes were expressed at extremely high levels in the arteritic lesions.[15] Immunohistochemical confirmation revealed that mitochondrial activation was particularly evident for macrophages in the medial layer. Considering the potential damage by oxygen radicals released in the medial layer, specific antibodies to lipid peroxidation products (4 hydroxy-nonenal) were used to examine smooth muscle cell membrane damage. Toxic aldehydes were detected on the surface of smooth muscle cells, often with widespread expression throughout the muscle cell layer. Inflammatory cells sitting between the smooth muscle cells were not entirely protected from oxidative attack.

Macrophages that served as the cellular source of reactive oxygen species were also shown to be specialized in the production of matrix metalloproteinase (MMP)-2.[15] Such macrophages coexpressing MMP-2 and high levels of mitochondrial antigens were localized directly adjacent to the disintegrated elastic lamina and may be pivotal in the formation of intimal hyperplasia.[16] They are equipped with two powerful mechanisms of tissue attack. They oxidize macromolecules, including matrix proteins, lipids, proteins, and DNA, thus mediating profound cellular damage. They also digest matrix molecules and cell membranes through the release of proteolytic enzymes. Such proteolytic enzymes are almost certainly needed to fragment the elastic membranes that separate the intima and the media and form a border between the media and adventitia. Fragmentation of the elastic lamina interna is often considered a histologic hallmark of GCA.

Myofibroblasts must be mobilized from their neighboring cells and matrix. On their path to the subendothelial space, they migrate through the tissue. An intact lamina elastica interna would be a barrier unless this membrane has been digested to allow for the passage of myofibroblasts. The MMP–and oxygen intermediates–producing macrophages in the media thus gain a key position in the events leading to the hyperplastic reaction of the intima.

■ PROTEIN NITRATION AS A MECHANISM OF TISSUE INJURY IN GCA

Most avenues of tissue destruction in GCA depend on macrophage effector functions, with T-cell derived cytokines controlling the activity/differentiation of such macrophages. Inflammation-related upregulation of macrophage products includes inducible nitric oxide synthase, NOS-2.[16] In vitro, NOS-2 can be induced by proinflammatory cytokines such as IFN-γ, IL-1, TNF-α, and IL-2. NOS-2 was found in wall-infiltrating macrophages, specifically in those homing to the hyperplastic intima. The presence of NOS-2 has raised the question whether protein nitration contributes to vascular disease damage in GCA. The reactive nitrogen intermediate, peroxynitrite, can nitrate tyrosine residues, leading to protein dysfunction. Expression of nitrated proteins was examined in temporal arteries (Borkowski A, Younge BR, Szweda L, Mock B, Björnsson J, Moeller K, Goronzy JJ, Weyand CM. Am J Pathol, in press). Surprisingly, little evidence for NOS-2–associated nitration was detected. Structures in the intima remained free of this protein modification. Nitrotyrosine was found on endothelial cells of microcapillaries in the media. Such capillaries emerge by inflammation-induced neoangiogenesis; under physiologic conditions, the media is avascular. We hypothesized that the selective nitration of endothelial targets in the media resulted from an interaction between reactive nitrogen and oxygen intermediates. Reactive oxygen species are characteristically present in the media. We depleted the cellular source of such radicals by treating temporal artery grafts in SCID mice with cell-specific antibodies. Elimination of macrophages disrupted nitrotyrosine formation, confirming that oxygen radical formation by macrophages was crucial in the process of nitration. Of note, these medial macrophages did not express NOS-2, and the NO derived from NOS-3–expressing endothelial cells. The functional consequences of nitration in medial endothelial cells need to be investigated. Tyrosine nitration may harm tyrosine phosphorylation pathways critically involved in intracellular signaling. Changes in adhesion molecule expression or alterations in the secretory activity of endothelial cells could be envisioned. The remarkable selectivity of nitration, sparing intimal and adventitial capillaries, suggests heterogeneity of microvessels in the disease.

■ MOLECULAR PATHWAYS COUNTERACTING TISSUE DAMAGE IN GCA

The inflammation-induced injury in GCA causes cell death, loss of microstructures, and functional impairment of the artery. It also initiates a response pattern of resident arterial cells that attempts to prevent and repair the damage.[1] Intimal hyperplasia is a maladaptive response-to-injury, intended to rebuild wall structures, but instead leading to excessive tissue production. Some of the reactions induced by the injury, however, will be protective and limit the negative consequences of inflammation. One such protective response, the upregulation of aldose reductase, has been studied in detail.[17]

Gene profiling of inflamed temporal arteries demonstrated abundant expression of aldose reductase. Transcription and tissue expression of aldose reductase occurred exclusively in arteries with characteristic inflammatory infiltrates. Aldose reductase is an oxidoreductase with broad substrate specificity for carbonyl compounds. Immunohistochemical studies revealed that the enzyme was produced by smooth muscle cells and by T cells and macrophages that infiltrated into the medial layer. A close relationship between the presence of lipid peroxidation products and aldose reductase was discovered. Given the substrate specificity of the reductase for carbonyl substrates, aldose reductase was examined as a possible detoxification system for toxic aldehydes formed during the process of lipid peroxidation. We experimentally approached this question by treating temporal artery-SCID mouse chimeras with aldose reductase inhibitors. After enzyme blockade, the amount of toxic aldehydes increased in the artery and the frequency of apoptotic medial smooth muscles cells increased threefold. We concluded that induction of aldose reductase is a protective

mechanism in that the enzyme metabolizes toxic aldehydes and protects cells from oxidative damage. This mechanism could prove therapeutically useful, particularly when considering that oxidative stress of the media is critical in initiating intimal hyperplasia.

TISSUE INJURY IN THE ATHEROSCLEROTIC PLAQUE – A NEW PARADIGM FOR ACUTE CORONARY SYNDROMES

Inflammation-associated degradation of tissue in arteritic lesions is expected, considering the intensity of the cellular infiltrate and the granulomatous reaction. Tissue damage in the arterial wall, mediated by T cells and macrophages, however, is not limited to frank vasculitis. Atherosclerosis, traditionally understood as a degenerative disease with mechanical factors dominating pathogenesis, is now emerging on the list of immune-mediated syndromes.[18] Compelling evidence has accumulated that the sudden rupture of the atherosclerotic plaque giving rise to atherothrombosis and luminal occlusion causes acute coronary syndromes. The precise events leading to the surface defect in the atherosclerotic plaque are not understood. However, detailed work has demonstrated that plaque rupture occurs in lesions that are infiltrated by inflammatory cells, including T cells and macrophages. Parallel to the scenario in GCA, it is to be expected that effector macrophages, by virtue of the wide spectrum of mediators they can release, have a role in injuring the cap overlying the atherosclerotic plaque. Metalloproteinases have been suspected to contribute to plaque destabilization. Oxidative damage could also participate in cellular and matrix damage, leading to erosion of the plaque cover. As in the arteritic infiltrate characteristic of GCA, the ultimate question relates to the signals that orchestrate macrophage recruitment, function, and activation.

We have examined T-cell populations recruited to the unstable plaque.[19] Plaque harvested by atherectomy or after fatal myocardial infarction contains T cells that have undergone clonal expansion and can be identified by the unique sequence of their T-cell receptor. The most interesting population so far contains CD4 T cells that have lost expression of the CD28 surface receptor. $CD4^+CD28^-$ T cells share with classic helper T cells the memory phenotype, yet they display a profile of surface receptors that distinguishes them from classic helper T cells. CD28-deficient CD4 T cells efficiently release IFN-γ. They expand to large clonal size and routinely circulate in the blood of the patient. By gene profiling, they were found to express the cytotoxic molecules perforin and granzyme B, which they use to induce death of target cells. $CD4^+CD28^-$ T cells display several defects in apoptosis, explaining why they expand to gigantic clonal size. High frequency $CD4^+CD28^-$ T cells is a biological marker for patients with unstable angina and is rarely found in patients with stable coronary artery disease.[20] The association between $CD4^+CD28^-$ T cells and plaque instability has given rise to the notion that these T cells may be directly involved in plaque inflammation and plaque erosion.

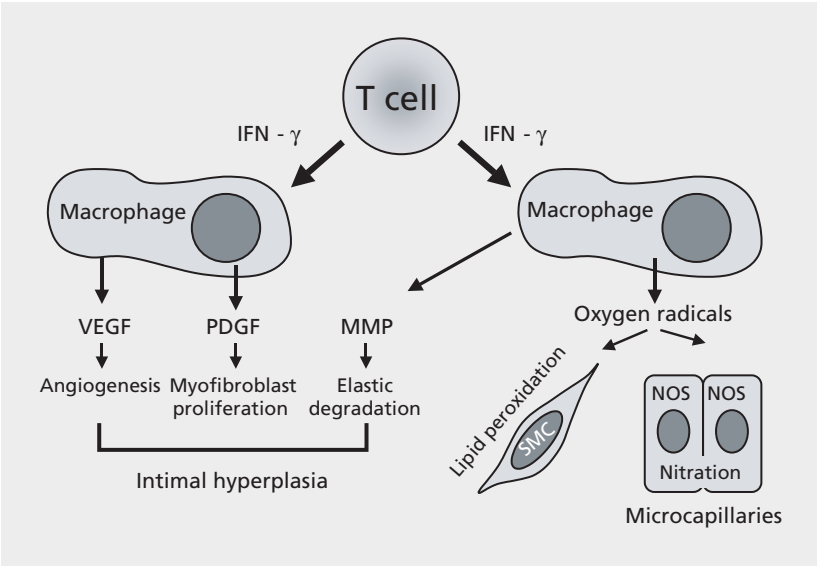

Figure 1. Mechanisms of vascular injury in GCA.

Potentially disease-relevant functions of $CD4^+CD28^-$ T cells in acute coronary syndromes have been carefully studied. In the peripheral blood, functional activity of $CD4^+CD28^-$ T cells seems to be predominantly related to their ability to secrete IFN-γ. Monocytes freshly isolated from patients with unstable angina have a molecular fingerprint of recent stimulation of the IFN-γ receptor with nuclear translocation of STAT-1 duplexes.[21] As a consequence, STAT-1–driven genes such as the chemokine IP-10 and the Fc receptor CD64 are upregulated.

Recent findings suggest that, in the arterial wall, $CD4^+CD28^-$ T cells may have yet other functional activities, those of cell-mediated cytolysis. In patients with unstable angina, $CD4^+CD28^-$ T cells assemble a cytolytic machinery and acquire cytotoxic capabilities. T-cell clones isolated from patients with acute coronary syndrome can effectively kill endothelial target cells. Endothelial injury is amplified in the presence of physiologic concentrations of CRP, suggesting a possible interaction between acute phase proteins and T lymphocytes in tissue damage.[22] How could endothelial death, induced by CD4 T lymphocytes, endanger the integrity of the atherosclerotic plaque?[23] As in GCA, atherosclerotic disease is associated with neoangiogenesis. Newly formed capillaries supply oxygen to the thickened wall. Injury to these microvessels could disrupt supply to the relatively hypoxic plaque tissue and induce ischemic damage.

SUMMARY

T lymphocytes, encountering stimulatory signals in the adventitia of medium-size arteries, emerge as the key players in inflammation-associated injury pathways. In GCA, all injury mechanisms have been related to effector

macrophages. Regulated by IFN-γ–producing T cells, macrophages commit to distinct avenues of differentiation and acquire a spectrum of potentially harmful capabilities **(Figure 1)**. Macrophages in the adventitia focus on production of pro-inflammatory cytokines. Macrophages in the media specialize in oxidative damage with lipid peroxidation attacking smooth muscle cells and matrix components. These macrophages also supply reactive oxygen intermediates that, in combination with nitrogen intermediates, cause protein nitration of endothelial cells. Production of oxygen radicals is complemented by the production of metalloproteinases, likely essential in the breakdown of elastic membranes. With the fragmentation of the internal elastic lamina, the intimal layer becomes accessible to migratory myofibroblasts that, driven by PDGF, form a hyperplastic intimal layer and cause occlusion of the vessel lumen. Expansion of the hyperplastic intima is accompanied by intense neoangiogenesis, supported by angiogenesis factors that again derive from specialized macrophages.

Similarities in injury pathways between GCA and another arterial disease, atherosclerosis, are beginning to be recognized. Specifically, activated T cells and macrophages are increasingly appreciated as key players in the process of instability and rupture of atherosclerotic plaque. A specialized subset of CD4 T cells, $CD4^+CD28^-$ T cells, are suspected to participate in tissue injury in the plaque. These T cells are equipped with cytolytic capabilities and release large amounts of IFN-γ. Comparative studies between patients with GCA and those with acute coronary syndromes should enhance our ability to define the principles of arterial wall inflammation, the specifics of injury in that microenvironment, and help in the identification of the eliciting signals.

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Implications for pathogenesis of patterns of injury in small- and medium-sized-vessel vasculitis

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Systemic vasculitis can be categorized as large-vessel vasculitis, medium-sized-vessel vasculitis, and small-vessel vasculitis based on the predominant vascular distribution of vasculitic lesions **(Figure 1)**.[1] Large-vessel vasculitis is chronic granulomatous arteritis that affects predominantly the aorta, major arteries to the extremities and head, and major visceral arteries. Medium-sized vessel vasculitis is necrotizing arteritis that affects predominantly major visceral arteries. Small-vessel vasculitis is necrotizing polyangiitis that has a predilection for capillaries and venules, but also may affect arterioles, arteries, and even veins.

The chronic granulomatous inflammation of large-vessel vasculitis is pathologically very different from the necrotizing inflammation that is the hallmark of the acute phase of medium-sized-vessel vasculitis and small-vessel vasculitis.[2] This implies that the pathogenesis of large-vessel vasculitis is quite different from the pathogenesis of medium-sized-vessel vasculitis and small-vessel vasculitis. Although medium-sized-vessel vasculitis and small-vessel vasculitis share some pathologic features, for example necrotizing arteritis, there are a number of overt or subtle pathologic features that indicate the engagement of different pathogenic mechanisms in specific categories of vasculitis within these larger groups. In order to shed light on their pathogenesis, this review will compare and contrast the pathologic features of different categories of necrotizing vasculitis.

POLYARTERITIS NODOSA VERSUS KAWASAKI DISEASE

Polyarteritis nodosa and Kawasaki disease are the two major categories of medium-sized-vessel vasculitis.[1,2] In the past, there was confusion about the relationship between polyarteritis nodosa and Kawasaki disease; in fact, the latter was once called infantile polyarteritis nodosa. However, by the late 1970s, polyarteritis nodosa and Kawasaki disease were recognized as separate forms of vasculitis, not only because of different clinical and epidemiologic features but also because of different pathologic features.[3,4] Both categories of vasculitis have acute necrotizing arteritis with inflammatory aneurysm formation; however, Kawasaki disease is clearly distinguished from polyarteritis nodosa by the presence of the mucocutaneous lymph node syndrome in the former but not the latter.[1]

Polyarteritis nodosa was first described by Kussmaul and Maier in 1866.[5] This category was initially used as a waste basket for all types of necrotizing arteritis, and thus would have been the diagnosis often used for patients with Kawasaki disease and necrotizing arteritis with aneurysms prior to Kawasaki's landmark publication in 1967.[6] Many other distinct forms of vasculitis that have a component of necrotizing arteritis also once were included in the polyarteritis nodosa category but now are recognized as pathologically and pathogenically distinct vasculitides. For example, as will be discussed in more detail later, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome were initially considered to be variants of polyarteritis nodosa but now are recognized as distinct from polyarteritis nodosa.[7]

Hints about pathogenesis of polyarteritis nodosa are more likely to be found in the acute rather than the chronic vascular lesions. Both polyarteritis nodosa and Kawasaki disease arteritis, as well as other forms of necrotizing arteritis, all enter a final common pathway of chronic inflammation and scarring. The transformation of the active acute inflammatory lesions to sclerotic lesions with a predominance of infiltrating T-lymphocytes and macrophages can occur as quickly as one or two weeks after the initiation of injury. Thus, very early lesions must be examined to identify evidence for primary pathogenic events.

Polyarteritis nodosa begins as a segmental necrotizing inflammation of arteries with conspicuous infiltration of neutrophils and monocytes, often with leukocytoclasia, and sometimes with superimposed thrombosis.[2,8,9] This

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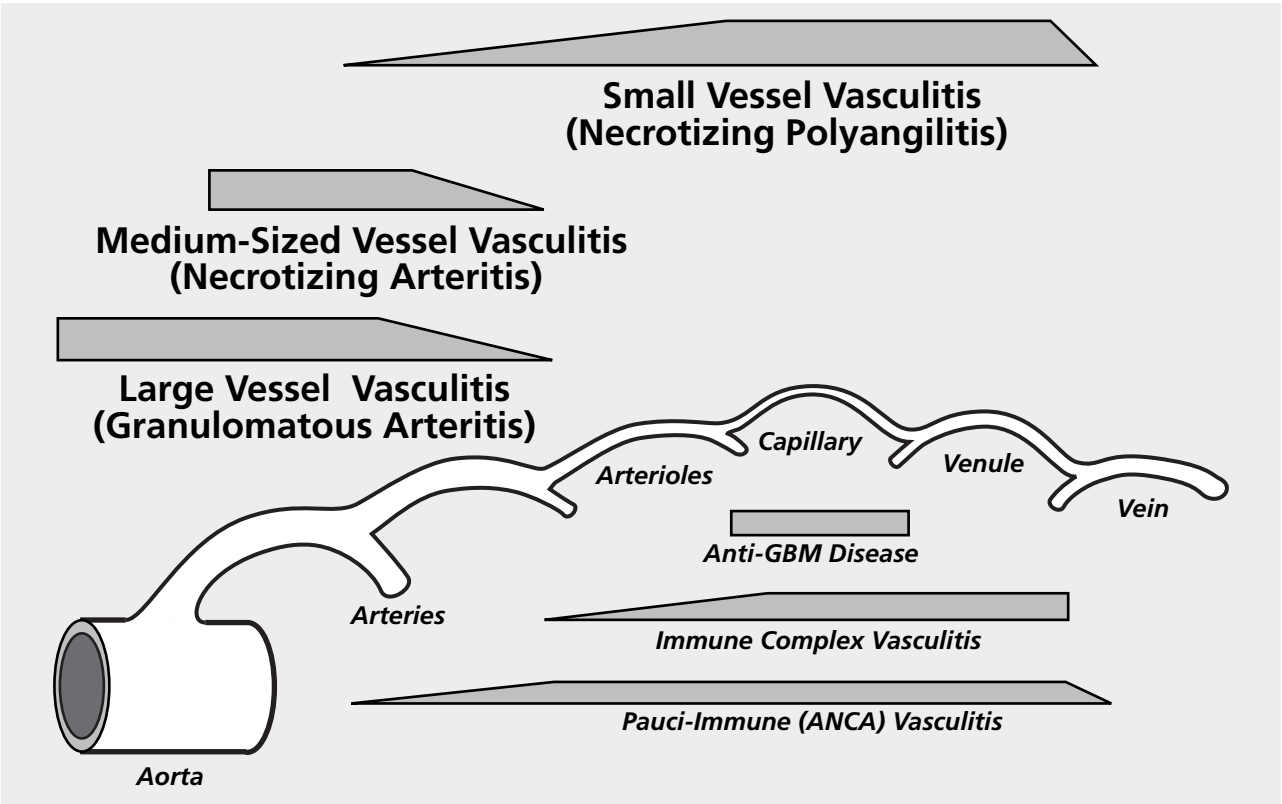

Figure 1. Diagram depicting the predominant vascular distribution of different categories of vasculitis. Note that large-vessel vasculitis, medium-sized-vessel vasculitis and small-vessel vasculitis can all affect arteries; however, only small-vessel vasculitis affects capillaries and venules. Anti-GBM disease, immune complex vasculitis and pauci-immune vasculitis all are types of small-vessel vasculitis, but they have different distributions of vessel involvement because of differences in their pathogenesis.

may progress to extensive circumferential, transmural fibrinoid necrosis **(Figure 2)**. Fibrinoid necrosis is a nonspecific pattern of acute necrotizing injury that is shared by all forms of necrotizing vasculitis.[10] Fibrinoid necrosis is characterized by the accumulation of plasma proteins, including coagulation factors that are converted to fibrin, at sites of tissue destruction. Over time, tissue matrix proteins infiltrate the fibrinous material, and it eventually is completely replaced by collagenous scar. If the necrotizing inflammation causes extensive destruction of the vessel wall and perivascular tissue, inflammatory aneurysms (pseudoaneurysms) will develop.

The histologic features of early lesions of polyarteritis nodosa suggest that focal activation of neutrophils and monocytes at the interface of blood and artery is an early pathogenic event and that leukocyte activation results in transmural infiltration of the artery wall and necrotizing injury. Once the injury enters the wall it may dissect longitudinally along the media or adventitia; thus, at some planes of section it may appear to be arising in the media or adventitia rather than adjacent to the lumen.

Kawasaki disease is characterized by the presence of the mucocutaneous lymph node syndrome, which was first described by Kawasaki in 1967.[6] The pathology of Kawasaki disease vasculitis was thoroughly described by the late 1970s.[11-14] The classic gross lesion is the inflammatory arterial aneurysm, often complicated by thrombosis. Although the coronary arteries are the most frequently involved vessels, arteries throughout the body may be involved, such as the iliac and femoral arteries, the renal arteries, arteries to the gut, and even veins. The inflammatory aneurysms are not optimum for studying the etiology and pathogenesis of Kawasaki disease because they occur relatively late in the evolution of acute injury. The earliest lesion, or at least one of the earliest lesions, is the focal accumulation of leukocytes beneath endothelial cells in arteries. These are predominantly monocytes and macrophages with some admixed neutrophils and T lymphocytes.[15,16] As the lesion progresses, there is transmural infiltration by mononuclear leukocytes and progressive edema and smooth muscle cell degeneration in the media **(Figure 3)**. Ultimately, the infiltrates extend completely through the media and into the adventitia. In the adventitia, as with polyarteritis nodosa, the infiltrates sometimes extend longitudinally along vessels. Thus, in some cross sections, the infiltrates appear to be adventitial (perivascular) rather than transmural. The infiltrating cells are predominantly macrophages with admixed T lymphocytes, predominantly CD8 T lymphocytes.[16] Although CD 20–positive B cells are infrequent, IgA-producing plasma cells may be present.[17] As the lesions progress there is more and more destruction of the media, infiltration by leukocytes, and eventually aneurysm formation if the injury is severe enough.

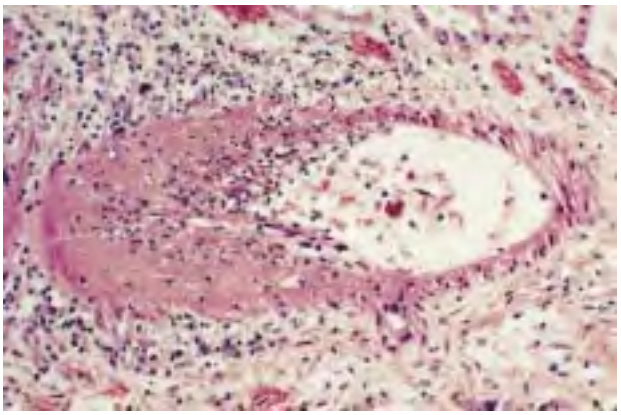

Figure 2. This necrotizing arteritis is consistent with a number of different categories of vasculitis, including polyarteritis nodosa, microscopic polyangiitis, and Wegener's granulomatosis. The wall of the artery on the right is relatively uninvolved, but the wall to the left has been completely replaced by fibrinoid material that contains focal accumulations of neutrophils and monocytes in varying stages of necrosis and apoptosis.

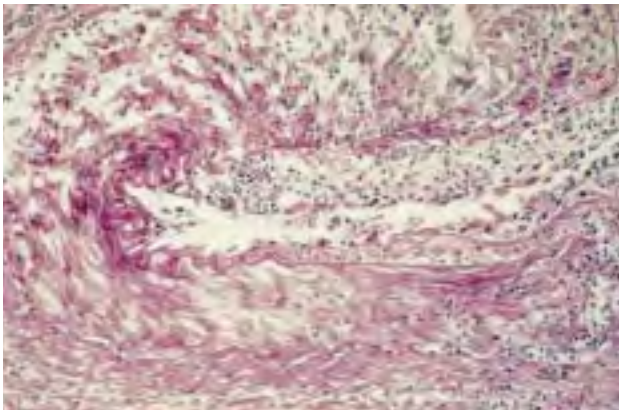

Figure 3. This necrotizing arteritis is from a patient with Kawasaki disease. The intima and muscularis of the upper half of the artery have marked edema with extensive infiltration by mononuclear cells. Note that there are no neutrophils and no fibrinoid necrosis. The different appearance of this arteritis compared to the arteritis in Figure 2 suggests a different pathogenesis.

Thus, the vasculitis of Kawasaki disease shares some pathologic attributes with polyarteritis nodosa; however, it has a number of distinctive characteristics that clearly set it apart, including the association with mucocutaneous lymph node syndrome, a strong predilection for the coronary arteries, and a pattern of inflammation characterized by marked edema and macrophage infiltration and little or no fibrinoid necrosis. This distinctive histology indicates that the pathogenesis of Kawasaki disease arteritis is different from that of polyarteritis nodosa. The numerous macrophages and T lymphocytes and the scarcity of neutrophils suggests that activation of T lymphocytes and monocytes plays a pivotal role in the pathogenesis of Kawasaki disease arteritis. This is supported further by the finding of high levels of monocyte chemoattractant proteins at sites of vasculitis and in the circulation of patients with Kawasaki disease[18] as well as by the presence of activated monocytes in the tissue and circulation.[19]

■ MEDIUM-SIZED-VESSEL VASCULITIS VERSUS SMALL-VESSEL VASCULITIS

As shown in **Figure 1**, a major distinction between medium-sized-vessel vasculitis and small-vessel vasculitis is the predilection of the latter for vessels other than arteries—especially capillaries and venules.[1,7] The arteritis of polyarteritis nodosa is not distinguishable from the arteritis of small-vessel vasculitis by histology alone. In the acute phase, both have fibrinoid necrosis with neutrophil and monocyte infiltration and leukocytoclasia. Polyarteritis nodosa tends to have less leukocyte infiltration and less leukocytoclasia, but this is not consistent enough to be a distinguishing feature. As described earlier, the necrotizing arteritis of Kawasaki disease not only is histologically different from polyarteritis nodosa but also from the necrotizing arteritis of small-vessel vasculitis.

The pathologic feature that most suggests different pathogenic mechanisms in medium-sized-vessel vasculitis versus small-vessel vasculitis is the difference in vascular distribution. Why does medium-sized-vessel vasculitis only affect arteries, and why does small-vessel vasculitis have a preference for capillaries and venules? The answers are unknown; thus any discussion is of necessity speculative. One reasonable conclusion is that the pathogenic events that cause medium-sized vessel vasculitis are operational only in arteries, whereas the pathogenic events that cause small-vessel vasculitis are most effective in very small vessels but occasionally can be accomplished in arteries. Structural or functional differences between small and large vessels, or both, could cause the differences in vascular distribution. Two of many possibilities is that high shear stress is required for the pathogenesis of medium-sized-vessel vasculitis, whereas close physical contact between vessel walls and leukocytes is required for small-vessel vasculitis.

Both polyarteritis nodosa and Kawasaki disease arteritis occur preferentially at arterial branch points.[9,14] For example, Kawasaki disease arteritis in a kidney may involve almost every junction between lobar arteries and arcuate arteries in the absence of any arteritis elsewhere in the kidney.[14] Branch points in arteries are sites of increased sheer stress. Increased sheer stress induces upregulation of endothelial inflammatory factors, such as adhesion molecules (eg, ICAM-1) and proinflammatory transcription factors (eg, NFκB).[20-23] There also are increased numbers of resident macrophages in the intima at sites of sheer stress.[24,25] This is true even in apparently normal arteries. For example, the coronary arteries of over 90% of children under 5 years old have increased numbers of macrophages in the intima of coronary arteries at points of bifurcation.[25] Thus, medium-sized vessel vasculitis may affect only arteries because sheer stress is required to initiate the pathogenic events, for example by producing susceptible foci of activated endothelial cells with underlying macrophages at bifurcation points in arteries. However, there is no proof for this speculation.

TABLE 1
MAJOR CATEGORIES OF VASCULITIS

- Large-vessel vasculitis (chronic granulomatous arteritis)
  - Giant-cell arteritis
  - Takayasu arteritis
- Medium-sized-vessel vasculitis (necrotizing arteritis)
  - Polyarteritis nodosa
  - Kawasaki disease
- Small-vessel vasculitis (necrotizing polyangiitis)
  - Pauci-immune small-vessel vasculitis (ANCA vasculitis)
    - Microscopic polyangiitis
    - Wegener's granulomatosis
    - Churg-Strauss syndrome
    - Drug-induced ANCA vasculitis
  - Immune complex small-vessel vasculitis
    - Henoch-Schönlein purpura
    - Cryoglobulinemic vasculitis
    - Rheumatoid vasculitis
    - Lupus vasculitis
    - Serum sickness vasculitis
    - Infection-induced immune complex vasculitis
    - Drug-induced immune complex vasculitis
    - Hypocomplementemic urticarial vasculitis
    - Behçet's disease
    - Goodpasture's syndrome
  - Paraneoplastic small-vessel vasculitis
  - Inflammatory bowel disease vasculitis
  - Others

Small-vessel vasculitis occurs preferentially in small vessels, especially vessels involved in substantial trafficking of fluid and cells between blood and tissue (eg, dermal venules), blood and urine (glomerular capillaries), or blood and air (pulmonary capillaries). Leukocytes are in close contact with endothelial cells in these small vessels, and the endothelium of these vessels is particularly responsive to proinflammatory signals. Stimulated neutrophils and monocytes are better able to adhere to endothelium in small vessels compared to large vessels once there has been upregulation of leukocyte and endothelial adhesion molecules.[26,27] Adherence is particularly likely if a leukocyte engages adhesion molecules on opposite sides of a vessel, which can only occur in very small vessels.[26] Even within a given microvascular bed, local hemodynamic factors result in some vessels that allow adherence of leukocytes and others that do not.[27] Because leukocyte adherence is probably a prerequisite for vasculitis, this could explain the focal nature of small-vessel vasculitis that is seen pathologically.

IMMUNE COMPLEX SMALL-VESSEL VASCULITIS VERSUS PAUCI-IMMUNE SMALL-VESSEL VASCULITIS

Within the category of small-vessel vasculitis, there are pathologic differences that correlate with different pathogenic events. With respect to vascular distribution, immune complex vasculitis has a more restricted distribution than pauci-immune vasculitis **(Figure 1)**. Pauci-immune vasculitis has a paucity or absence of vessel wall staining for immunoglobulin, and often is associated with anti–neutrophil cytoplasmic autoantibodies (ANCA) in the circulation.[28] Anti–glomerular basement membrane (anti-GBM) disease is a special form of in situ immune complex disease that causes necrotizing vascular injury virtually restricted to glomerular or pulmonary capillaries, or both.

Immune complex vasculitis can be categorized on the basis of clinical and pathologic characteristics into many distinct categories **(Table 1)**. Most immune complex disease has a predilection for glomerular capillaries, dermal venules, and other small vessels, for example arterioles in the intestinal wall. This is the basis for the frequent clinical features of nephritis, purpura, and abdominal pain with many types of immune complex small-vessel vasculitis, for example Henoch-Schönlein purpura, cryoglobulinemic vasculitis, and serum sickness vasculitis.[7] Pauci-immune small-vessel vasculitis, which usually is ANCA small-vessel vasculitis, also has a predilection for capillaries and venules. However, much more often than immune complex vasculitis, it also affects arterioles, arteries, and even veins.[7]

The venulitis and arteritis caused by immune-complex–mediated and pauci-immune small-vessel vasculitis are relatively similar pathologically, although immune complex vasculitis may have identifiable aggregates of immune complexes by light microscopy, especially cryoglobulinemic vasculitis. By immunohistology, immune complex vasculitis has identifiable vessel wall immunoglobulin and complement deposits, whereas, by definition, pauci-immune vasculitis has little or no vessel wall staining for immunoglobulin.[7] The most striking differences in the pathology of immune complex vasculitis versus anti-GBM and ANCA-vasculitis are in the glomerular lesions.[2]

ANCA-vasculitis and anti-GBM disease frequently cause rapidly progressive glomerulonephritis. ANCA-glomerulonephritis and anti-GBM glomerulonephritis are histologically indistinguishable from each other and are characterized in the acute phase by focal segmental lysis of glomerular tufts with disruption of basement membranes and matrix and accumulation of fibrinoid material. Variable numbers of neutrophils and monocytes, often undergoing leukocytoclasia, are seen at the sites of necrosis. As the lesion progresses, over 90% of patients develop crescents in Bowman's spaces as a result of spillage of inflammatory mediators across the ruptured glomerular capillaries, accumulation of macrophages, and proliferation of epithelial cell. Non-necrotic glomerular segments often are remarkably normal histologically. This is in contrast to the immune complex glomerulonephritis that is a component of immune complex vasculitis, such as Henoch-Schönlein purpura or cryoglobulinemic vasculitis. Most immune complex glomerulonephritis has no necrosis or crescent formation, and when crescents are present, they usually affect less than 50% of glomeruli. The localization of immune complexes typically causes glomerular hypercellularity resulting in mesangioproliferative, proliferative, or membranoproliferative glomerulonephritis. Of course, these distinctive differences are most apparent in early lesions. Ultimately, as glomerular inflammatory in-

jury of any type progresses toward resolution or sclerosis, the predominant inflammatory cells are macrophages and T lymphocytes, as is true of any type of chronic inflammation. This can occur relatively quickly. For example, in animal models of necrotizing glomerulonephritis, acute necrotizing lesions with fibrinoid necrosis can transform into sclerotic lesions with no fibrinoid material in less than two weeks.

Immune complexes are thought to mediate vasculitis by activating leukocytes and endogenous glomerular cells to cause inflammatory injury and cell proliferation. Anti-GBM antibodies complexed with collagen in the walls of glomerular and pulmonary capillaries could activate neutrophils and monocytes as the leukocyte surface projections come in contact with these complexes through the fenestrations that are present in glomerular and pulmonary endothelial cells, possibly by Fc receptor or complement receptor engagement. ANCA may first activate neutrophils and monocytes in the circulation by interacting with proteinase-3 or myeloperoxidase on the surface or in the microenvironment around the cells.[29] This could be through Fc receptor engagement or through direct binding to the targets on the cell surface. Activation of leukocytes in the circulation would first cause injury to endothelial cells. This is supported by pathologic findings by electron microscopy indicating that the earliest vascular lesion of pauci-immune small-vessel vasculitis is endothelial injury with subendothelial accumulation of fibrin[30,31] and intravascular lysis of leukocytes.[30]

Something about the pathogenesis of immune complex localization in glomeruli causes predominantly endocapillary cell proliferation and influx of leukocytes without extensive necrosis, whereas something about the pathogenesis of anti-GBM and ANCA disease causes severe necrotizing injury to glomerular capillaries and other small vessels with marked lysis of collagenous matrix material. The basis for this is unknown. However, the remarkable degree of local cell death and lysis of matrix material suggests that major amounts of cytotoxic and proteolytic enzymes are released or activated in a very confined space. One likely mechanism would involve release of oxygen metabolites and enzymes from activated neutrophils and monocytes that were able to act locally but were effectively neutralized beyond the site of injury. The oxidants, in addition to being cytotoxic, would provide a local shield against anti-proteinases and also would activate matrix metalloproteinases. Serine proteinases would neutralize tissue inhibitors of metalloproteinases.[32] Serine proteinases (eg, elastase and proteinase 3) and metalloproteinases (eg, collagenase and gelatinase) would then be able to cause unfettered lysis of vessel wall matrix at the site of leukocyte activation, but the mediators of this injury would be neutralized by antioxidants and antiproteinases away from the site.[32]

In summary, in anti-GBM and ANCA vasculitis, the pathologic finding of focal, very lytic necrotizing injury suggests very effective local activation of neutrophils and monocytes with release of oxidants and proteases that are neutralized beyond the site of injury. The predilection for small vessels suggests that some element of the pathogenesis, most likely adhesions between leukocytes and endothelial cells, is dependent on close proximity of leukocytes to endothelial cells.

ANCA-vasculitis is accompanied by necrotizing granulomatous inflammation in patients with Wegener's granulomatosis and Churg-Strauss syndrome.[1,7] This inflammation does not look like the inflammation that results primarily from T-lymphocyte induced granulomatous inflammation. In the acute phase, there is no dense accumulation of lymphocytes and macrophages, but rather there is a very lytic process with zones of necrosis containing numerous neutrophils and monocytes undergoing apoptosis and necrosis. A few multinucleated giant cells are scattered within the inflamed tissue. This ANCA-associated necrotizing granulomatous inflammation could be caused by ANCA-induced activation of neutrophils and monocytes within extravascular interstitial tissue via mechanisms similar to those that cause activation in vessels. This would not occur with anti-GBM disease or immune complex disease, because the pathogenic complexes between antibodies and antigens are located exclusively or predominantly in vessel walls rather than in the interstitium. In patients with circulating ANCA, these autoantibodies would be in the interstitial fluid as well as the blood. If ANCA can activate neutrophils and monocytes in blood vessels, they should be able to activate them in interstitial tissue. Activation in the vessels would cause necrotizing vasculitis. Activation in the tissue would cause necrotizing tissue inflammation. The pathologic appearance of acute extravascular tissue injury in Wegener's granulomatosis and Churg-Strauss syndrome are consistent with extensive activation of neutrophils and monocytes in extravascular tissue.

■ SUMMARY

The different pathologic features of different types of necrotizing vasculitis indicate that there are different pathogenic mechanisms causing the injury. The pathogenic mechanisms for medium-sized-vessel vasculitis are most effective at causing injury in arteries and are not effective at causing injury in smaller vessels. The predilection of medium-sized-vessel vasculitis for bifurcations may relate to the increased expression of adhesion molecules and increased numbers of intimal macrophages at these sites. The preferential involvement of small vessels by small-vessel vasculitis may relate to the requirement for close apposition between leukocytes and endothelial cells for the pathogenic mechanisms to be operational. The pathology of the necrotizing vasculitis of Kawasaki disease is most consistent with a primary role for monocytes/macrophages and T lymphocytes in the acute injury. The pathology of the necrotizing vasculitis of polyarteritis nodosa and small-vessel vasculitis, including ANCA-vasculitis, is most consistent with a primary role for neutrophils and monocytes in the acute injury.

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SUPERANTIGENIC ACTIVATION OF T LYMPHOCYTES AND ENDOTHELIAL CELLS: A MECHANISM FOR SUPERANTIGEN-INDUCED VASCULITIS

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Introduction: Superantigens (SAgs) are potent stimulators of T cells bearing specific Vβ T cell receptors (TCR), and although controversial, may have a pathogenetic role in Kawasaki disease (KD) and other childhood systemic vasculitides. We examined a novel mechanism of SAg-induced T cell/endothelial cell activation, and specifically investigated the hypothesis that the endothelial cell may operate as a "non-professional" SAg-presenting cell for T cells bearing specific Vβ TCRs.

Methods: To assess the ability of the endothelial cell to present SAg to T cells, human umbilical vein endothelial cells (HUVECs) with and without pretreatment with γ-interferon (to upregulate MHC Class II) were co-cultured for 4 hours in the presence or absence of purified allogeneic T cells with SEB, or TSST-1. After staining of the co-cultured cells with fluorescent conjugated monoclonal antibodies, flow cytometric analysis was performed on the HUVECs and T cells to examine surface expression of endothelial cell activation markers (cell adhesion molecules), Vβ-specific T cell activation (CD69), and Vβ-specific T cell adherence to the endothelial cell monolayer in vitro.

Results: Co-culture of purified T cells (CD3+, <0.8% expressing HLA-DR) with HLA-DR expressing HUVECs and TSST-1 or SEB resulted in Vβ-restricted CD4 and CD8 activation as determined by surface expression of the T cell activation marker CD69 (Vβ2 activation for TSST-1; Vβ3 and 12 activation for SEB). Additionally, there was CD4 T cell (but not CD8 T cell) Vβ-restricted adherence at 4 hours to the HUVEC monolayer. ICAM-1 and E-selectin expression was upregulated only on the HLA-DR expressing HUVECs following exposure to TSST-1 or SEB in the presence of CD3+ T cells.

Conclusion: In vitro, in the presence of the Th-1 cytokine γ-interferon, the endothelial cell becomes a competent SAg-presenting cell. This results in massive T cell activation and CD4 adherence to the endothelium, consequently resulting in endothelial cell activation. If this mechanism is operational in Kawasaki disease or other childhood vasculitides, it may be possible to block SAg-mediated vascular injury with SAg-peptide antagonists, providing a novel, specific, and potentially nontoxic therapy.

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ANALYSIS OF AUTOANTIBODY REPERTOIRES IN SMALL AND MEDIUM SIZED VESSEL VASCULITIS: EVIDENCE FOR DISEASE-SPECIFIC PERTURBATIONS IN CLASSIC POLYARTERITIS NODOSA (PAN), MICROPOLYANGIITIS (MPA), CHURG-STRAUSS SYNDROME (CSS) AND WEGENER'S GRANULOMATOSIS (WG)

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Objective: To analyze autoantibody repertoires in patients with small and medium sized vessel vasculitis.

Method: Using a quantitative immunoblotting technique on extracts of normal human kidney, liver, lung, muscle and medium sized artery tissues, we analyzed the reactivities of serum IgM, serum IgG and purified serum IgG from patients fulfilling the ARA and Chapel Hill criteria for the diagnosis of classic PAN, WG, MPA or CSS. Blood samples were obtained from 20 patients with PAN, 10 patients in each other group at the time of diagnosis and before treatment, and 60 age- and sex-matched healthy controls. Sera were tested at the same IgG (200 μg/ml) and IgM (20 μg/ml) concentrations.

Results: Repertoires of reactivities of purified serum IgG and of serum IgG of patients with WG, MPA and CSS significantly differed from those of controls and other patients, as assessed by multivariate statistics. IgM repertoires from PAN and MPA but not of WG and CSS patients significantly differed from those of controls and other patients. Antibody reactivities specific to PAN patients were directed toward muscle, liver and/or artery 30 and 40 Kda antigens; one antibody reactivity directed toward 85 Kda antigen in lung was specific to CSS patients.

Conclusion: Autoantibody repertoires from patients with PAN, WG, CSS and MPA are disease specific. Two IgG reactivities directed toward muscle, liver and artery antigens in the case of PAN and one IgG reactivity directed toward lung antigens in the case of CSS were identified.

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Granuloma formation, implications for the pathogenesis of vasculitis

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The term *granuloma* refers to any nodular organized aggregation of mononuclear inflammatory cells or collection of modified macrophages, usually surrounded by a rim of lymphocytes and often containing multinucleated giant cells. Some granulomas may also contain eosinophils and plasma cells. Granuloma formation occurs in response to chronic inflammatory stimuli such as infection with certain intracellular pathogens (eg, mycobacteria and fungi) or the presence of inert material (eg, silica, beryllium). This pattern of inflammation is initiated and maintained by sensitized CD4 T cells that exhibit a T helper type 1 (TH-1) pattern of cytokine production.[1] In infectious diseases, granulomas form a focus that isolates the pathogen and promotes the development of protective immunity by allowing cross-talk between T lymphocytes and macrophages. The persistence of granulomatous inflammation in the setting of infection is dependent on the continuous presence of the microbial pathogen and the lesion generally resolves after eradication of the organism. If the host response fails to sterilize the lesion, the persistent granuloma serves to contain the microorganism and prevent dissemination of the infection. Granulomatous inflammation may also occur in response to noninfectious agents (silicosis, berylliosis) and from unknown causes (sarcoidosis, Wegener's granulomatosis).

Vasculitis is a clinicopathologic process characterized by inflammation and necrosis of blood vessels which leads to vessel occlusion and ischemia of tissues supplied by the affected vessel. The primary systemic vasculitis syndromes are generally thought to be mediated by immunologic mechanisms. However, the primary immunopathogenic events that initiate the process of vascular inflammation and blood vessel damage are still largely unknown. Granulomatous inflammation involving the vessel itself, the adjacent tissue, or distant sites is a feature of several systemic vasculitis syndromes. In these syndromes, the granulomatous inflammation occurs in the absence of any identifiable exogenous agent. In this review I will first summarize the current understanding of the immunologic events responsible for granuloma formation and then consider how these immunopathologic mechanisms may relate to the pathogenesis of selected systemic vasculitis syndromes.

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PATHOGENESIS OF GRANULOMA FORMATION

As indicated above, granulomatous inflammation is a normal host response to infection with certain intracellular pathogens such as mycobacteria and fungi. Most of the knowledge about the pathogenesis of granuloma formation comes from murine models of infection with intracellular organisms such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*. In these model systems, granulomatous lesions appear to be initiated by nonspecific inflammatory signals arising from the interaction of tissue macrophages with microbial products. Within this inflammatory environment, tissue dendritic cells take up microbial antigens, migrate to regional lymph nodes, and present processed antigens to naive CD4 T cells.[2] These activated CD4 T cells leave the lymph node and migrate to the focus of infection where they secrete soluble mediators that play a central role in initiating and sustaining granuloma formation (see below).

Studies in gene-disrupted mice and evidence from human diseases support the primacy of CD4 T cells in initiating and maintaining granuloma formation. Mice rendered CD4-deficient by MHC II or CD4 gene disruption exhibit delayed, poorly organized granuloma formation and increased mortality in response to intravenous infection with *M tuberculosis*.[3] In human HIV infection, selective depletion of CD4 T cells by this virus is associated with increased susceptibility to both tuberculosis and disseminated infection due to *Mycobacterium avium* complex. In HIV-infected patients with mycobacterial infections, the extent of granuloma formation is correlated with peripheral CD4 T cell counts in that patients with low CD4 counts exhibit defective granuloma formation.[4,5]

Inflammatory phagocytes are attracted to the site of microbial invasion in a process mediated by chemokines and cytokines which cause up-regulation of adhesion molecules on both leukocytes and endothelial cells. The exact pattern of chemokine expression that controls the recruitment and extravasation of leukocytes remains incom-

pletely understood. Murine models of tuberculosis suggest that the adhesion molecule ICAM-1 and the chemokines RANTES, MIP1-α, MCP-1, and IL-8 are likely involved in the processes (reviewed by Flynn and Chan[6]). Studies in human sarcoidosis also suggest that the chemokines RANTES, MIP1-α, MCP-1, and IL-8 are involved in the recruitment of mononuclear cells into the sarcoid granuloma.[7-9]

Following the accumulation and activation of macrophages, the inflammatory lesion begins to take on a granulomatous form. With the arrival of antigen-specific T cells, the lesion transforms into a mature granuloma where activation of macrophages by interferon-gamma (IFN-γ) and tumor necrosis factor (TNF) results in inhibition of microbial growth. Eventually, the granuloma becomes encapsulated by a fibrotic rim and, in the case of infections, the center becomes necrotic. These tissue reactions function to protect the host by promoting microbial containment and reducing the nutrient supply to the pathogen.

■ CYTOKINES IN GRANULOMA FORMATION: CENTRAL ROLE OF IL-12, IFN-γ, AND TNF

Cytokines serve as crucial signal transmitters between cells in granulomatous lesions and are required for the recruitment of lymphoid cells and efficient activation of macrophages. Although up-regulation of a number of cytokines is seen in animal models of granulomatous inflammation,[9-11] available evidence suggests that the TH-1 cytokines IFN-γ, IL-12, and TNF are required for normal granuloma formation and maintenance. In animal models of infectious granulomatous inflammation, IFN-γ is produced early in the infection by NK cells and later by TH-1 T cells. IFN-γ has a number of effector functions relevant to granuloma formation, including activation of macrophage bactericidal mechanisms, induction of TNF secretion by macrophages, activation of the endothelium to promote CD4 T cell adhesion, and promotion of TH-1 differentiation.[1] Studies in gene disrupted (knockout) mice have clearly shown that IFN-γ is required for normal granuloma formation in response to experimental infection with mycobacteria. In contrast to wild-type mice, IFN-γ or IFN-γ receptor knockout mice do not develop mature granulomas or protective immunity following experimental infection with mycobacteria.[12-14] The granulomas in these knockout mice are poorly formed with increased neutrophilic infiltration and necrosis.

IL-12 is a heterodimeric cytokine produced primarily by antigen presenting cells. In addition to enhancing proliferation and cytotoxicity of NK cells and cytolytic T cells, IL-12 is a potent inducer of TH-1 differentiation and hence IFN-γ production. When mice with disruption of IL-12 p40 or IL-12 receptor β1 gene are experimentally infected with mycobacteria they exhibit increased mortality and abnormal granulomas.[15-17] As was the case with IFN-γ knockout mice, the granulomatous lesions in IL-12 knockout mice are poorly formed with increased neutrophilic infiltration and necrosis. Taken together, these studies indicate that IL-12-induced IFN-γ production is essential for normal granuloma formation in these animal models.

Analysis of humans with rare genetic mutations in the IFN-γ receptor, IL-12 receptor β1, or IL-12 p40 genes also support a central role for IL-12 and IFN-γ in granulomatous inflammation and immunity to intracellular pathogens. Individuals with mutations in either the IFN-γ receptor 1 or IFN-γ receptor 2 genes exhibit complete absence of IFN-γ responsiveness and develop disseminated infections with environmental mycobacteria at a young age.[18-20] Histological examination of infected tissues from these patients reveals granulomas that are poorly circumscribed and poorly formed, suggesting that IFN-γ is required for normal granuloma formation in humans with mycobacterial infections.

Patients with severe mycobacterial infections and mutations in the genes encoding IL-12 p40 or IL-12 receptor β1 have also been described.[21-23] Like the patients with IFN-γ receptor mutations, these individuals exhibited disseminated infections with environmental mycobacteria or BCG. However, their clinical course appears to be less severe and these patients exhibited well formed granulomatous lesions. This latter finding suggests that in humans, IL-12 dependent IFN-γ production is not required for mature granuloma formation.

TNF is another TH-1 cytokine that appears to be crucial for normal granuloma formation. The major source of TNF is mononuclear phagocytes, although T cells are also capable of producing substantial amounts of this cytokine. TNF is a potent cytokine with a broad range of activities including upregulation of adhesion molecules on endothelium, activating macrophages to kill intracellular bacteria, and induction of cellular apoptosis. Studies in gene knockout mice have provided useful information about the role of TNF in normal granuloma formation. Mice with targeted disruption of the TNF gene have been generated, and their response to experimental infection has been analyzed.[24,25] As was the case in mice with disruption of the IL-12 or IFN-γ genes, TNF-knockout ($TNF^{-/-}$) mice exhibit increased mortality when experimentally infected with mycobacteria.[24] Interestingly, $TNF^{-/-}$ mice infected with *M tuberculosis* exhibit levels of antigen-specific T cell proliferation, IFN-γ production, and macrophage activation that are comparable to wild-type mice.[24] However, granuloma formation in $TNF^{-/-}$ is retarded and markedly abnormal. Granulomas were poorly formed, contained large numbers of organisms, and exhibited extensive necrosis and pronounced neutrophilic infiltration.[24] In contrast to wild-type mice, T lymphocytes in $TNF^{-/-}$ mice were confined to the perivascular and peribronchial areas and were not seen within the inflammatory lesions. This latter finding plus the extensive neutrophilic influx and necrosis seen in $TNF^{-/-}$ mice suggests that TNF plays an important role in controlling local cellular traffic in granulomatous lesions. TNF may function to limit the influx of neutrophils that cause tissue damage, while promoting the recruitment and migration of T lymphocytes into granulomas where they can interact with macrophages.

Further insights into the role of TNF in regulating granulomatous inflammation can be found in the response of $TNF^{-/-}$ mice to injection with heat-killed *Corynebacterium parvum*. An advantage of using a heat-killed organism as

an inflammatory stimulus is that the effects of active microbial infection are eliminated. When normal mice are injected with heat-killed *C parvum* they develop hepatosplenomegaly with abundant granuloma formation and prominent extramedullary hematopoiesis in the spleen.[25] This response peaks at 10 to 14 days, with progressive reversion to normal morphology by 40 days. In contrast, when $TNF^{-/-}$ mice are injected with heat-killed *C parvum* they exhibit little or no granuloma formation or extramedullary hematopoiesis at day 14. However, by days 40 to 80 the $TNF^{-/-}$ mice develop hepatosplenomegaly and ascites and die. The livers and spleens of these mice show sheetlike infiltrates of monocytes with focal areas of necrosis and poorly formed granulomas.[25] Thus, $TNF^{-/-}$ mice injected with heat-killed *C parvum* show little granulomatous inflammation at 10 to 14 days, but develop a florid, fatal inflammatory response at a time when the inflammatory lesions in normal mice have resolved. These results are consistent with TNF exerting a pro-granulomatous effect during the initial phase of an inflammatory response and an anti-inflammatory effect after the inflammatory stimulus has been localized within the granuloma.

IMPLICATIONS FOR THE PATHOGENESIS OF VASCULITIS

One of the histologic hallmarks of vascular lesions in Wegener's granulomatosis (WG), giant cell arteritis (GCA), and Takayasu's arteritis is the presence of granulomatous inflammation with multinucleated giant cells. Although granulomatous inflammation is a characteristic host response to infections with certain intracellular pathogens (eg, mycobacteria and fungi), attempts to isolate an infectious agent from lesional tissue in these syndromes have failed. These observations are compatible with the possibility that vascular injury in WG, GCA, and Takayasu's arteritis may be initiated by a TH-1 type cell-mediated immune response directed against an antigen present in the vessel wall.

In vitro studies in GCA and WG provide some evidence that aberrant TH-1 responses play a role in the pathogenesis of these syndromes. With regard to GCA, Weyand and colleagues[26,27] derived a series of CD4+ T cell clones from temporal artery lesions of patients with GCA. Using RT-PCR techniques they analyzed the sequences of the variable (V) region of the β chain (Vβ) of the TCR from these clones and found that individual TCR specificities were present in multiple copies, indicating clonal expansion. In addition, CD4+ T cell clones with identical TCR β chains were isolated from anatomically distinct lesions of the same or contralateral temporal artery in the same patient. These T cell clonotypes represented only a small fraction of the tissue-infiltrating T cells and were not detectable in the peripheral blood of these patients. The finding of multiple CD4+ T cell clones with identical TCR specificities in anatomically distinct lesions provides strong indirect evidence that the cellular infiltrate in giant-cell arteritis represents a localized, antigen-driven immune response. These same investigators also demonstrated that T cells in arterial lesions from patients with GCA produce IL-2 and IFN-γ.[26] This profile of cytokine expression is typical of TH-1 CD4+ helper T cells that stimulate predominantly cell-mediated immune responses. Taken together, these data provide indirect evidence that the inflammatory reaction in GCA is mediated by TH-1 CD4+ T cells that recognize an antigen residing in the arterial wall.

Studies in patients with WG have also yielded indirect evidence that an excessive TH-1 type response is central to the pathogenesis of this syndrome. Ludviksson et al found that peripheral blood lymphocytes from patients with active WG produce 10 to 20-fold higher levels of IFN-γ compared with normal controls. Increased production of TNF by CD4 T lymphocytes and IL-12 by purified monocytes were also noted, but production of IL-4, IL-5, and IL-10 was not increased. The addition of recombinant IL-10 to cell cultures suppressed the overproduction of IFN-γ by WG T cells in a dose-dependent manner.[28] A predominant TH-1 type response was also found by Csernok et al, who analyzed cytokine expression by T cell clones or polyclonal T cells derived from peripheral blood, nasal biopsies, or bronchoalveolar lavage of patients with WG.[29]

Based on these observations, it can be hypothesized that patients with WG have an immunoregulatory defect, which leads to excessive production of TH-1 cytokines (TNF and IFN-γ) in response to environmental insults (such as infections) and/or autoantigens. Dysregulation of monocyte IL-12 secretion may be the underlying immunoregulatory defect that accounts for this unbalanced TH-1 response. The excessive production of TNF and IFN-γ could serve to initiate and perpetuate the granulomatous inflammatory vascular lesion that is characteristic of WG.

These findings have important therapeutic implications since inhibitors of IFN-γ, TNF, and other pro-inflammatory cytokines are currently available and may be effective in the treatment of WG, GCA, and related vasculitic syndromes. However, as studies in gene disrupted mice have shown, blocking individual cytokines in a complex inflammatory response may have unanticipated effects. Carefully conducted clinical trials with cytokine antagonists will be needed to determine if these agents have a role in the treatment of WG, GCA, and related vasculitic syndromes.

SUMMARY

The pathogenesis of granulomatous inflammation is complex and involves a variety of mechanisms acting in concert to bring about an inflammatory lesion that is able to contain and destroy intracellular pathogens. While this process is crucial to host defense, it is also a two-edged sword in that excessive or inappropriate granulomatous inflammation results in considerable damage to normal tissue. In recent years, there has been significant progress in dissecting the immunologic events involved in granuloma formation and maintenance. A better understanding of these events will allow us to more precisely modulate the granulomatous inflammatory response to the benefit of patients with both infectious and autoimmune diseases.

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8-052

CHEMOKINE RECEPTOR EXPRESSION ON CD4+ AND CD8+ MEMORY T-CELLS AND IN GRANULOMAS IN WEGENER'S GRANULOMATOSIS

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Objective: Upregulated expression of receptors for inflammatory chemokines enables T cells to enter sites of inflammation. We analyzed whether peripheral blood and tissue T cells express chemokine receptors suggestive of their capability to respond to chemotactic gradients and of coordinated T-cell migration in Wegener's granulomatosis (WG).

Methods: Patients with biopsy-proven localized WG (n=5), generalized WG (n=16) and age- and sex-matched healthy controls (HC, n=13) were analyzed. PBMC were isolated and labeled with fluorochrome-conjugated monoclonal antibodies for cell surface antigens or appropriate negative (isotype) controls. Expression of chemokine receptors CCR3, CCR5 and CXCR3 was determined by four-color flow cytometric analysis (FACS). Lymphocytes were gated for analysis based on light-scattering properties and on CD45, CD4 and CD8 staining. Positively and negatively stained populations were calculated by quadrant dot plot analysis determined by isotype controls. CD3 and CCR5 staining of granulomas was done using immunohistochemistry.

Results: The fractions of CCR5+ and CCR3+ cells within the CD4+CD45RO+ and CD8+CD45RO+ T-cell population were significantly expanded in localized and generalized WG as compared to healthy controls. The ratio of CCR5/CCR3 expression on CD4+ and CD8+ memory T-cells and on CD28- T-cells was higher in localized WG compared to generalized WG. CCR5 was also expressed in granulomas on T-cells.

T-cell subset/ mean %	Localized WG	Generalized WG	HC
CD4+CD45RO+CCR5+	15%	4%	1.5%
CD8+CD45RO+CCR3+	50%	5%	2.8%
CD4+CD45RO+CCR5+	7%	5%	1.2%
CD8+CD45RO+CCR3+	10%	4%	1.4%

Conclusion: Upregulated CCR5 and CCR3 expression on memory T cells indicates activation and homing capability of CD4+ and CD8+ memory T cells in WG. Together with CCR5 expression on T cells in granulomas, these findings suggest that expanded Th1 and Th2 effector memory T cell populations home to the pathogenic site with apparent differences between localized and generalized WG.

9-016

GENETIC RESISTANCE TO WEGENER'S GRANULOMATOSIS—A ROLE FOR CCR5 IN PATIENTS WITHOUT ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

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Background: During inflammation, chemokines control emigration of leukocytes via their G-protein-coupled cell surface receptors. CC chemokine receptor 5 (CCR5) is the receptor for the β-chemokines including RANTES, MIP-1α and MIP-1β. CCR5 is expressed mainly on macrophages, Th1 T cells and dendritic cells. CCR5 Δ32, a naturally occurring variant of CCR5, has a 32 bp deletion (Δ32) that results in a nonfunctional receptor. Leukocytes from individuals heterozygous for CCR5 Δ32 express significantly lower levels of CCR5.

Objective: To investigate whether the expression of CCR5 and its ligands is altered in affected tissues and whether genetic variations in genes for CCR5 and its ligands confer susceptibility to WG.

Patients: One hundred eighteen Caucasian patients with WG and 127 ethnically matched healthy controls were included in the genetic analysis. Four lung biopsies that had classical features of WG were examined for protein levels of CCR5 and its ligands.

Measurement: Genomic DNA samples were amplified using PCR-based method. CCR5 Δ32, RANTES –28 and –401 polymorphisms were determined by either specific primers or direct sequencing. Tissue protein levels of CCR5, RANTES, MIP-1α and MIP-1β were examined using immunohistochemistry.

Results: $CCR5^+$ cells were enriched in lung lesions from patients with WG. Among patients in whom circulating antineutrophil cytoplasmic antibodies (ANCA) were repeatedly absent, none were found to carry the CCR5 Δ32 allele. The significant under-presentation of CCR5 Δ32 in patients without ANCA (0/25, 21.9% of WG cohort) suggests that CCR5 signaling exerts an important and perhaps critical role, with maximal impact in WG patients in whom the influence of ANCA is minimal. Among all patients, patient subsets and controls, there was no significant difference in the frequency of polymorphisms located in the promoter regions of the gene encoding RANTES. Enhanced protein levels of three CCR5 ligands RANTES, MIP-1α and MIP-1β were all noted in WG lung lesions, indicating redundancy of ligands for CCR5 in affected tissue. Taken together, these results demonstrate a critical role for CCR5 in tissue inflammation and destruction in WG.

Endothelial cell biology, perivascular inflammation, and vasculitis

MARIA C. CID, MD

1. INTRODUCTION

Endothelial cells are among the most dynamic and biologically active cellular components of blood vessels and play a crucial role in the pathogenesis of systemic vasculitis. The participation of endothelial cells in the pathogenesis of vascular inflammation is complex. On one hand, vascular endothelium may be the main target for injury. On the other hand, endothelial cells may actively participate in amplifying and maintaining the inflammatory process. The role of endothelial cells as a target for injury seems to be more prominent in small-vessel vasculitis, namely hypersensitivity vasculitis and vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA). In large-vessel vasculitis, endothelial cells are crucial protagonists of what we have called vascular response to inflammation, a complex constellation of changes that occur in the vessel wall in response to inflammatory mediators released by infiltrating leukocytes.[1,2] Vascular response to inflammation leads to the amplification of the inflammatory response, vessel remodeling and repair, and eventually, vessel occlusion, source of some of the most severe complications in patients with systemic vasculitis.

2. ENDOTHELIAL CELL AS A TARGET FOR INJURY

2.1 Vasculitis triggered by infectious agents

Although most of the infection-related vasculitides are immune-complex–mediated (i.e., hepatitis B virus [HBV]-related polyarteritis nodosa, and hepatitis C virus [HCV]-associated cryoglobulinemia), some pathogens are able to directly infect the endothelial cell. Rickettsiae and Herpesvirus family members, particularly cytomegalovirus, are the best documented.[3,4] Serious infections by these agents frequently include vasculitic lesions.

2.2 Immune-complex–mediated endothelial cell injury

In immune-complex–mediated vasculitis, endothelial cell morphology is altered and the luminal endothelium is eventually destroyed. Complement-mediated lysis as well as neutrophil-mediated endothelial cell damage are the main mechanisms of endothelial cell injury in these processes.[5] The membrane attack complex C5b-9, final product of the complement activation cascade, has been detected in necrotizing vasculitis of polyarteritis nodosa type.[6]

2.3 ANCA-mediated vasculitis

ANCA stimulate many neutrophil functions resulting in endothelial cell damage. ANCA may recognize myeloperoxidase (MPO) or proteinase-3 (PR3) translocated to the neutrophil membrane by the effects of cytokines such as tumor necrosis factor (TNFα) or interleukin-8 (IL-8) or may bind to Fc receptors through their Fc portion. Both interactions, specific and Fc-mediated, appear to be functionally relevant.[7,8] Experimental work by several groups has demonstrated that ANCA binding to neutrophils may stimulate or amplify many neutrophil functions including respiratory bursts with generation of reactive oxygen intermediates,[7] degranulation and protease release,[7] nitric oxide production,[9] and chemotactic activity.[10] ANCA binding also stimulates integrin expression and integrin-mediated homotypic adhesion and adhesion to endothelial cells, partially through an Fc-mediated mechanism.[11-13] Studies with blocking monoclonal antibodies have shown that enhancement of TNF-induced neutrophil activation by ANCA is, at least, partially dependent on homotypic interactions mediated by neutrophil integrins.[14]

In several experimental settings it has been demonstrated that ANCA-stimulated neutrophil function results, indeed, in an augmentation of neutrophil-mediated endothelial cell injury.[15,16] ANCA-stimulated neutrophils are able to produce endothelial cell detachment and lyse endothelial cells previously damaged by other mediators.[15,16] In addition, in an inflammatory microenvironment, enzymes released by activated neutrophils, including MPO and PR3, may induce endothelial cell apoptosis.[17]

2.4 Anti-endothelial cell antibodies

Circulating anti-endothelial cell antibodies have been detected in several vasculitides including Wegener's granulomatosis, microscopic polyangiitis, Kawasaki disease, thromboangiitis obliterans, Behçet's disease, and

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Takayasu's arteritis.[18-20] Antigens recognized by anti-endothelial cell antibodies seem to be highly heterogeneous and have not been well characterized. Some anti-endothelial cell antibodies, such as those detected in Kawasaki disease, recognize cytokine-inducible molecules,[21] whereas others, such as those detected in Wegener's granulomatosis and microscopic polyangiitis, recognize constitutive endothelial cell antigens.[18]

In vitro studies have shown that some anti-endothelial cell antibodies may trigger complement activation or antibody-dependent cellular cytotoxicity.[5,18,22] Therefore, anti-endothelial cell antibodies might contribute to endothelial cell damage in systemic vasculitis. However, their precise pathogenic role has not been fully characterized.

3. THE ENDOTHELIAL CELL AS AN INFLAMMATION AMPLIFIER

Rather than being passive spectators of leukocyte infiltration, vessel wall components, particularly endothelial cells, actively and dynamically react to the products released by infiltrating leukocytes. Endothelial cells are able to amplify the inflammatory response by three main mechanisms: adhesion molecule expression, cytokine production, and angiogenesis.

3.1 Endothelial adhesion molecules

Vessel infiltration by leukocytes requires finely regulated interactions among leukocytes, endothelial cells, and the underlying matrix mediated by adhesion molecules.[23]

3.1.1. Immunopathogenic mechanisms of vessel damage and adhesion molecules. Most of the primary immunopathogenic mechanisms which are thought to play a role in the pathogenesis of blood vessel inflammation in vasculitis have been shown to influence adhesion molecule expression or function.[23]

In vitro studies have shown that complement activation products induce adhesion molecule expression by cultured endothelial cells. C1q induces E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1),[24] and C5a has been shown to up-regulate P-selectin expression.[25] Adhesion molecule expression and function are required for immune-complex– and complement-mediated vessel damage in vivo.[26,27]

Recent studies have shown that ANCA binding to endothelial cell membrane-associated PR 3[28] or related epitopes on the endothelial cell surface[29] may induce E-selectin and VCAM-1 expression by endothelial cells.[30,31] In an inflammatory context, PR3 released by neutrophils in the vicinity of endothelial cells is able to induce endothelial cell ICAM-1 expression.[32] In vitro studies have shown that anti–endothelial cell antibody binding to endothelial cells also induces endothelial adhesion molecule expression.[33]

In vasculitis, activated lymphocytes and macrophages actively produce IL-1, TNFα, and interferon γ,[34,35] the main inducers of endothelial adhesion molecules. Topographical relationship between inducer cytokines and endothelial adhesion molecule expression has been demonstrated in tissue samples from patients with microscopic polyangiitis.[36]

3.1.2. Tissue expression of endothelial adhesion molecules. Expression of endothelial adhesion molecules in lesions has been investigated in sizeable and homogeneous series of patients with cutaneous leukocytoclastic vasculitis, Kawasaki disease, classical polyarteritis nodosa, and giant-cell arteritis.[37-40] In all of them, expression of inducible adhesion molecules E-selectin and VCAM-1 by endothelial cells can be detected at some point and constitutive expression of ICAM-1 is usually up-regulated. In glomerular lesions of Wegener's granulomatosis and microscopic polyangiitis, as well as in ANCA-associated necrotic and crescentic glomerulonephritis, VCAM-1 and ICAM-1 expression can be observed at the glomerular tuft as well as in tubular epithelial cells and peritubular capillaries.[41-43]

In small-vessel vasculitis, endothelial adhesion molecule expression occurs in the luminal endothelium.[37] However, in medium-sized vasculitis such as classical polyarteritis nodosa, the luminal endothelium only expresses constitutive or inducible adhesion molecules at early stages. As the inflammatory process proceeds, the luminal endothelium is damaged and the vascular lumen is occluded. Endothelial adhesion molecules are then strongly expressed by adventitial neovessels.[39] In kidney lesions of ANCA-associated vasculitis, glomerular expression of ICAM-1 and VCAM-1 also declines in sclerotic glomeruli.[43] In large-vessel vasculitis such as giant-cell arteritis, adhesion molecule expression occurs in neovessels at the adventitia and within the inflammatory lesions, mainly at the intima/media junction. These observations suggest that, in large- and medium-sized vessels, infiltrating leukocytes do not come from the vascular lumen. Rather, inflammatory cells penetrate the vessel wall through the adventitial vasa vasorum and neovessels.

3.1.3. Functional relevance of endothelial adhesion molecules in vasculitis. Immunohistochemical studies usually disclose a close topographical relationship between endothelial expression of adhesion molecules and expression of their ligands by infiltrating leukocytes, suggesting that interactions mediated by adhesion molecules actively participate in the development of inflammatory infiltrates in vasculitis.[39,40] The functional relevance of interactions mediated by adhesion molecules in the pathogenesis of vessel inflammation has been investigated in in vitro studies exploring adhesion of T lymphocytes to glomeruli in tissue sections from patients with renal vasculitis,[44] and in animal models. In a murine model of systemic vasculitis induced by immunization against *Mycobacterium butyricum*, the administration of blocking monoclonal antibodies and the application of vital microscopy have demonstrated the important participation of interactions mediated by selectins and by α4 integrins in leukocyte adhesion and transmigration through postcapillary venules.[45] Similarly, ICAM-1 deficiency considerably reduces the development of vasculitis in MRL/lpr mice,[46] and blocking E-selectin ligands or α4 integrins prevents the development of β-glucan–induced granulo-

matous vasculitis.[47,48] Although none of these models satisfactorily represents specific human vasculitic syndromes, these findings underline the functional importance of interactions mediated by adhesion molecules in the development of vascular inflammation.

3.1.4. Effects of treatment on endothelial adhesion molecule expression. In vitro studies have shown that corticosteroids may suppress endothelial cell adhesion molecule expression induced by endotoxin or by cytokines.[49] In addition, corticosteroids inhibit the production of proinflammatory cytokines which are the main inducers of adhesion molecule expression.[50]

The effect of treatment on adhesion molecule expression in patients with vasculitis is not well defined. Immunoglobulin therapy decreases endothelial cell adhesion molecule expression in skin samples from patients with Kawasaki disease.[38] Preliminary cross-sectional studies show a substantial decrease in E-selectin and VCAM-1 expression in lesions from patients with giant-cell arteritis treated with corticosteroids for up to one month, but some expression still persists,[40] indicating a persistent exposure of endothelial cells to an inflammatory microenvironment. A decrease in endothelial adhesion molecule expression in synovial biopsies from patients with polymyalgia rheumatica treated with corticosteroids has also been observed.[51] Corticosteroid and immunosuppressive treatment of patients with polyarteritis nodosa for just a few days does not substantially modify adhesion molecule expression.[39]

3.2 Cytokine production

Endothelial cells have the potential to produce a variety of cytokines, chemokines and growth factors in an inflammatory microenvironment. Through the production of IL-1α and IL-6, endothelial cells may contribute to the systemic acute-phase reaction which is characteristically prominent in many systemic vasculitides compared with other immune-mediated diseases.[1]

Endothelial cells are able to produce colony-stimulating factors and these may be able to prolong the half-life of infiltrating leukocytes as suggested by in vitro studies.[52] In fact, the occurrence of leukocytoclastic vasculitis in association with granulocyte colony-stimulating factor therapy has been reported.[53]

Several chemokines such as IL-8, RANTES, Gro α, and SLC, among others, can be produced by endothelial cells.[54] Chemokines selectively attract leukocyte subpopulations bearing specific receptors. Chemokine production by endothelial cells may contribute to tissue targeting in systemic vasculitis, and by attracting additional leukocytes may perpetuate and amplify vessel inflammation.[55]

As for adhesion molecules, ANCA binding to endothelial cells,[30,31] some anti–endothelial cell antibodies,[24] and cytokines released by infiltrating cells[52] stimulate endothelial cell production of cytokines and chemokines such as IL-8. PR3 binding to endothelial cells may also increase endothelial cell production of IL-8 and monocyte chemoattractive protein-1 (MCP-1).[32]

3.3 Angiogenesis

Angiogenesis, new vessel formation, is a relevant phenomenon in systemic vasculitis. Immunohistochemical studies have shown that, in vasculitis, extensive neovascularization occurs in inflammatory lesions, particularly in the adventitial layer or surrounding tissues.[56,57] In large-vessel vasculitis, neovessels also appear within the inflammatory infiltrates, particularly at the intima/media junction.[40]

We have proposed that angiogenesis may play a dual role in systemic vasculitis. On one hand, in medium-sized and large-vessel vasculitis such as giant-cell arteritis and polyarteritis nodosa, newly formed vessels intensively express adhesion molecules for leukocytes and provide new sites through which leukocytes may invade the vessel wall.[39,40] In addition, new vessels provide a wider endothelial cell surface and provide a new source of cytokines, chemokines, and growth factors, amplifying and perpetuating the inflammatory process.

On the other hand, in small-vessel vasculitis and at distal sites supplied by large or medium-sized vasculitis, angiogenesis may be a compensatory mechanism to avoid ischemia. The relevance of angiogenesis as a compensatory mechanism is illustrated by the fact that interferon α, a potent angiogenesis inhibitor, may worsen cryoglobulinemia-related ischemic complications.[58] Similarly, in giant-cell arteritis, the magnitude of the angiogenic response measured in temporal artery samples inversely correlates with the development of ischemic complications.[59] Even though giant-cell arteritis is considered a large-vessel vasculitis, we have shown that small cranial arteries are frequently involved and, in fact, characteristic ischemic complications such as blindness or scalp necrosis usually occur in territories supplied by small arteries.[57] These observations suggest that angiogenic activity might have a compensatory function in giant-cell arteritis. Detection of neovessels by imaging techniques may be of clinical interest in assessing disease activity. In this regard, preliminary studies suggest that, in Takayasu's disease, intramural neovascularization can be detected by computed tomography after bolus injection of contrast material, and this may reflect active inflammation.[60]

Angiogenesis results from a delicate balance between the influx of angiogenic and anti-angiogenic factors and the regulation of the expression and function of their respective receptors. A large variety of molecules may exhibit angiogenic activity. These include growth factors, chemokines, thymosins, acute-phase proteins and extracellular matrix protein fragments.[61] Several angiogenic factors such as vascular endothelial cell growth factor (VEGF), fibroblast growth factor (FGF-2),[62] IL-8, and thymosin β4 (Cid et al, unpublished) have been detected in temporal artery lesions from patients with giant-cell arteritis but their functional relevance is incompletely understood. Other factors such as TNFα and transforming growth factor beta (TGFβ), also produced in giant-cell arteritis lesions,[63] may also have angiogenic activity in vivo, probably through indirect mechanisms requiring the participation of additional cell types.

4. ENDOTHELIAL CELLS AND VESSEL OCCLUSION

Vascular inflammation frequently leads to vessel occlusion with the ensuing ischemia of supplied tissues. Ischemic complications often result in organ dysfunction and major disabilities in patients with vasculitis. Major contributors to vessel occlusion are thrombosis and intimal hyperplasia. Thrombosis is more frequently seen in small/medium-sized vessel vasculitis, whereas in large-vessel vasculitis lumen reduction usually occurs as a consequence of intimal hyperplasia.[1]

Several cytokines and growth factors produced in inflamed vessels have prothrombotic and fibrogenic effects. IL-1 and TNFα have procoagulant activity by inducing endothelial expression of tissue factor.[52] However, both IL-1 and TNFα can also induce prostacyclin synthesis, which is a potent inhibitor of platelet aggregation, and TNFα may increase the production of plasminogen activators.[64,65] The final impact of these opposite interactions on the coagulability status is complex and is probably determined by many interactions in the inflammatory microenvironment at a given time point.

Endothelial cells may produce fibrogenic factors able to stimulate myointimal cell proliferation and matrix deposition leading to intimal hyperplasia. These include IL-1α, FGFs, TGFβs and platelet-derived growth factors (PDGFs), among others.[52] However, in large-vessel arteritis, macrophages, rather than endothelial cells, are probably the main producers of fibrogenic growth factors,[66,67] and the fibrogenic impact of endothelial cells is probably less relevant in these diseases.

5. CONCLUDING REMARKS

Endothelial cells have a relevant and complex participation in vasculitis pathogenesis, both as target for injury and as active protagonists of the inflammatory process. Endothelial cell response to inflammatory mediators may be both harmful and beneficial, given that endothelial cells have proinflammatory functions and may actively participate in vessel remodeling and repair. A better understanding of the endothelial response to inflammation may lead to new therapeutic approaches in the future.

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10-023

NOVEL EFFECTS OF INFLAMMATORY CELL PROTEASES ON VASCULAR ENDOTHELIA

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Leukocytes secrete enzymes upon stimulation, which are key effectors of vascular inflammation. In particular, the T-cell protease granzyme B can directly activate target cell caspases responsible for apoptotic execution. The neutrophilic pseudoprotease azurocidin has been linked with increased vascular permeability and edema. The studies herein reveal novel functions of two additional neutrophilic proteases, proteinase 3 and elastase, extending their role in inflammatory disease beyond the described functions of extracellular matrix degradation. Because leukocytes release millimolar amounts of proteases at sites of inflammation, we investigated the effects of neutrophil serine proteases, proteinase 3 and elastase, on the function and survival of human umbilical vein endothelial cells (HUVEC).

We report that endothelial cells internalize proteolytically active proteinase 3 into endosomal-like vesicles. Once internalized, PR3 cleaves NF-κB (p65) in the N-terminal region, generating a fragment of ~56kDa that is dysfunctional as a transcription factor. Protein sequence analysis of the N-terminal amino acids of the PR3 generated fragment showed cleavage at the $VGKDC^{95}$ -R^{96} motif of p65, two amino acids upstream of the reported caspase 3 site. We found that elastase also inactivates NF-κB function through direct cleavage in the C-terminal domain. Caspase 3 inhibitors did not block this cleavage. Treatment with proteinase 3 or elastase results in apoptosis. However, NF-κB cleavage alone is not sufficient to induce death pathways. To determine the signaling pathways utilized by proteinase 3 and elastase in the activation of apoptosis, signaling molecules of known stress pathways were examined using antibodies specific for the active forms. Our data indicate that both p38 MAPK and JNK pathways are responsive to protease treatment. Inhibition of JNK or p38 with the inhibitor SB203580 reduced proteinase 3-induced apoptosis by ~75%.

Direct cleavage of NF-κB by proteinase 3 and elastase as reported here, combined with reports of cleavage of IL-1β and Sp1, indicates that these proteases possess caspase-like functions. Proteinase 3 and elastase are noncaspase proteases secreted by neutrophils at sites of acute inflammation that have the capacity to mediate intercellular caspase-like functions.

Understanding the pathogenesis of ANCA: Where are we today?

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In the intervening years between the ANCA workshop in Groningen, The Netherlands, and the current proceedings in Cleveland, Ohio, there have been substantial advances in understanding the pathogenesis of ANCA small-vessel vasculitis (SVV). There have been and still are several hypotheses generated on the basis of both in vivo and in vitro observations. These include the possibilities that: 1) ANCA induce the activation and degranulation neutrophils and monocytes in the circulation or, after adherence, to the endothelium; 2) ANCA bind to target antigens on the surface of endothelial or epithelial cells that synthesize and express these antigens or to target antigens that are deposited on the surfaces; 3) ANCA antigens have direct effects on the vascular endothelium; 4) there is no relationship between ANCA, their target antigens, and SVV.

It is most likely that some or all of these mechanisms are involved. So too, it is likely that more than a "single hit" is required for disease generation and proliferation. Thus, in any given patient it is plausible that the antibody forms in susceptible patients, and that there are genetic factors and environmental pressures (ie, especially silica or infectious diseases with superantigen formation) that conspire to cause an inflammatory reaction.

ANIMAL MODELS

Some of the most dramatic progress has been made in elucidating an animal model of ANCA SVV. There are currently several models of SVV in animals. Some of these models rely upon the spontaneous induction of vasculitis associated with a polyclonal autoantibody response. One such model is in a rat strain susceptible to autoimmune syndrome in response to mercuric chloride.[1] These animals develop autoantibodies to myeloperoxidase (MPO), but also to a host of other proteins and nuclear antigens. The model provides some intriguing clues about the potential role of infection in antibody-induced vasculitis. For instance, treatment with antibiotics in these animals diminished mercuric chloride-induced vasculitis. Unfortunately, these animals never developed necrotizing glomerulonephritis. Furthermore, the range of autoantibodies that occurs precludes any possibility of establishing a prominent role for any particular antibody as a driving force in vasculitis. Similarly, the SCG/Kj mice in which anti-MPO antibodies are found develop a crescentic glomerulonephritis and necrotizing vasculitis.[2] However, this anti-MPO response is only part of a polyclonal immune response.

There have been two examples of anti-MPO–induced crescentic glomerulonephritis requiring pretreatment with anti–glomerular basement membrane antibodies. First, Kobayashi and then Heeringa treated rats with subnephritogenic anti–glomerular basement membrane disease and then either administered rabbit anti–rat MPO antibodies or induced the development of an anti-MPO response in rats. In the Heeringa model, rats developed lesions characterized by fibrinoid necrosis and crescentic formation.[3]

Recently, Hong Xiao made use of a model in which MPO knockout mice are immunized with murine MPO. As reported in these proceedings, these mice developed a brisk anti-MPO antibody response. When splenocytes from these animals are transferred to RAG2 mice, SVV and a necrotizing and crescentic glomerulonephritis develops. When mice are immunized with control antigen such as bovine serum albumin and splenocytes transferred, no lesion occurs. Interestingly, both experimental and control mice have a baseline immune complex deposition in their glomeruli. In preliminary observations, transfer of anti-MPO antibodies alone, derived from immunized MPO knockout mice, into RAG2 mice results in a necrotizing glomerulonephritis and crescentic glomerulonephritis.

These observations would suggest that the anti-MPO antibody alone is capable of creating a necrotizing glomerulonephritis. It is most likely that the transfer of the splenocytes results in more aggressive crescentic glomerulonephritis, providing insights into the stimulatory roles of T cells in this process. Much work remains to be done with respect to the relative roles of T and B cell constituent stimuli as a granulomatous angiitis and to the contributing components of the antibodies and their receptors used for anti-MPO–induced damage.

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TABLE 1
BIOLOGICAL ACTIVITIES OF PR3

Regulation of differentiation
- Truncation of NF-κB[24]
- Hydrolyzation of Hsp 28[25]
- Truncation of Sp1[26]
- Component of leukemia-associated inhibitor[27]

Impact on cytokine network
- Conversion of IL-8 to active form[28]
- Conversion of TNF-α to active form[29]
- Conversion of IL-1β to active form[29]
- Activator of latent TGFβ1[30]
- Enhances IL-8 production by endothelial cells[31]

Other substrates and physiological functions
- Cleavage of C1 inhibitor[32]
- Cleavage and inactivation of the thrombin receptor[33]
- Cleavage of matrix macromolecules (elastin, fibronectin, laminin, vitronetin, type IV collagen)[34]
- Activator of MMP-2[35]

Effect on endothelial cells
- Internalization into cells[4]
- Induction of apoptosis[4]
- Activates signaling molecules[36]
- Stimulates tissue factor production[37]

TABLE 2
BIOLOGICAL ACTIVITIES OF MPO

- Bactericidal through enzymatic production of hypochlorous acid[38]
- Functions as a peroxidase to produce free radicals causing lipid peroxidation of low-density lipoproteins[39]
- Produces oxidants that activate cell-signaling pathways[40]
- Produces hypochlorous acid activates NF-κB transcription factor[41]
- Produces advanced glycation end products at sites of inflammation[42]
- Internalized by endothelial cells causing increased free radical production[4]
- Tyrosine nitration of vascular ECM proteins[38]

■ ANCA ANTIGENS ARE MORE DESTRUCTIVE

Another area of substantial research in this field pertains to the roles of the target antigens MPO and proteinase 3 in the development of endothelial injury. It has been demonstrated that both MPO and proteinase 3 were capable of entering endothelial cells of many types.[4] This process of antigen entry into cells most likely is a consequence of receptor-mediated endocytosis. The exact nature of the receptor remains controversial, although Dr. Daha suggested previously that 111 kb protein was an important ligand.[5] Whether there are multiple receptors including the soluble protein C receptor for proteinase 3 is not clear at this time.[6] However, once proteinase 3 or MPO enters a cell, there are many potential consequences. It is already known that proteinase 3 has numerous effects other than that of just a destructive enzyme **(Table 1)**. Similarly, once inside the cell, MPO has a number of effects as well **(Table 2)**. We now know that entry of proteinase 3 into endothelial cells pushes the balance of cellular signaling pathways toward a proapoptotic event through JNK and p38MAPK pathways. Although these signals result in a pro-apoptotic pathway under some circumstances, they may have proliferative effects under other conditions. Once inside the cell, proteinase 3 cleaves p65 NF-κB at a site in the vicinity of a caspase 3 site, rendering it dysfunctional. These results have implications not just for vasculitis, but for inflammation in general, as it would suggest that proteinase 3 may function in a manner analogous to granzyme B released from lymphocytes.[7,8] While the substrates are different, the killing effect of these proteases is quite similar.

Myeloperoxidase and proteinase 3 are cationic in nature. These enzymes bind ionic proteins on endothelial cell surface. As such, these antigens may be the source of ANCA binding that would result in local cell injury. Whether the antibody antigen interaction on the surface of the endothelial cell is a prime effector arm of endothelial cell injury remains controversial.[9] There is really no good evidence for linear or granular staining of immune reactives along the endothelial cells in vivo.

There still remains substantial controversy as to whether proteinase 3 and MPO are expressed by cells other than those of myeloid lineage. Most evidence suggests that proteinase 3 is not made by endothelial cells,[10,11] although there are data on both sides of this controversy.[12] Of note, proteinase 3 message may be expressed by glomerular epithelial cells and may be associated with crescent formation.[13] Proteinase 3 protein and message were found in distal tubular epithelial cells and glomerular epithelial cells in normal kidney and in patients with crescentic glomerulonephritis. In fact, glomerular proteinase 3 RNA expression was associated with the percentage of cellular crescents.

Over the years, ANCA have been shown to activate leukocytes in an ever-expanding number of ways, including induction of reactive oxygen production, degranulation, activators of 5-lipoxygenase pathway, and stimulation of cytokine message and protein, including IL-8 and IL-1β. Each of these effects may be involved in direct tissue damage as well as the recruitment of new leukocytes to areas of acute then chronic inflammation.

■ ANCA ACTIVATE LEUKOCYTES

There is controversy as to the mechanism by which ANCA induce neutrophil and monocyte activation. What is the relative contribution of the Fcγ receptor engagement versus $F(ab')_2$ antigen binding in activation? Part of the controversy likely stems from the "readout" or outcome variable studies. Several studies have used superoxide anion production or release of a granule protein. We have recently studied the effect of the whole antibody versus $F(ab')_2$ fragment on the stimulation of transcription of a distinct set of genes in leukocytes from healthy

donors. Interestingly, some changes in gene expression were unique to whole IgG, some unique to $F(ab')_2$ fragments, and some to both. We concentrated on a gene called "differentiation-dependent gene 2 (DIF2)," also known as "IEX-1," investigating both message and protein levels. Levels are increased in leukocytes activated by ANCA $F(ab')_2$ and by the whole immunoglobulin. Similarly, we have looked at the transcription of IL-8 and COX-2, and we found that there are differences between ANCA IgG and their respective $F(ab')_2$. This would suggest that in some circumstances one gene may be more responsive to particular signals than another. Whether these in vitro phenomena have any in vivo correlate has recently been studied by looking at RNA and protein levels in circulating leukocytes of ANCA patients with active disease when compared to ANCA patients in remission and disease controls (systemic lupus and IgA nephropathy). The corresponding increase of genes in vivo found in active disease that are stimulated in vitro gives credence to these in vitro observations.

The mechanisms by which ANCA alter neutrophils and monocytes seem to require ANCA binding to the antigen. It is likely that the $F(ab')_2$ signal is different than the combined signals stimulated by the whole endeavor. Signals to activate transcription of certain genes may originate from a different portion of the antibody, while binding to the Fc receptor may predominantly signal leukocyte activation. Neutrophils respond to the physical cues of ANCA by upregulating transcription of IL-1β and IL-8.[14-18] Some evidence has linked ANCA with protein kinase C activation and IP3 generation.[19] ANCA-induced signaling can synergize with arachidonic acid pathways,[20] and with TNF-α signaling pathways.[21] A major function of signaling networks is to place a value on a signal such that it is either dissipated or converted into further biochemical events. Consequently, in the hierarchical framework of signaling networks, the strongest signal prevails.[22] It is possible that there are multiple signals that are "integrated" through several different pathways that result in leukocyte activation or leukocyte production of phlogistic effectors or in the development of an apoptotic signal. The point that $F(ab')_2$ binding engages signaling components was further confirmed by Harper et al,[23] who reported that neutrophils from ANCA patients had a greater degree of apoptosis that correlated with higher concentrations of surface proteinase 3. Moreover, once the cells became apoptotic, they became unresponsive to ANCA binding and signaling, indicating that ANCA require an intact signaling network to mediate a response.

ANCA signaling is most likely a consolidation of signals produced by both ANCA-$F(ab')_2$ and ANCA-Fc engagement. These signals are probably not mutually exclusive, and the complexity of outputs results in a variety of neutrophil and monocyte functions. We still have much to learn about the consequences of ANCA on the clinical and pathologic phenotype of ANCA vasculitis.

SUMMARY

The role of ANCA, ANCA antigens, endothelial cell damage, genetic and environmental pressures, and the "activatability" of leukocytes will probably prove to be important variables in human ANCA vasculitis. The advent of a reliable animal model may open new areas of investigation and treatment of these vasculitic conditions.

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11-027

TNF-α-ACCELERATED APOPTOSIS ABROGATES ANCA-MEDIATED NEUTROPHIL RESPIRATORY BURST BY A CASPASE-DEPENDENT MECHANISM

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Apoptosis is important for terminating inflammation. However, constitutive PMN apoptosis was also shown to upregulate membrane expression of autoantigens, including proteinase 3 (PR3) and myeloperoxidase (MPO). TNF-α is increased in patients with active ANCA vasculitis, primes PMN for an ANCA-induced respiratory burst, but also rapidly induces apoptosis. We investigated the effect of TNF-α-induced apoptosis on ANCA antigen expression and on ANCA-induced superoxide generation in human PMN. PMN were brought to apoptosis by 10 ng/ml of TNF-α or a combination of TNF-α and 2.5 μg/ml the protein synthesis inhibitor cycloheximide, or cycloheximide alone for 3 h. Apoptosis and ANCA antigen expression were assessed by FACS and microscopy. Superoxide was determined with the ferricytochrome C assay. TNF-α with cycloheximide for 3 h caused apoptosis in 87% PMN compared to 2% in untreated controls (n=18; $p<0.01$). Accelerated apoptosis was associated with an increase in ANCA-antigen expression for both proteinase 3 and myeloperoxidase ($p<0.05$). Nevertheless, apoptosis was paralleled by a decreased PR3 and MPO ANCA-induced respiratory burst ($p<0.05$). Blocking caspase-3 activity prevented apoptosis in TNF-α with cycloheximide-treated cells (83% to 2%) and prevented compromised respiratory burst in response to ANCA. Also, caspase-3 inhibition abrogated apoptosis-mediated ANCA antigen upregulation (PR3 141.6 ± 34.1 MFI to 33.9 ± 7.8; MPO 48.3 ± 12.9 MFI to 11.9 ± 3.2, n=6, $p<0.05$). We conclude that TNF-α-accelerated apoptosis is associated with increased ANCA antigen expression but with downregulated respiratory burst activity in response to ANCA. Specific inhibition of apoptosis by caspase-3 blockade prevented the increase in ANCA-antigen expression and preserved the capability of generating superoxide, thereby establishing a causative role for apoptosis. We suggest that TNF-α exhibits dual actions by both priming and terminating ANCA-mediated activation of human PMN.

12-071

MEMBRANE EXPRESSION OF NEUTROPHIL PROTEINASE 3 (PR3) IS ASSOCIATED WITH RELAPSE IN PR3-ANCA RELATED VASCULITIS

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Background: The highly specific presence in serum of autoantibodies directed against intracellular neutrophil proteins such as PR3 (PR3-ANCA) suggests a pathophysiological role of these autoantibodies in patients with necrotizing small-vessel vasculitis. A stable but interindividually highly variable membrane expression of PR3 has been found on resting neutrophils. We hypothesized that, in patients with PR3-ANCA related vasculitis, a higher expression of PR3 on neutrophil membrane would lead to more interaction with PR3-ANCA and could thereby influence the extent or course of the disease.

Methods: PR3 expression on unstimulated isolated neutrophils from patients with PR3-ANCA related vasculitis was determined by FACS analysis using the anti-PR3 murine mAb 12.8. Patients were divided according to the distribution of neutrophil membrane PR3 in 3 groups: low, bimodal, and high. Disease extent at diagnosis was scored with the Birmingham Vasculitis Activity Score (BVAS). Actuarial relapse-free survival was calculated from diagnosis to the first relapse and compared between groups with the log rank test.

Results: 89 patients (age 49 ± 16.6; 47 male/42 female) with PR3-ANCA related vasculitis followed at our department were included. At diagnosis, renal involvement was present in 52 (58%) and pulmonary involvement in 49 (55%) patients, BVAS was 23 ± 10.5. During follow-up (81 ± 67 months) 50 patients had one or more relapse. Age at diagnosis, organ involvement and BVAS at diagnosis were not different between patients with low (n=32), bimodal (n=26), and high (n=31) neutrophil membrane PR3 expression. However, median relapse-free survival was 104.5 months in patients with low PR3 expression as compared to 36.6 and 30.8 months in patients with bimodal and high PR3 expression, respectively ($p=0.023$). Clinical manifestations at first relapse of vasculitis were not different between these groups.

Conclusion: The level of individual PR3 expression on resting neutrophils is significantly associated with risk for relapse in patients with PR3-ANCA associated vasculitis, but not with disease extent or manifestations at diagnosis or relapse. These data support the hypothesis that interaction in vivo of ANCA with PR3 expressed on membranes of neutrophils plays a role in the pathophysiology of PR3-ANCA related vasculitis.

ANCA subsets: Influence on disease phenotype

ULRICH SPECKS, MD

Over the last decade a large body of evidence has accumulated linking antineutrophil cytoplasmic autoantibodies (ANCA) to the pathogenesis of the small vessel vasculitides Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA). The concept of how ANCA directly and indirectly contribute to vascular injury is derived mostly from in vitro studies **(Figure 1)**; it is not only plausible, but also consistent with clinical and animal model observations. Nevertheless, significant gaps in our knowledge remain, and the controversy whether ANCA cause disease manifestations or are merely a marker of the disease has yet to be settled. Answers to the following questions would represent major advances in our understanding of the pathogenesis of ANCA-associated vasculitis. 1) What triggers and what causes persistence of the ANCA response? 2) Why, of all ANCA, are only those directed against proteinase 3 (PR3) and myeloperoxidase (MPO) unequivocally linked to pauci-immune small vessel vasculitis? 3) Why does not every patient with PR3-ANCA or MPO-ANCA suffer from active vasculitis? The goal of this overview is not to provide answers to these questions, but to set the stage for the hypothesis that the answers will be found by unraveling the molecular (and functional) interactions between ANCA and their specific target antigens.

ANCA AND THE PATHOGENESIS OF VASCULITIS

ANCA were first described in the early 1980s as a cause of diffuse granular cytoplasmic immunofluorescence staining (C-ANCA) on ethanol-fixed neutrophils in association with glomerulonephritis, vasculitis and Wegener's granulomatosis (WG).[1,2] PR3 was subsequently identified as the principal target antigen for these ANCA.[3-6] At the same time, ANCA reacting with myeloperoxidase (MPO) causing a perinuclear immunofluorescence staining pattern on ethanol-fixed neutrophils (P-ANCA) were found in patients with MPA, its renal-limited variant, pauci-immune glomerulonephritis, and, less frequently, in WG.[7] Multiple other neutrophil granule constituents have since been identified as potential targets for ANCA in a variety of disorders (reviewed in reference 8). However, many large clinical studies have confirmed that only C-ANCA reacting with PR3 and P-ANCA reacting with MPO have a high specificity for the autoimmune vasculitides WG and MPA (reviewed in references 8 and 9).

Because of their high disease specificity and other clinical observations, PR3- and MPO-ANCA have been suspected to be more than an epiphenomenon. ANCA levels frequently correlate with disease activity, even though this correlation may not always be apparent in every individual patient (reviewed in reference 10). Prospective studies have confirmed the persistence or recurrence of ANCA and significant ANCA titer rises as independent risk factors for clinical relapses; also, a relapse of vasculitis activity in the absence of ANCA is extremely unusual.[11,12] ANCA-negative patients with biopsy-proven WG usually have a good prognosis and do not develop systemic vasculitic complications until there is ANCA seroconversion.[13] Patients receiving drugs such as propylthiouracil, hydralazine or allopurinol, which are known to induce autoantibodies and clinical autoimmune syndromes, may develop high titers of MPO-ANCA and small vessel vasculitis.[14] These clinical observations all support a significant contributory role of ANCA for the development of small vessel vasculitis.

Over the past decade many in vitro experiments and some animal model studies have been performed to better understand the pathogenic role of ANCA and to identify specific mechanisms by which ANCA may lead to vascular injury (reviewed in references 15 to 17). Many proinflammatory effects of ANCA on neutrophils, monocytes, and endothelial cells which enhance and perpetuate endothelial cell and tissue damage have been well documented. The pathogenic role of ANCA for the development of vasculitis is also supported by animal models of MPO-ANCA–associated vasculitis (reviewed in reference 16). They clearly indicate that ANCA contribute directly to the development of vasculitis and glomerulonephritis and that the interaction of ANCA with its target antigen is required for the development of lesions. Furthermore, the localization of lesions is determined by the site of this interaction. At the same time, animal models support the significance of genetic determinants for the

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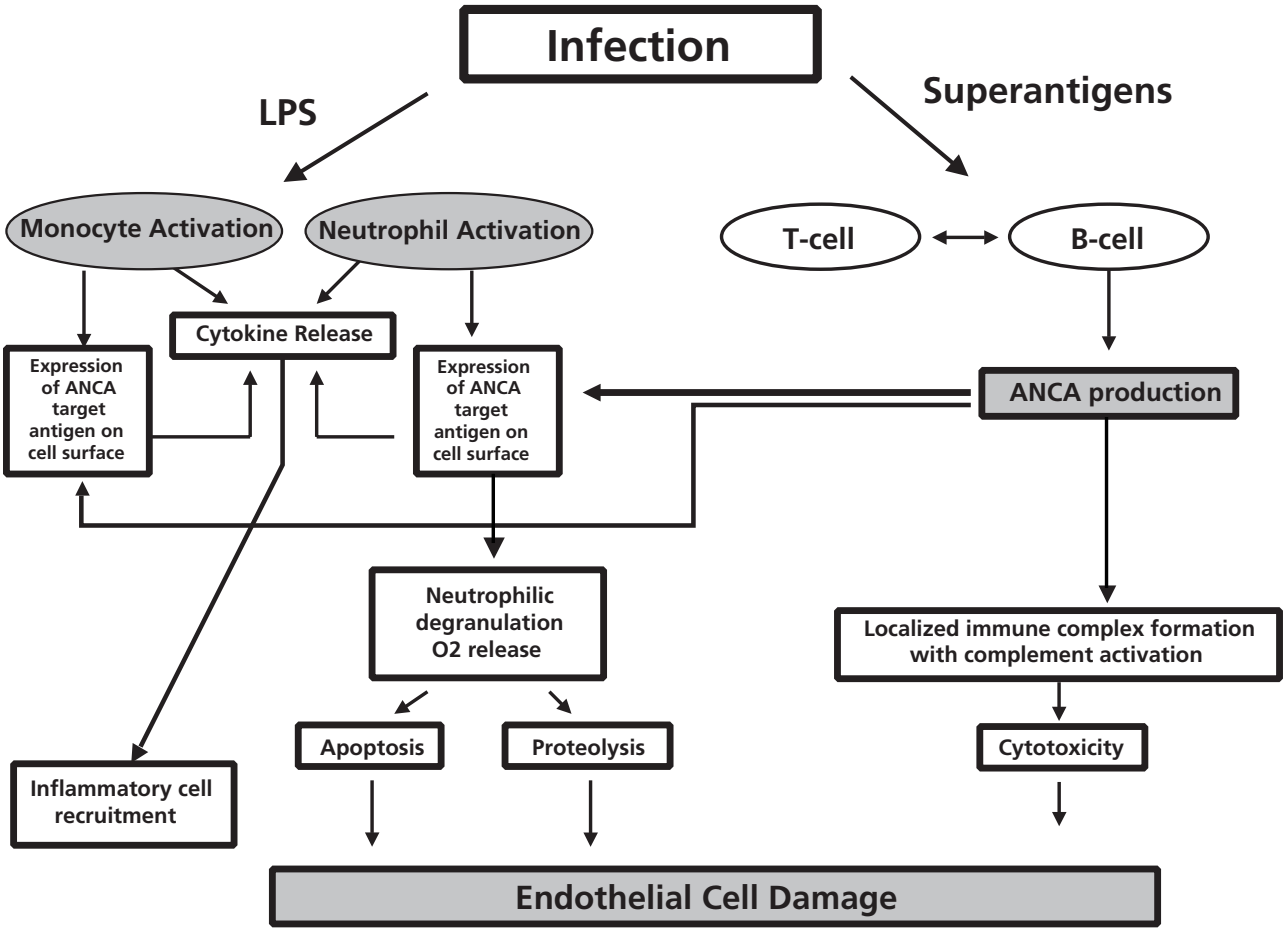

Figure 1. Schematic concept of proinflammatory effects of ANCA leading to vasculitis. Cofactors such as infections can trigger an inflammatory response leading to cytokine-mediated priming of monocytes and neutrophils. This results in cell surface expression of ANCA target antigens (such as PR3), which then may become a target for circulating ANCA. ANCA production may be the result of interactions between T cells and B cells, activated by microbial superantigens. Stimulation of primed neutrophils and monocytes by ANCA induces degranulation with protease release, and a respiratory burst with release of radical oxygen species. These effects result in direct injury to endothelial cells. Invoked mechanisms include cytotoxicity and apoptosis, but also localized immune complex formation with complement activation. LPS = lipopolysaccharide; ANCA = antineutrophil cytoplasmic antibodies.

development of autoimmunity, vasculitis, and a specific phenotype with characteristic organ involvement and histopathologic features. Finally, animal model studies indicate that infections may be significant disease modifiers.[18,19] Thus, the data from existing animal models of ANCA-associated vasculitis are fully consistent with the pathogenic concept of ANCA depicted in **Figure 1**.

Does the type of ANCA matter?

The mechanisms of ANCA induction and of their subsequent persistence remain unknown. ANCA directed against a variety of target antigens have been reported in infections with bacterial, mycobacterial, fungal, viral and parasitic organisms (reviewed in reference 8). However, positivity for c-ANCA reacting with PR3 and for p-ANCA reacting with MPO is highly specific for small vessel vasculitis and extremely uncommon in infections. Nevertheless, c-ANCA/PR3-ANCA have been reported in amebiasis as well as subacute bacterial endocarditis (SBE).[20,21] In both instances the ANCA disappeared with appropriate antimicrobial therapy, suggesting that ANCA may occur transiently in the setting of infection, and that the persistent ANCA response in patients with vasculitis may be the result of molecular mimicry in susceptible hosts.[22] Subsequent diversification of T- and B-cell responses ("epitope spreading") may lead to responses against different epitopes on the same target molecule (intramolecular spreading) or extend to other molecules (intermolecular spreading).[23]

It is therefore an attractive hypothesis that infections may trigger an initial ANCA-response. If it happens to be directed against the "right" molecules, ie, PR3 or MPO, and is allowed to persist, the stage is set for the subsequent development of small vessel vasculitis. Even though it is currently unclear what makes these two antigens so unique among all the described ANCA target antigens that only ANCA against these two are tightly associated with small vessel vasculitis, the ANCA target antigen

specificities seem to have a bearing on clinical disease manifestations. Whereas WG is mostly associated with c-ANCA/PR3-ANCA,[24,25] the majority of MPA patients have p-ANCA/MPO-ANCA.[26] A direct comparison of clinical features of patients categorized by their ANCA-status revealed that extra-renal manifestations, granuloma formation, and relapse were more frequent in patients with PR3-ANCA than those with MPO-ANCA.[27] In patients with pauci-immune glomerulonephritis and MPA, the relative risk of death was 3.78 times greater in patients with c-ANCA than in those with p-ANCA. Most individual histopathologic lesions, and particularly the capillaritis of the lung and the focal segmental necrotizing glomerulonephritis, do not allow a distinction between MPO-ANCA–associated disease and PR3-ANCA–associated disease per se. Yet the characteristic necrotizing granulomatous ("geographic necrosis") inflammation with giant cells of WG is rarely encountered in patients with MPO-ANCA.[28,29] A careful analysis of renal biopsy specimens of 173 patients obtained at the time of diagnosis suggests that active and chronic renal lesions are more abundant in MPO-ANCA–positive patients than in PR3-ANCA positive patients,[30] confirming previous observations made by others in a smaller cohort of patients.[31] Thus, despite substantial overlap, there appear to be clinical and pathologic differences between patients with PR3-ANCA and MPO-ANCA that may reflect different pathogenic interactions between ANCA, their target antigens, and the target organ environment at the molecular level.

Why does not everybody with PR3- or MPO-ANCA have active vasculitis?

The clinical observation that not everybody with a PR3-ANCA or MPO-ANCA develops a small vessel vasculitis appears to be at odds with the concept of the pathogenicity of these ANCA. Even the best-standardized ANCA detection methods occasionally identify false-positive results. The best-documented false-positive C-ANCA/PR3-ANCA are the already mentioned 7 patients with SBE (only one of whom also had proven vasculitis).[21] Another important clinical observation is that not every vasculitis patient with ANCA relapses when ANCA recur or persist.[12,32] This and the fact that the ANCA of the SBE patients disappeared promptly after successful antibiotic therapy might suggest that "pathogenic" ANCA need to persist long enough for subsequent exposure of the patient to cofactors (such as infection or wound healing) that via neutrophil and monocyte priming allow for ANCA to interact with their target antigen on the effector cell surface and trigger the cascade leading to endothelial cell damage **(Figure 1)**.

Another possible explanation is that ANCA subsets, which are not differentiated by routine methods of ANCA detection, interact differently with their target antigens, resulting in variable functional consequences. Competition studies using different monoclonal antibodies have shown that sera from patients contain variable amounts of PR3-ANCA subsets recognizing different epitopes.[33] Furthermore, some PR3-ANCA interfere with the complexation of PR3 with its inhibitor, alpha 1-PI, and some directly inhibit the enzymatic activity of PR3. In these small patient series, PR3-ANCA with these properties seemed to be associated disease activity.[34-36] The notion that some PR3-ANCA recognizing specific epitopes may be more relevant for the pathogenesis of vasculitis than others is also supported by our observation that PR3-ANCA encountered in cocaine abusers with midline destructive lesions, but no vasculitis, seem to be different from those found in WG patients.[37] Furthermore, we have shown that PR3-ANCA subsets recognizing conformational epitopes on the pro-form of PR3 may be more sensitive to changes in disease activity than PR3-ANCA reacting with epitopes only accessible on the mature form of PR3.[38] ANCA recognizing different epitopes on MPO have also been reported,[39,40] and certain epitope recognition profiles seem to be related to clinical disease manifestations.[41] Finally, ANCA of different IgG subclass may affect the clinical disease manifestations. Enrichment of IgG3 subclass ANCA has been observed in patients with active disease.[12,42,43] IgG3 is the most effective complement activator and binds with high affinity to Fc receptors on neutrophils and monocytes.

These observations support the hypothesis that specific ANCA subsets may contribute to the heterogeneity of clinical manifestations via their different functional effects on their target antigens and inflammatory cells. At the same time, ANCA subsets may also explain apparent incongruences between ANCA test results and clinical findings. More definitive studies aimed at the identification of specific ANCA subsets, their functional impact and clinical correlations in large well-characterized patient populations allowing detailed statistical analysis with sufficient power are needed.

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13-096

SEQUENTIAL EPITOPE MAPPING OF THE MYELOPEROXIDASE ANTIGEN

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A limited percentage of small-vessel vasculitis patients are shown to have a p-ANCA staining pattern revealing the presence of autoantibodies specific to proteins found in the azurophilic granules of neutrophils. Of these proteins, myeloperoxidase (MPO) seems to be the most commonly bound by p-ANCA, which suggests that these autoantibodies may in some way contribute to the propagation of vasculitic disease. Antibody responses to MPO have been found most frequently in patients with microscopic polyangiitis, crescentic glomerulonephritis, and in Churg-Strauss syndrome. In addition, anti-MPO antibodies have also been found in a wide variety of diseases ranging from rheumatoid arthritis to inflammatory bowel disease. Despite this, no specific causative role for these autoantibodies and vasculitis has yet been identified.

In this study, we seek to characterize the sequential antigenic determinants of MPO in patients with p-ANCA–positive vasculitis. We have screened p-ANCA–positive patient sera for reactivity with the maximally possible decapeptides of MPO on derivatized, polyethylene solid phase supports using a modified ELISA assay. Patient serum samples, shown to have p-ANCA by indirect immunofluorescence, were tested on INNOVA anti-MPO ELISA kits to identify patients with anti-MPO antibodies. Ten such patients were identified and were assayed on the decapeptide ELISA's to further establish what epitopes p-ANCA most commonly bound to on MPO. Six epitopes were found to be the most commonly bound by the ten patients at statistically relevant levels (four standard deviations above the normal mean). Epitope 1 (VLTPAQLNVL) was bound by thirty percent of the patients, epitope 2 (EQDKYRTITG) by thirty percent of the patients, epitope 3 (YEDGFSLPYG) by thirty percent of the patients, epitope 4 (SARIPCFLAG) by thirty percent of the patients, epitope 5 (LPLVLGPTAM) by thirty percent of the patients, and epitope 6 (LPALNLASWR) by forty percent of the patients. This study shows that the anti-MPO response found in p-ANCA–positive patients bind most commonly at six sequential areas of the protein, a fact which may help evaluate the pathogenic potentials and potential etiological triggers of anti-MPO antibodies.

Genetics of ANCA-associated vasculitides

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Within the spectrum of vasculitides, antineutrophil cytoplasmic autoantibodies (ANCA), particularly those directed to proteinase 3 (PR3) and myeloperoxidase (MPO), are strongly associated with the idiopathic pauci-immune small-vessel vasculitides.[1] Data from in vitro and in vivo experimental studies suggest that ANCA are involved in the pathophysiology of those disorders.[2] Although the etiopathogenesis of the ANCA-associated vasculitides has not yet been elucidated, current knowledge strongly points to an autoimmune background of the diseases. It is becoming increasingly clear that genetic factors are involved in the expression of autoimmune diseases. In systemic lupus erythematosus (SLE), multiple genes seem instrumental in disease induction and expression.[3] Also in the ANCA-associated vasculitides, genetic factors may be operative. A number of familial cases have been described,[4-9] but no systematic approach has been undertaken to document increased familial segregation of this group of diseases. The genes supposedly involved in disease induction and expression have not yet been identified for the ANCA-associated vasculitides, but skewing in polymorphisms of both immune response genes and genes encoding for PR3 and its inhibitor alpha-1-antitrypsin (α_1-AT) have been documented. This short report discusses data that point to a role of those particular genes in the ANCA-associated vasculitides.

HLA-GENES

Products of the very polymorphic HLA-genes are involved in immune recognition by T-cells. Class I gene products present peptides to CD8-positive cytotoxic T-cells, and class II gene products are responsible for presentation of antigenic peptides to CD4-positive T-cells. In particular, class II genes are, by their extreme polymorphism, supposed to influence immune responses to specific antigens, both qualitatively and quantitatively. Therefore, associations have been sought between HLA-gene polymorphisms and diseases that are thought to be based on specific autoimmune responses. Also for the ANCA-associated vasculitides many groups have studied possible associations with HLA-genes. Although some negative and positive associations have been found, no consistent and significant associations came out of these studies. In a study on 59 patients, 34 with Wegener's granulomatosis (WG) and 25 with microscopic polyangiitis (MPA), Spencer et al found an increased frequency of HLA-DQ7 and a decreased frequency of HLA-DR3.[10] These findings could not be confirmed by Hagen et al[11] studying 224 patients with ANCA-associated vasculitis. They found a decreased frequency of HLA-DR13DR6, but did not observe differences in the distribution of HLA-antigens between patients with WG and MPA, between anti-PR3- and anti-MPO–positive patients, and between patients with and without relapsing disease. Another study on 37 Greek patients with WG showed a weak association with DR1.[12] Taken together, the data presently available do not point to a specific MHC profile in ANCA-associated vasculitides and, so, not to a particular (exogenous?) antigen involved in disease induction.

OTHER IMMUNE RESPONSE GENES

It has become increasingly clear that not only HLA-genes but almost every gene in an outbred human population shows polymorphism resulting, in many cases, in functionally (slightly) different products. As for genes involved in immuneresponsiveness polymorphisms in genes encoding for Fcγ-receptors (Fcγ-Rs), complement factors C3 and C4, integrins such as CD18, and TNFα have all been studied in the ANCA-associated vasculitides.

As mentioned, ANCA are thought to be involved in the pathogenesis of ANCA-associated vasculitides, mostly by their potential to activate primed neutrophils.[2] This process is clearly dependent on interaction with Fcγ-Rs on neutrophils, although $F(ab')_2$-fragments of ANCA can induce some activation as well. As Fcγ-Rs show functional polymorphism, it is understandable that Fcγ-R polymorphism has been studied in the aforementioned diseases in relation to disease induction and disease expression. As for the individual phenotypes of FcγRIIa, FcγRIIIa, and FcγRIIIb, no significant skewing was observed in patients with WG[13] or ANCA-positive systemic vasculitis,[14-16] although the NA_1 allele of the FcγRIIIb seemed overrepresented in patients with MPO-ANCA–positive systemic vasculitis.[14] Combining haplotype frequencies, it proved that the presence of the homozygous FcγRIIa-H/H131 in combination with the homozygous FcγRIIIa-V/V158 polymorphism constituted a significant risk factor for WG (RR 4.6, CI 1.4-15.2).[13] Individuals with FcγRIIa-H/H131

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bind more IgG3 and are capable of binding IgG2 compared to individuals homozygous for the R/R131 form of FcγRIIa, and individuals with the V/V158 form of FcγRIIIa bind more IgG1 and IgG3 than individuals with the F/F158 form of FcγRIIIa. So, the observed skewing is consistent with a stronger interaction of ANCA with neutrophils. In addition, Dijstelbloem et al[13] showed that WG patients homozygous for both the R131 form of FcγRIIa and the F158 form of FcγRIIIa had an increased risk for relapse of WG (RR 3.3, CI 1.6-6.8). They hypothesized that the presence of these phenotypes with low binding capacity for IgG-subclasses increased the risk for infection or colonization with *Staphylococcus aureus*, a well-known risk factor for relapse.

Persson et al[17] studied complement C3 and C4 allotypes in patients with ANCA-positive vasculitis. Although the role of the complement system in ANCA-positive vasculitis is not as clear as in SLE, immune complexes and complement might be involved in the initial stages of the former diseases.[18] Persson et al found the C3F allele increased in PR3-ANCA–positive patients, but the clinical significance is not clear. They also observed an increased frequency of the C4A3 allele in patients with ANCA-positive vasculitis but, again, the functional significance of this finding is not directly apparent.[17]

Gencik et al[15,19,20] studied a number of other polymorphisms in relationship to immune responsiveness. They detected 10 single nucleotide polymorphisms in the integrin molecule CD18, and 4 of these were associated with MPO-ANCA–positive vasculitis. One of these four was localized in an alternate transcription initiation site and may potentially influence CD18 gene expression and, so, the adhesion cascades of leukocytes. No differences in the distribution of polymorphic phenotypes were detected for a number of other immunoregulatory proteins such as TNFα, IL-2, and IL-5 receptorα.

Also, Huang et al did not find an association of particular polymorphisms of TNFα or IL-1β with WG.[21] They did find an association of a microsatellite of CTLA-4 with WG.[21] As CTLA-4 is involved in T-cell activation, this association is supportive of the role of T-cells in the pathogenesis of WG.

■ POLYMORPHISM IN ALPHA-1-ANTITRYPSIN AND ANCA-ASSOCIATED VASCULITIS

The target antigen of ANCA in WG is PR3 in most of the cases. PR3 is a serine protease from the azurophilic granules of neutrophils released upon degranulation. PR3 is a lytic enzyme and the body is protected against its destructive action by anti-proteases. Amongst these, α_1-AT is of major importance. α_1-AT is encoded by a highly polymorphic gene at the protease inhibitor (Pi) locus. The products are functionally classified as normal (M) or deficient (Z) resulting in, based on its codominant expression, homozygous and heterozygous phenotypes. The Pi MM-variant functions normally, the Pi MZ-phenotype is slightly deficient, and the Pi ZZ-phenotype is severely deficient. One might hypothesize that functional deficiency of α_1-AT results in more severe disease expression or even in induction of anti-PR3 once active PR3 persists in an inflammatory environment. Indeed, Esnault et al[22] observed an increased prevalence of the Pi ZZ and Pi MZ phenotypes in anti-PR3–positive patients. An increased frequency of the Pi Z allele in patients with anti-PR3 has been confirmed by many other groups (reviewed in 23). Reversely, the presence of the Pi Z allele in a random population was not associated with an increased incidence of ANCA-associated vasculitis,[24,25] as far as could be studied in a relatively small population of 47 and 191 individuals being homozygous for Pi ZZ, respectively. Nevertheless, these data show that the Pi ZZ phenotype of α_1-AT is not a major risk factor for ANCA-associated vasculitis but may contribute to disease induction.

■ GENETIC FACTORS RELATING TO PROTEINASE 3 EXPRESSION

PR3 itself, the major target of ANCA in WG, has been shown to be expressed in polymorphic forms. Gencik et al[26] screened the entire coding and promoter sequences of the PR3 gene for polymorphisms. Besides one amino acid change at position 119, they found 7 single-nucleotide polymorphisms, one 84 bp insertion/deletion, and a microsatellite. Comparing PR3 from patients with WG with that of healthy controls they observed the A-564 G polymorphism, localized in the promoter region of PR3, to be overrepresented in WG. The functional significance of this polymorphism is, however, not clear.

Possibly more interestingly, Witko-Sarsat et al demonstrated that PR3, which is mainly localized in the azurophilic granules of PMNs, is present, at least in a subset of PMNs, in the secretory vesicles as well, and can be easily mobilized to the cell membrane.[27,28] The same authors showed that the percentage of PMNs with membrane expression of PR3 on resting cells is constitutively determined for each individual and appears to have a genetic background.[29] Based on family studies they suggest membrane expression of PR3 as a new polymorphism with three different phenotypes: low expression (on <20% of PMNs), intermediate expression (on ±50% of PMNs), and high expression (on ±75% of PMNs).[29]

As stated, membrane expression of PR3 was found present on resting neutrophils. Previous studies had already shown that priming of PMN as well as PMN apoptosis result in surface expression of PR3,[2] a prerequisite for PMN activation by ANCA. However, both "low" and "high PR3 expressing" PMNs can be activated by stimulants such as fmlp, although a comparison has not been made with respect to their, possibly differential, degree of activation by ANCA.

The frequency of the "high expression" phenotype of resting PMNs was increased in patients with systemic vasculitis as well as in patients with rheumatoid arthritis, but not in patients with cystic fibrosis and diabetes, compared to healthy controls.[29] The "high expression" phenotype was not restricted to patients with PR3-ANCA–associated vasculitis but was seen also in MPO-ANCA–positive and ANCA-negative vasculitis patients.[29] The functional significance of this supposed polymorphism is not clear. We recently could confirm increased membrane expression of PR3 on resting PMNs in patients with WG com-

pared to healthy controls, although the difference was of minor significance.[30] When surface expression of PR3 on resting PMN was categorized as low, bi-modal, and high in patients with WG, it proved that bi-modal and high expression was associated with a significantly increased risk for relapse. So, increased membrane expression of PR3 on resting PMN, assumed to be genetically determined, may have functional significance with respect to (ANCA-induced?) neutrophil activation.

CONCLUSION

ANCA-associated systemic vasculitis may develop by the interplay of exogenous and endogenous factors. Within the latter category, genetic factors play a primary role. Genetic factors related to immune response genes seem to be involved as in other autoimmune diseases. In PR3-ANCA–associated vasculitis, genetic polymorphism in the PR3-molecule, as well as in its expression in and on the leukocyte, may influence induction and clinical course of WG, a disease strongly associated with PR3-ANCA. In the same line, functional differences in antiproteases, such as deficiency in α_1-AT, may determine the clinical expression of PR3-ANCA–associated disease. The pathways from genetic risk factors to overt disease still should be elucidated.

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14-028

MEMBRANE EXPRESSION OF PROTEINASE 3 IS GENETICALLY CONTROLLED

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Proteinase 3 (PR3) is present on the surface plasma membrane of a subset of PMN. A large PR3-positive subset favors the development of ANCA vasculitis. Earlier studies suggested that PR3 membrane expression is influenced by genetic variance. We tested the hypothesis that membrane PR3 expression on PMN is genetically influenced. We recruited 11 pairs of identical twins (mz; 4m 18f, 31 ± 10 years old) and 7 pairs of fraternal twins (dz; 5m 9f, 26 ± 6 years old). PMN were isolated and PR3 membrane expression was assessed by FACS using a mab to PR3 (CLB-7). In addition, PR3-positive and PR3-negative cells were separated by cell sorting and the intracellular PR3 content was estimated by Western blot analysis. Repetitively FACS analysis performed in 15 healthy subjects showed an excellent correlation in 2 independent investigations (r = 0.91). Assaying the percentage of PR3-positive PMN, we found a highly significant correlation of r=0.991 within mz twins. In contrast, no correlation at all was detected within dz twins (r=0.05). Furthermore, we studied whether or not the difference in membrane expression was the consequence of a different intracellular PR3 content. Western blotting showed similar intracellular PR3 amounts in membrane PR3-positive and membrane PR3-negative cells separated by FACSsort. In summary, our data from a small twin analysis clearly demonstrate that the membrane expression of PR3 is genetically influenced. Furthermore, our data suggest that PR3 membrane expression is not a function of the intracellular PR3 content.

15-015

GENETIC POLYMORPHISMS IN TNF, IL-1, IL-6 AND CYTOTOXIC T LYMPHOCYTE-ASSOCIATED ANTIGEN 4 (CTLA-4) IN WEGENER'S GRANULOMATOSIS (WG)

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Background: Although the precipitating event(s) that trigger WG are unknown, evidence for genetic predisposition has been suggested by the occurrence, albeit infrequent, of familial cases. Cytokines and co-stimulators control the quality and intensity of immune responses. Thus they are relevant candidates for the study of immune dysregulation in WG that may have either an acquired or inherited basis.

Patients and Methods: Using PCR-based, genomic DNA genotyping, this study investigated the polymorphisms located in the genes encoding proinflammatory cytokines such as TNF-α, IL-1 and IL-6, and CTLA-4 (a negative co-stimulator for T cell activation), in 117 American patients with WG and 123 ethnically matched healthy controls.

Results: A significantly lower percentage of patients homozygous for the shortest allele 86 in the microsatellite polymorphism (AT)n located in the 3'-untranslated region of exon 3 of CTLA-4 was found as compared with healthy controls (47% versus 70%, p = 0.0005). Examination of a bi-allelic exonal polymorphism in CTLA-4 did not show skewing in patients. Significant differences in the allelic and genotypic frequencies of polymorphisms in the other proinflammatory cytokine genes studied were not found between patients and controls.

Conclusion: The CTLA-4 (AT)n 86 allele has been previously demonstrated to be crucial for maintenance of normal levels of CTLA-4 expression and T cell activation. Our results confirm findings from a Scandinavian cohort, suggesting that this observation is not limited to WG patients who are ethnically unique. The (AT)n polymorphisms in the CTLA-4 gene may represent a WG-related susceptibility mutation and account for increased T cell activation and clonal expansion in WG patients. Blockade of T cell costimulation using CTLA-4Ig might be a useful therapeutic intervention, providing an alternative or complementary approach to conventional immunosuppressive agents.

Classification of anti–endothelial cell antibodies into antibodies against microvascular and macrovascular endothelial cells: The pathogenic and diagnostic implications*

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Antibodies that react with endothelial cell (EC) structures (known as anti–endothelial cell antibodies [AECA]) were first reported in the early 1970s during an immunohistochemical study of kidney biopsy specimens. The examined sera were from patients with various rheumatic diseases, including systemic lupus erythematosus (SLE) and scleroderma.[1,2] Since then, several methodologic approaches have demonstrated the existence and potential pathogenic role of AECA in a wide variety of inflammatory diseases (for review, see ref. 3). The AECA bind to different structures on endothelial membranes, mainly through the $F(ab)_2$ portion of the immunoglobulin.[3] IgG, IgM, and IgA isotypes of those antibodies have been reported. The EC target antigens for AECA have not yet been defined, but it is clear that there are likely to be multiple target antigens.[4-7] Accordingly, sera positive for AECA have been shown to display a broad reactivity against EC obtained from different human anatomic sources: from large arterial (aorta) or venous (umbilical cord vein, saphenous vein) vessels as well as from small vessels such as renal, skin, omental, and brain microvasculature.[8-10] In addition, AECA are apparently not species specific, since they cross-react with human, bovine, and murine EC (for review, see refs. 3 and 11).

In this respect, one can speculate that AECA are nonspecific antibodies that are directed against endothelial antigens and commonly expressed on the majority of vascular tissue. Based on our previous observations that AECA in large-vessel diseases such as *Takayasu's arteritis* (TA) bind to and activate *macrovascular human umbilical vein endothelial cells* (HUVEC), and not microvascular EC,[12] we advocate the attractive hypothesis that AECA from different sources recognize different types of EC target molecules, which is correlated with the origin of the disease. Therefore, classification of AECA into antibodies that are directed against microvascular and macrovascular EC should shed more light on this complex group of antibodies. This lecture will summarize the results of some of our previously published studies as well as the current literature on this subject, which would support a rationale for proposed classification of AECA.

CLASSIFICATION OF AECA AS ANTIBODIES AGAINST MICROVASCULAR AND MACROVASCULAR EC

ANCA-positive necrotizing and crescentic glomerulonephritis

The first reports of disease-specific vascular bed involvement came from studies on autoantigens of ANCA in necrotizing and crescent glomerulonephritis (NCGN).[13] ANCA-positive sera of patients with NCGN reacted with glycoproteins from a membrane preparation of polymorphonuclear neutrophil granulocytes, designated as gp170/80-110 and verified to be identical with human lysosomal-associated membrane protein 2 (h-lamp-2). Unexpectedly, these sera also cross-reacted with 130-kD EC membrane glycoprotein (gp130) of the renal microvasculature. Gp130 is also present on the surface of EC of intestinal capillaries and placental capillaries but not on EC of arteries and arterioles.[13] Accordingly, both monoclonal antibody against gp170/80-110 and rabbit anti-gp130 failed to bind unstimulated and IL-1–treated HUVEC. Interestingly, 14 of 16 patients with NCGN had IgG specific for gp130 and gp170/80-110. The relation between gp130 and h-lamp-2 has not been established yet. A possible explanation is that the 130-kD antigen shares one or several epitopes with h-lamp-2. It is also possible

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TABLE 1
PREVALENCE OF ANTI–ENDOTHELIAL CELL ANTIBODIES AMONG DIFFERENT IMMUNOLOGICALLY MEDIATED DISEASES

Disease	Prevalence (%)	References*
Primary autoimmune vasculitis		
Wegener's granulomatosis/microscopic polyangiitis	55-80	8, 9, 17, 19, 20
Kawasaki disease	Up to 72	28-31
Takayasu's arteritis	95	32
Giant-cell arteritis	Up to 50	11
Idiopathic retinal vasculitis	35	33
Behçet's disease	Up to 50	10
Thromboangiitis obliterans	25-36	11, 34
Churg-Strauss disease	50	11
Systemic autoimmune diseases		
Systemic lupus erythematosus	Up to 80	4, 14, 17, 35, 36
Antiphospholipid syndrome	64	40
Rheumatoid arthritis with vasculitis	Up to 65	17, 45, 46
Rheumatoid arthritis without vasculitis	Up to 30	17, 45, 46
Systemic sclerosis	20-80	6, 47, 48
Mixed connective tissue disease	45	17
Polymyositis/dermatomyositis	44	53
Transplantation		
Heart and kidney allografts	Up to 71	15, 54, 55
Miscellaneous		
Inflammatory bowel disease	Up to 55	11, 62-64
Hyperprolactinemia in women	76	65
Hemolytic uremic syndrome	93	66
Thrombotic thrombocytopenic purpura	100	66, 67
Heparin-induced thrombocytopenia	100	68
Multiple sclerosis	23-75	11, 83
IgA nephropathy	32	75
Diabetes mellitus	26-75	11, 16, 76, 77
Hypoparathyroidism (autoimmune)	100	11
Acute pre-eclampsia	50	78
Rocky Mountain spotted fever	50	79
Viral infection	Up to 18	11
Borderline hypertension	None reported	80
Hepatitis C virus mixed cryoglobulinemia	41	81

* Reference numbers apply to references in the original full-length review from which this article is excerpted (Arth Rheum 2001; 44:1484–1494).

that the 130-kD membrane protein is an isoform of h-lamp-2, which differs primarily in its carbohydrate side chain.[14]

SLE and primary APS

Recent analysis of AECA specificity in primary antiphospholipid syndrome (APS) and SLE revealed differences in both the pattern of antibody binding and band intensity between membrane antigens on HUVEC and human microvascular EC (HMEC).[15] In fact, of 17 primary APS sera, antibody binding to HUVEC membranes was found in 9 sera and to HMEC membranes in 7 sera. Binding at 72 to 79 kD was confined to HUVEC. Among 32 SLE sera, binding to HUVEC and HMEC membranes was detected in 17 and 22 sera, respectively, with binding at 135 to 155 kD being confined to HMEC. As mentioned, some anti-EC reactivity in APS may be directed to epitopes on phospholipid-binding proteins, especially β_2GPI.

Takayasu's arteritis (TA)

In a previous study we were able to show entirely differential binding and activation of microvascular and macrovascular EC in various vasculitic conditions and diverse autoimmune disorders. With this regard, we obtained monoclonal anti-EC antibodies (mAbs) from a patient with TA.[12] Six mAbs were selected, the mixture of which produced 100% inhibition of binding of the original IgG from the patient with TA to HUVEC. All mAbs possessed high activity against macrovascular EC (ie, anti-HUVEC activity), but none had significant anti–microvascular EC (anti–human bone marrow EC:HBMEC) activity. Four of the 6 mAbs activated EC, which was manifested by increased IL-6 and von Willebrand factor secretion. The 4 mAbs induced EC expression of adhesion molecules and increased adhesion of monocytes to EC. In addition, these mAbs stimulated the nuclear translocation of the nuclear factor κB transcription factor. Moreover, the immunohistochemistry studies demonstrat-

ed considerable anti–human-aortic EC activity of the mAbs, while anti–microvascular EC antibodies (from patients with heparin-induced thrombocytopenia) or normal human IgG did not react with human aorta. Again, the distinct predilection of the AECA mAbs to macrovascular antigens is compatible with the pathological characteristics of TA, which exclusively affects large arteries.

Other diseases

Behçet's disease is preferentially a small-vessel disease, although large vessel involvement is observed in 15% to 35% of patients.[16] However, when the same patients' sera were exposured to microvascular (omental) or macrovascular (HUVEC) EC employing a cyto-ELISA, binding to microvascular EC was seen in 43% of patients' sera, whereas 26% of patients' sera recognized HUVEC.[10]

Progress on the isolation and culture of various EC has allowed comparison of biochemical and physiologic properties of EC from the micro- and macrovasculature. These cells share certain common features, including monolayer formation, production of factor VIII, and prostacyclin as well as the presence of Weibel-Palade bodies. EC from small capillaries, however, differ from the EC of large arteries and veins in their nutritional requirements and in their responses to growth and migration stimuli.[17] The antigenic heterogeneity of vascular endothelium was further elucidated employing immunocytochemical methods on different human vascular beds.[18] According to the results of this study, capillary EC strongly express MHC class I and II, ICAM, and OKM5, which are variably weak to undetectable on large vessels. In contrast, large vessels strongly express von Willebrand factor and appear to constitutively express E-selectin. The authors anticipated that the capillary EC may be more efficient at antigen presentation or more susceptible to immune attack in vivo.

The concept of vascular bed–specific hemostasis and hypercoagulable states, recently presented by Rosenberg and Aird,[19] sheds additional light on phenotypic and functional differences between various EC. According to this concept, the endothelium integrates different extracellular signals and responds differently to the same endogenous (eg, local changes in blood flow) or exogenous injurious agents in different regions of the vascular tree.

■ CONCLUSION

The differences between EC from large and small vessels and the consequent reactivity of AECA in small- and large-vessel diseases underscore the importance of using cells derived from vessels of appropriate size when studying macro- or microangiopathies. The wide range of AECA frequencies in a defined disease, as shown in **Table 1**, may be therefore attributed to the various sources of EC used for detection of AECA. Some sera apparently negative for AECA may be reactive if EC of appropriate types were employed. Consequently, it is rational to classify AECA into one of two groups of antibodies, against either microvascular or macrovascular EC.

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16-029

HMG-COA REDUCTASE INHIBITORS DECREASE ANCA-MEDIATED ACTIVATION OF HUMAN NEUTROPHILS

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HMG-CoA reductase inhibitors (statins) may modulate cellular inflammatory functions independent of their cholesterol-lowering action. We tested the hypothesis that statins decrease respiratory burst activity of human PMN in response to ANCA. Superoxide generation was measured by the ferricytochrome C assay and the nitroblue tetrazolium (NBT) test. Pretreatment with either cerivastatin or simvastatin inhibited respiratory burst activity of TNF-α-primed PMN to ANCA dose-dependently (1-25 μM). Both statins also inhibited the response to human ANCA. PR3-ANCA resulted in 18.6 ± 3.9 nmol O_2^- /0.75 × 10^6 PMN/45 min; this amount was decreased to 7.6 ± 1.8 nmol by preincubation with 10 μM simvastatin ($p<0.01$). For MPO-ANCA these numbers were 22.6 ± 2.8 nmol for controls versus 16.7 ± 3.1 nmol with simvastatin ($p<0.01$). The inhibitory effect was confirmed using the NBT test. We next investigated whether or not the inhibition could be reversed by mevalonic acid (MVA). TNF-α-primed neutrophils released 26.7 ± 2.8 nmol O_2^- and 10 μM simvastatin reduced this amount to 18.0 ± 2.1 nmol. The inhibitory effect could not be reversed in the presence of 500 μM MVA (18.0 ± 2.2 nmol O_2^-). By FACS, we demonstrated that simvastatin resulted in a small but significant decrease in TNF-α-mediated ANCA antigen translocation (from 219 ± 33 to 180 ± 35 MCI for PR3 and 24.0 ± 2.4 to 18.3 ± 1.1 MCI for MPO). Finally, we studied the effect of simvastatin on MAPK, since we found earlier that p38 MAPK and ERK control TNF-α-mediated priming. Western blotting demonstrated that simvastatin inhibited TNF-α-induced ERK phosphorylation, but had no effect on p38. These findings demonstrate that HMG-CoA reductase inhibitors decrease respiratory burst activity of human PMN in response to ANCA. This effect was independent of mevalonate but involved inhibition of ERK activation during TNF-α priming. Our data suggest that treatment of patients with HMG-CoA reductase inhibitors may help to limit inflammatory responses caused by ANCA-activated neutrophils.

Kawasaki disease: Etiology, pathogenesis, and treatment

KARYL S. BARRON, MD

Kawasaki disease is an acute febrile illness of childhood and is the primary cause of acquired heart disease in children in the United States and Japan. Initially described by Kawasaki in 1967,[1] the syndrome was thought to be a benign, self-limited febrile illness. It is now known to be a systemic vasculitis occurring predominantly in small and medium-sized muscular arteries, especially the coronary arteries. Cardiac sequelae account for most of the morbidity and mortality of the disease, and treatment is based on prevention of aneurysm formation.

EPIDEMIOLOGY

Eighty percent of cases of Kawasaki disease occur in children younger than 5 years of age. The peak incidence is in children 2 years of age and younger, with boys affected 1.5 times as often as girls. Recurrences occur in 2% to 4% of cases,[2,3] and familial incidence is approximately 2%.[4]

Although all racial groups are represented, children of Asian ancestry continue to predominate, with the incidence in the Japanese being highest at approximately 50 to 200 per 100,000 children younger than 5 years of age.[3,5] In the United Sates, reports of incidence range from six to 15 per 100,000 children younger than 5 years of age,[6] with Asian Americans being proportionately overrepresented and white Americans being proportionately underrepresented. New cases occur throughout the year in North America, with larger numbers occurring in late winter to early spring.

The occurrence in siblings is rare. In a nationwide study in Japan, the overall second-case rate for siblings was 2.1% compared with an overall incidence of approximately 0.19% in the general population of children 0 to 4 years of age.[4] More than half of the second cases developed 10 days or fewer after the first case occurred.

CLINICAL FEATURES

The principal diagnostic criteria include: 1) fever lasting more than 5 days; 2) conjunctival injection; 3) oropharyngeal changes including erythema, swelling and fissuring of the lips, diffuse erythema of the oropharynx or strawberry tongue; 4) peripheral extremity changes including erythema of the palms and soles, induration of the hands and feet, desquamation of the skin of the hands and feet, or Beau's lines (transverse grooves in the nails); 5) polymorphous rash; 6) cervical lymphadenopathy, usually a single node >1.5 cm. Five of the six criteria, with fever being an absolute, must be present for diagnosis. "Atypical" cases may be diagnosed with fewer criteria when coronary artery aneurysms are noted by echocardiography or angiography. There are a number of associated manifestations that may aid in the diagnosis of Kawasaki disease. Among these are: irritability, sterile pyuria, meatitus, perineal erythema and desquamation, arthralgias, arthritis, abdominal pain, diarrhea, hepatitis, obstructive jaundice, hydrops of the gallbladder, pulmonary infiltrates, pleural effusions, uveitis, sensorineural hearing loss, and cardiovascular manifestations.[7,8]

CARDIAC DISEASE

Cardiac abnormalities manifested during the acute stage include pericardial effusions in approximately 30% of cases. Myocarditis is also common in the acute phase and is manifested by tachycardia and gallop rhythm. Congestive heart failure and atrial and ventricular arrhythmias can occur. Electrocardiogram findings include decreased R-wave voltage, ST segment depression, and T-wave flattening or inversion. Slowed conduction can also occur with PR or QT prolongation.[9] Mitral regurgitation may be present in approximately 30% of patients, although it is usually mild.[10] Aortic valve involvement has also been described.[11]

Coronary artery lesions are responsible for most of the morbidity and mortality of the disease. They developed in approximately 15% to 25% of patients prior to the widespread use of intravenous immune globulin (IVIG), but now occur in less than 10%. Aneurysms usually appear from 1 to 4 weeks after onset of fever, and it is rare to detect new lesions after 6 weeks. Aneurysms are most easily detected by transthoracic two-dimensional echocardiography. Aneurysms are described as small (<4 mm), medium (4 to 8 mm), or giant (>8 mm), and are more commonly proximal than distal. Ectasia of the vessels (vessel size larg-

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er than in age-matched controls) is also a common finding. Small and medium-sized aneurysms generally regress radiographically within 5 years of follow-up[12]; however, these vessels most likely remain abnormal, because response to pharmacologic dilation may remain impaired.[13] Pathologically regressed aneurysms may reveal abnormal intimal proliferation[14] and in fact may also be associated with narrowed lumens and calcified arterial walls, despite the fact that these changes may not be apparent on arteriography.[15] Therapy with IVIG has decreased the incidence of giant aneurysms,[12] which rarely regress and frequently develop complicating thromboses, stenosis, or total occlusion. Myocardial infarction may result; when it occurs, it is most likely to be in the first year, with 40% occurring in the first 3 months of illness.[16] There are, however, reports of young adults suffering myocardial infarctions more than a decade after their initial disease and others with coronary artery aneurysms who were not known to have had Kawasaki disease as children.

■ ETIOLOGY

Despite years of intensive investigation, the etiologic agent of Kawasaki disease remains elusive. Many of the clinical features suggest an infectious etiology, including the fever, exanthem, conjunctival injection, cervical adenopathy and self-limited nature of the illness. In addition, the epidemiologic features including well-defined epidemics with winter-spring predominance and a geographic clustering of cases suggest an infectious etiology.

The peak incidence in early childhood and the virtual absence of Kawasaki disease in adults suggests the causative agent(s) is a ubiquitous microbe that causes an asymptomatic infection in most individuals with acquired immunity by adulthood. The rarity of illness in infants less than 3 months of age suggests passive protection via maternal antibody. The search for an etiologic agent could cover an infectious disease textbook. Because most ubiquitous microbes enter the host via the respiratory or gastrointestinal tracts, one or both of these portals of entry would be likely for the putative agent(s).

Bacteria?

Kawasaki disease shares features with a number of well-characterized bacterial or rickettsial infections, including:

- Staphylococcal or streptococcal toxic shock syndrome
- Rheumatic fever
- Scarlet fever
- Staphylococcal "scalded skin" syndrome
- Rocky Mountain spotted fever
- Leptospirosis

However, in the more than 30 years since the initial report of this disease, none of these can claim responsibility. In an interesting report from Shibata in 1999,[17] nested PCR was used to amplify bacterial ribosomal DNA from PBL obtained from patients with Kawasaki disease. Analysis of a sequence obtained in 3/20 patients revealed a new *Corynebacterium* species. With the exception of *C diphtheriae*, corynebacteria have been considered unimportant as the cause of human diseases. Coryneform bacteria are members of the normal flora of skin and cutaneous membranes; thus, corynebacteria would have been easily missed in the bacterial culture of the nasopharynx from patients with Kawasaki disease. However, increasing evidence shows pathogenic roles for several corynebacteria, and it will be interesting to see future studies.

Virus?

Using techniques of viral isolation and/or serologic confirmation, the following viruses have been incriminated as possible etiologic agents of Kawasaki disease:

- Measles virus
- Epstein-Barr virus
- Adenovirus
- Parainfluenza virus
- Rotavirus
- Influenza virus
- Herpesvirus 6 (HHV6)
- Parvovirus

The wide variety of agents encountered and the failure to demonstrate any unique serologic relationship suggest that these viruses are likely to be either incidental or to play some sort of a "helper" role in the pathogenesis of KD. Furthermore, Chua[18] used PCR to determine whether Kawasaki could be the result of infection by parvovirus B19, human herpesvirus 8, TT virus, GB virus C/hepatitis G virus, or *Chlamydia pneumoniae*. The data do not support an etiologic association between Kawasaki disease and infection with any of these agents.

Retroviruses held a place on the front page of the news as a possible etiologic agent for a number of years. In 1986, DNA polymerase activity was reported from cultured peripheral blood mononuclear cells (PBMC) in patients by 2 independent groups. Burns[19] reported that retrovirus-associated reverse transcriptase (RT) activity was found in culture supernatants of PBMC from 14 patients but not in febrile controls. Shulman and Rowley[20] demonstrated RT activity in 8/18 patients but in only 1/18 controls using co-cultivated PBMC/lymphoblastoid cell supernatants. Melish[21] was unable to demonstrate significant RT activity or other evidence of involvement of retrovirus in the etiology of Kawasaki disease. It was later considered that these initial findings were typical of DNA-dependent DNA polymerase rather than viral RT.[22]

Epstein-Barr virus and Kawasaki disease. EBV generally infects asymptomatically in the vast majority. However, in some instances it causes or relates to the development of a wide spectrum of diseases such as infectious mononucleosis, lymphoproliferative disorder (which occurs in immunologically compromised individuals), hemophagocytic syndrome, chronic active EBV, Burkett's lymphoma and nasopharyngeal carcinoma. Infection occurring in conjunction with an imbalance or deficiency in the normal immune response is considered to increase the risk of development of a range of EBV-associated disorders, as listed above. Kikuta[23] reported detection of EBV genome in 3 cardiac tissue samples and one aortic tissue sample examined by PCR obtained from 3 patients with chronic, active EBV infection associated with Kawasaki-like coronary artery aneurysms. However, none of these patients

had clinical hallmarks of Kawasaki disease. However, Culora[24] was unable to detect EBV-encoded RNA by in situ hybridization in postmortem sections of a coronary artery and myocardium of a patient suspected of having Kawasaki disease. In addition, Marchette[25] found no serologic evidence of EBV infection in Kawasaki patients. For the time being, EBV may be taken off the "most wanted list."

Chlamydia has also taken a lead in the popularity race over the years. C *pneumoniae* is a common respiratory pathogen and is a plausible infectious trigger for Kawasaki disease because it has been linked to endocarditis and myocarditis in children and to an increased risk of atherosclerosis and heart attacks in adults. There is intriguing molecular mimicry between the outer membrane protein of chlamydiae and cardiac alpha myosin, thus providing a mechanism by which *Chlamydia* infection might trigger an immune response to the myocardium. Serologic studies indicate that C *pneumoniae* infections occur most commonly among children 5 to 15 years old. However, serologic studies may underestimate the prevalence of C *pneumoniae* in young children because infected children do not always mount an antibody response. The majority of infections in children are mild or asymptomatic and rarely present as pneumonia. In a study by Normann,[26] C *pneumoniae* was detected by immunohistochemistry in heart tissue specimens from 2 children who died of Kawasaki disease. (Note, however, that this association was based on a small sample size and control tissues from children without Kawasaki disease were not examined.) However, Schrag et al[27] analyzed blood, urine, and pharyngeal specimens from Kawasaki patients for evidence of recent C *pneumoniae* infection by culture, PCR, and serology and found no evidence of current C *pneumoniae* infection in Kawasaki patients. Furthermore, Strigl et al[28] found no difference in the prevalence of anti-chlamydial IgG, IgM, and IgA between Kawasaki patients and controls.

An association with mycobacterial antigens has been suggested because of the inflammatory change that occurs at the site of a previous bacillus Calmette-Guérin (BCG) immunization in children with Kawasaki disease[29,30] and the temporarily positive response to mycobacterial HSP antigens in children with acute Kawasaki disease.[31,32] Whether these responses represent a specific reaction to mycobacterial antigens, or represent a more general response to bacterial or other heat-shock proteins that are cross-reactive, is not clear.

Noninfectious causes that must be considered include:

- Infantile polyarteritis nodosa
- Mercury toxicity (acrodynia)
- Stevens-Johnson syndrome
- Erythema multiforme
- Adverse drug reactions
- Systemic juvenile rheumatoid arthritis
- Association with rug shampoo

In the 1980s, dust mites and rug shampoo were high on the list of possible etiologic agents. In a report in *Lancet* in 1982, Patriarca[33] indicated that the application of rug shampoo during the month before onset of disease was associated with the occurrence of Kawasaki disease. Daniels[34] evaluated the published case-control studies and found 2 studies with significant associations and 3 studies with no association. Rickettsia-like particles were reportedly found in the digestive tracts of house dust mites obtained from the homes of patients.[35] While there was a flurry of publications regarding dust mites and rickettsia-like particles in the 1980s, current literature is devoid of its mention. With the passage of time, the possible association of rug shampoo and Kawasaki disease has faded from popularity.

Superantigen theory

Features of Kawasaki disease are similar to those found in certain illnesses that are caused by toxin-producing bacteria, such as toxic shock syndrome and scarlet fever. Staphylococcal enterotoxins and streptococcal exotoxins are prototypic superantigens that stimulate large populations of T cells in a class II MHC-dependent, yet unrestricted manner. These toxins bind directly to conserved amino acid residues outside of the antigen-binding groove on class II MHC molecules and selectively stimulate T cells expressing particular β-chain variable gene segments.

Other variable elements (Dβ, Jβ, Vα, Jα) of the TCR contribute much less to the recognition of these superantigens. All T cells possessing a specific sequence on the TCR are activated by the MHC-superantigen complex, and this may represent as many as 20% of circulating lymphocytes. The result is an unusually large amount of cytokines from activated T cells, hence the name "superantigen." One hallmark of T-cell activation caused by a toxin with superantigenic activity is an increase in the number of T cells expressing a specific TCR Vβ region. (In contrast, conventional peptide antigens usually require all 5 variable elements for T-cell recognition and therefore stimulate only a low frequency of T cells).

A report in 1992 first described selective expansion of Vβ 2+ T cells and to a lesser extent Vβ 8.1+ T cells in patients with acute Kawasaki disease[36] similar to that seen in patients with toxic shock syndrome. During the convalescent phase, the overrepresentation of the Vβ2+ and Vβ8.1+ T cells returned to normal, indicating that the increase occurs after the onset of Kawasaki disease and is not a marker of susceptibility. (Note: TCR Vβ expression was assessed after in vitro cultivation of T cells in the presence of anti-CD3 antibodies.) Since the release of this report, a flurry of reports, either supporting or denouncing this claim, have surfaced. The jury is still out deliberating whether the superantigen theory is still plausible. While we are waiting for the verdict, I will present evidence for both sides of the argument.

Positive evidence of superantigens

- **Abe 1993**[37]—Confirmed that the increase in Vβ2-bearing T cells occurred primarily in the CD4 cell subset. Sequence analysis of TCR β chain genes of Vβ2 and Vβ8.1 expressing T cells from acute KD patients showed extensive junctional region diversity, supporting the concept of a polyclonal expansion.
- **Leung 1993**[38]—Bacteria were cultured from throat, rectum and groin of 16 patients with untreated

acute KD and 15 controls. Bacteria-producing toxins were isolated from 13/16 KD patients but only 1/15 controls. TSST-secreting *Staphylococcus aureus* was isolated from 11/13 toxin-positive cultures, and streptococcal pyrogenic exotoxins (SPE) B and C were found in the other 2.

- **Curtis 1995**[39]—using monoclonal antibodies to TCR Vβ2, 5, 8, 12, 19, found the mean percentage of Vβ2 expressing T cells in KD patients was increased. Did **not** find a selective increase in Vβ8 bearing T cells (as did Abe[36]).
- **Leung 1995**[40]—KD patients demonstrated elevated levels of TCR Vβ2+ and to a lesser extent Vβ8.1+ T cells in comparison to cells from normal donors and control patients with other febrile illnesses. During convalescence, the proportion of Vβ2+ and Vβ8.1+ T cells returned to normal levels.
- **Yamashiro 1996**[41]—the occurrence of Vβ2+T cells was found to be selectively increased in the small intestinal mucosa of 12 patients with acute KD compared to controls. No significant difference in the occurrence of Vβ+ T cells was noted in the jejunum compared to controls. Did NOT find TCR Vβ significant expansion of T cells in the peripheral blood compared to controls.
- **Masuda 1998**[42]—investigated peripheral T-cell response to superantigens by measuring proliferation and IL-2 production to determine whether there is T-cell anergy induced by superantigens in KD patients. T cells from patients in acute or convalescent stage showed significantly lower proliferation and IL-2 production than did T cells from healthy control subjects following stimulation by SPE-C, but not SPE-A or TSST-1. The T-cell response to SPE-C normalized within 1 year. These results may indicate that the transient low T-cell response to SPE-C in patients may have been related to superantigen-induced anergy or disappearance of SPE-C responding cells from the circulation. They did not examine evidence of invasion of SPE-C or the presence of Strep-producing SPE-C in patients and thus did not confirm a direct role for SPE-C in the etiology.
- **Yoshioka 1999**[43]—the mean percentage of Vβ2- or Vβ6.5-bearing T cells in PBMC in the acute phase was significantly higher than that of patients in the convalescent phase of KD or in healthy donors. This expansion was polyclonal because DNA sequences in the complementarity-determining region 3 of Vβ2 and Vβ6.5+ cDNA clones were all different from each other.

Negative evidence of superantigens

- Group A β-hemolytic Strep has not been consistently isolated from patients; ASO titer is not raised; lack of response to antibiotics.
- **Sakaguchi 1993**[44] found no difference in percentage of Vβ2+ or Vβ8.1+ T cells among patients with acute, convalescent KD, age-matched controls and adults.
- **Pietra 1994**[45]—Using flow cytometry, reported no expansion of any Vβ family in acute KD.
- **Marchette 1995**[46]—Found no evidence of etiological association between exposure to TSST-1 and development of KD.
- **Tristani-Firouzi 1995**[47]—selective expansion of Vβ2 and Vβ8.1 families was not observed.
- **Abe 1995**[48]—Could not confirm that TSST-1 secreting *S aureus* is specifically associated with Kawasaki disease nor that the superantigen produced by the staphylococcal isolates is related to the change of TCR repertoire. They postulated that an unknown etiologic agent stimulates Vβ2 positive T cells or that the Vβ2 expansion in PBMC may be caused by an unknown immunopathological process.
- **Nishiyori 1995**[49]—No expansion of Vβ2- and Vβ8.1-expressing T cells. No increase in anti-TSST-1 titer.
- **Todome 1995**[50]—There were no noticeable differences between *S aureus* strains from KD patients and control children in the production of staphylococcal exotoxins A-E, coagulase serotype, hemolysis of sheep erythrocytes, and tryptophan auxotrophy. The pathological or etiological role of a new TSST-1 secreting *S aureus* clone in patients with KD was not confirmed. Group A Strep could not be isolated from either KD patients or controls.
- **Terai 1995**[51]—Culture supernatants of bacterial isolates from patients did not support involvement of toxin-producing staphylococci in KD.
- **Deresiewicz 1996**[52] found no evidence of an *S aureus* strain or TSST-1 sequence uniquely associated with KD.
- **Morita 1997**[53] examined serum antibody responses to superantigens in paired acute/convalescent sera from KD patients and found a very low frequency of detection of anti-superantigen antibodies and no marked IgG seroconversion.
- **Choi 1997**[54] found clonal expansion of TCR Vβs in some patients, suggesting that the antigen that induced the expansion of T cells may have belonged to the conventional antigens rather than to the superantigens. (These clonal expansions were found mainly in the CD8+ T cells.)
- **Mancia 1998**[55]—No abnormal usage of any TCR Vβ family was found, neither acutely nor during convalescence compared to a group of healthy children.
- **Nomura 1998**[56] examined 25 Vβ families. Selective expansion of the Vβ family in KD was not observed. The pattern of increased Vβs did not show the specific pattern that indicates a particular superantigen.

The jury is still out. After reviewing the gamut of infectious (and non-infectious) etiologies, the etiologic agent is still elusive. The longer that a single infectious agent cannot be identified as the cause of Kawasaki disease, the more the possibility must be considered that multiple agents, each of which can lead to a common pathway, may result in this clinical syndrome. It is possible that the disease is triggered by infection (early acute

stage) and thereafter rheumatic manifestations (immunologically mediated reaction to the initiating infection) follow. Alternatively, the agent may have already been cleared from the sampled compartment at the time of specimen collection.

The close temporal association of KD with acute infection with multiple common agents suggests 3 possibilities: the association may be entirely coincidental; Kawasaki disease represents a cascade of host responses that can be triggered by infection with more than one agent; or common infectious agents infecting acutely ill Kawasaki patients may be acting in a "helper" role to activate or enhance pathogenic expression of the real causative agent. While the stereotyped nature of Kawasaki disease makes the multiple pathogenesis hypothesis unlikely, no primary agent has been identified despite extensive search. Another plausible explanation for the largely negative results may be that a previously unidentified microbial agent causes Kawasaki disease.

Immune abnormalities

Laboratory findings in acute Kawasaki disease reflect the marked degree of systemic inflammation. Early in the course of disease, laboratory evaluation reveals a leukocytosis with a left shift and elevation of acute phase reactants, as measured by the erythrocyte sedimentation rate, serum α1-antitrypsin and quantitative C-reactive protein measurements. A global lymphocytosis follows with a predominance of B cells.[57] Despite evidence of a polyclonal B-cell activation, the antibody repertoire in these patients is poorly defined. Sera from patients with acute KD do not contain the usual antibodies frequently associated with other collagen vascular diseases—no RF, ANA, anti-DNA, or ANCA. In the subacute stage of the disease, platelet count increases and frequently reaches 1,000,000 per microliter or greater by the 3rd week of illness.

B cells are not alone in this state of activation. There is evidence of T-cell activation as well, with increased numbers of CD4+ and CD8+ cells bearing MHC class II antigens[57] and increased levels of soluble interleukin-2 receptors.[58-60]

Striking immunologic perturbations occur in the acute stage of KD. Patients demonstrate cutaneous anergy with delayed-type hypersensitivity reaction on skin testing, supporting the notion of a global dysfunction of circulating T cells.[57]

There is evidence of cytokine cascade activation and endothelial cell activation. Circulating levels of a number of cytokines—tumor necrosis factor-α (TNF-α),[58,60-66] interferon-γ (IFN-γ),[58,63,67,68] interleukin 1 (IL-1),[69] IL-6,[60,64,65,70,71] and IL-8[60,65,72]—have been reported. Peripheral blood mononuclear cells (PMC) from patients with acute, but not convalescent KD spontaneously produce high levels of IL-1[73] and TNF-α.[74,75] These cytokines elicit an overlapping set of proinflammatory and prothrombotic responses in endothelial cells. The acute phase of KD is associated with the appearance of circulating antibodies that are cytotoxic against vascular endothelial cells pre-stimulated with IL-1, TNF-α[76] or IFN-γ,[77] but to a lesser extent or not at all against unstimulated endothelial cells. Successful treatment of KD patients with IVIG plus ASA is associated with a reduction in their cytokine production and endothelial cell activation.[78] In contrast, patients treated with aspirin alone have prolonged T- and B-cell activation.

In a series of reports, anti-endothelial antibodies have been demonstrated in the sera of acute KD patients.[73,76,77,79-81] These antibodies lysed human umbilical vein endothelial (HUVE) and human saphenous vein endothelial (HSVE) cells pretreated with cytokines, including TNF or interferon-gamma. One can postulate that Kawasaki disease is associated with cytokine-mediated endothelial cell activation, possibly including the intimal surfaces and the vasa vasorum of medium-sized arteries. This activation may be associated with the expression of new endothelial cell antigens and functional endothelial cell changes and culminates in an immune response directed against the abnormal stimulated vascular endothelial cell with influx of inflammatory cells into the media with consequent weakening of the vessel wall and predisposition to aneurysm formation.

Adherence of leukocytes to endothelial cells is a key event in the sequence of an inflammatory response. As an initial event during inflammation, leukocytes in the blood stream roll along endothelial cells with loose contact mediated by E-selectin, P-selectin, and L-selectin. In the second phase of inflammation, activation of leukocyte integrins occurs with expression of immunoglobulin-like adhesion proteins on endothelial cells, including intercellular adhesion molecules (ICAM) and vascular cell adhesion molecule (VCAM). Leukocyte traffic across the vascular endothelium is dependent on interaction between leukocytes and endothelial cells mediated by a variety of cell adhesion molecules. Elevated levels of soluble forms of these molecules have been found in conditions associated with endothelial cell activation and inflammation and are thought to be formed by cleavage of the membrane-bound form and release into the circulation of the extracellular domain. Levels of circulating ICAM are also increased in the acute phase of KD.[82-85] ICAM-1 and E-selectin expression on endothelial cells has also been detected in biopsy samples of skin from patients with acute KD.[73]

■ PATHOLOGY

The pathology of acute KD reveals a panvasculitis of the small and medium-sized muscular arteries with endothelial edema, necrosis, desquamation, and leukocyte infiltration of the arterial wall. Inflammatory cells are initially neutrophils but rapidly change to mononuclear cells (paralleling what is seen in the periphery). Infiltration of macrophages and activated T cells has been observed in the vascular lesions of KD.[85] The inflammatory process frequently involves the entire vascular wall. Edema and necrosis cause the wall to lose its structural integrity, leading to formation of aneurysms. One to two months later, the inflammatory cells are less apparent, and fibrous connective tissue begins to form within the vessel wall. The intima proliferates and becomes thickened. Eventually the wall may become stenotic or occluded by either stenosis or thrombosis.

The IgA plasma cell story

A new twist to the Kawasaki story was launched in 1997 when Rowley et al[86] reported the presence of IgA plasma cells in the vascular wall of patients with KD. This was a surprising finding as blood vessels in other disease states have not been found to be infiltrated by immunoglobulin-producing B cells, or, even more surprising, IgA-producing plasma cells. These IgA plasma cells were noted primarily in the adventitial layer in an artery with mild vasculitis but in all 3 layers of the coronary arteries when panarteritis was present. They postulated that the inflammatory reaction in the vascular wall in Kawasaki disease is initiated in the adventitial layer, around the vasa vasorum, and ultimately progresses to involve all 3 layers of the vascular wall in more severely affected arteries, indicating that the coronary artery lumen endothelial cell is not the primary site of vessel damage. It was speculated that IgA-producing cells migrate to the vascular tissue and myocardium in KD by the following pathway. An etiologic agent with a gastrointestinal or respiratory portal of entry is processed in the gut-associated lymphoreticular tissue or in the bronchus-associated lymphoid tissue. B cells undergo switching to IgA-precursors, leave the gut-associated lymphoreticular tissue or bronchus-associated lymphoid tissues, enter the general circulation, and migrate to the vascular wall and myocardium.

In a larger series of patients, plasma cell infiltration was prominent in the proximal respiratory tract, especially the submucosal glands of the trachea and large bronchi (with relative sparing of the distal lungs) as well as pancreatic ducts and kidneys.[87] The infiltration by IgA plasma cells was independent of the vasculitis in the respective organ. These data suggest a respiratory portal of entry of the KD etiologic agent with subsequent spread through the blood stream.

In a recent study, Rowley[88] examined the clonality of the IgA response by sequencing the CDR3 region of the α genes isolated from the vascular tissue of fatal acute KD patients. The IgA produced in acute KD is oligoclonal, consistent with an antigen-driven immune response.

Animal models of Kawasaki disease

A number of animal models have been described:

- *Candida albicans* extracts injected into mice induce systemic angiitis.[89]
- An experimental allergic angiitis in rabbits with horse serum injection leading to serum sickness hypersensitivity and coronary arteritis has been used in combination with a dietary model of atherosclerosis. A relationship was identified between the migration of smooth muscle cells into thickened intima and premature atherosclerosis.[90,91]
- Juvenile polyarteritis syndrome in beagles occurs spontaneously.[92] The treatment of these dogs with prednisone resulted in a rapid clinical improvement accompanied by a decrease of IL-6 activity.
- Lehman et al developed a murine model of coronary arteritis with a single intraperitoneal injection of *Lactobacillus casei* cell wall fragment.[93] Histopathologic study revealed mononuclear cells in the adventitia of the coronary arteries, followed by focal asymmetrical invasion of the vessel wall and later circumferential lesions with a marked proliferation of the intima/media, narrowing/obstruction of the lumen, and proliferation of adventitial fibrous tissue. Dense fibrous tissue around the coronary arteries, luminal narrowing from intimal proliferation, and recanalization after thrombosis followed. This model has been used to investigate various modalities to suppress coronary arteritis, which may provide clues to the pathogenesis of the early inflammatory response.[94]

The issue with all these models is how applicable they are to KD. Data must be accumulated to show how similar they are to KD to assess whether information can be extrapolated to KD. A true model of disease would involve transmission of an (infectious) agent from a child with KD to a susceptible host with duplication of the clinical and laboratory findings in the animal model. Attempts by us as well as others to transmit an agent from KD specimens to mice, rabbits, guinea pigs and macaques have been unsuccessful. At this meeting we will report our attempt to transmit Kawasaki disease to young chimpanzees.

■ TREATMENT AND LONG-TERM FOLLOW-UP

Treatment in the acute phase of the disease is aimed at limiting inflammation. Treatment with IVIG in various regimens has been shown to significantly reduce myocardial inflammation and the incidence of coronary artery aneurysm formation, as well as lead to a rapid defervescence and more rapid normalization of acute phase reactants.[95-97] In the United States, the standard of care is 2 g/kg IVIG as a single infusion. The efficacy of IVIG has been studied only during the first 10 days of illness. No controlled studies have been conduced regarding treatment later than the 10th day of illness, although therapy is recommended for such children with continued fever and features of acute inflammation, as persistent or recurrent fever is a major risk factor for the development of coronary abnormalities in patients with KD.

Aspirin is administered for both its anti-inflammatory and antithrombotic effects. It is given concurrently with the IVIG at a dosage of 80 to 100 mg/kg/d. The pharmacokinetic properties of aspirin are altered in children with acute KD, with decreased absorption and increased clearance of drug.[98] Therefore, most children with acute KD do not achieve therapeutic serum salicylate concentrations (20-30 mg/dL) despite the administration of high doses of aspirin. Once the patient has been afebrile for 48 to 72 hours, the dose can be lowered to 3 to 5 mg/kg/d, which is sufficient for the antiplatelet effect. This dose is continued until the platelet count and other indicators of inflammation (sedimentation rate) return to normal (about 8 weeks of illness). Although long-term, low-dose aspirin is recommended for any child in whom persistent coronary artery abnormalities have been detected, the efficacy of this approach for the prevention of coronary thrombosis has not been documented by controlled studies.

Treatment with high-dose IVIG and aspirin has be-

come the standard of care in the United States. However, even when high-dose IVIG is administered within the first 10 days of illness, approximately 5% of children with KD develop at least transient coronary artery dilation and 1% develop giant aneurysms.[99] Approximately 10% of children treated with IVIG have persistent or recurrent fever despite IVIG treatment.[97] If the fever persists, a second dose of IVIG at 1 g/kg may result in defervescence, although it is unknown whether retreatment prevents the development of coronary artery lesions.[100,101]

A subgroup of patients with Kawasaki disease is resistant to IVIG therapy; these patients are at greatest risk of development of coronary artery aneurysms and long-term sequelae of the disease. No effective treatment for these patients with refractory disease has been established. Although corticosteroids are the treatment of choice in other forms of vasculitis, their use in Kawasaki disease has been controversial. Reluctance to use steroids in acute KD derived from an early study by Kato et al,[102] demonstrating an extraordinarily high incidence of coronary artery aneurysms (11 of 17 patients) in a group that received oral prednisolone at a dose of 2 to 3 mg/kg/d for at least 2 weeks, followed by 1.5 mg/kg/d for an additional 2 weeks. What is often overlooked is that in the same study a smaller group of 7 patients received prednisolone plus aspirin, and none developed aneurysms. Following this report, many physicians were reluctant to administer steroids to children with KD. In a randomized trial in 100 children treated with intravenous prednisolone (followed by an oral taper) versus low-dose IVIG (300 mg/kg/d for 3 days), Nonaka et al[103] reported shorter fever duration in the steroid-treated group, but no significant difference in the prevalence of coronary aneurysms. Other studies have suggested that both oral[104] and intravenous[105] steroids may have a beneficial effect on coronary outcome. Wright et al[106] described preliminary results showing that some patients with IVIG-resistant Kawasaki disease can be treated safely with intravenous pulse steroid therapy. Shinohara reported that treatment with prednisolone was associated with a significantly shorter duration of fever after institution of treatment, as well as with a lower prevalence of coronary artery aneurysms.[107] In view of intriguing initial data suggesting that steroid therapy may be beneficial and that its adverse effects with short-term use are low, the efficacy of steroid administration in the treatment of KD should be assessed with randomized controlled studies.

Additional therapeutic options have been suggested; however, no formal consensus as to efficacy has been achieved. In response to reports of elevated TNF-α and soluble TNF receptor levels in acute Kawasaki disease,[44,61,66] pentoxifylline (thought to block TNF-α production) has been tried in combination with IVIG.[108] This preliminary study, which reported efficacy in reducing the incidence of coronary artery lesions when administered early in the course of Kawasaki disease, awaits confirmation by other investigators. A logical extension to the concept of reducing the effect of TNF-α is the treatment with one of the newer anti-TNF agents, such as etanercept or infliximab. To date, there has been no controlled study evaluating the efficacy of this type of treatment in Kawasaki disease.

The duration, frequency, and best imaging methods for the long-term follow-up are still a matter of debate. Of greatest concern are those children with initial coronary artery aneurysms, because thrombosis or segmental stenosis may occur in the chronic phase of the disease. The American Heart Association has recommended guidelines for long-term follow-up.[99] Children with multiple aneurysms, giant aneurysms, or known coronary artery obstruction require close follow-up and possible long-term anticoagulation therapy. Stress testing in the adolescent years is important, especially in those patients with a history of coronary artery involvement, because abnormalities may require limitations in physical activity and may indicate the need for angiography to assess the degree of coronary artery stenosis or obstruction.

Severe coronary artery complications of Kawasaki disease have been treated by a variety of coronary artery bypass procedures.[109-111] There is a small population of patients in whom revascularization procedures are not successful or not possible because of the extent of their disease. This has led to consideration of cardiac transplantation in these patients.[112] The most common indication for transplantation is ischemic left ventricular dysfunction, occurring usually later than 1 year after onset of Kawasaki disease.

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Epidemiology of giant-cell arteritis

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Although a case of giant-cell arteritis (GCA) was clearly described by Hutchinson[1] in the late 1880s, it was not until the description by Horton and co-workers[2,3] in the 1930s that this vasculitis came to the attention of most physicians. After their reports, GCA was still considered an uncommon illness. The spectrum of clinical findings associated with GCA, including visual loss, occlusive changes in the large branches of the proximal thoracic aorta, and musculoskeletal symptoms, were described over the following years in case reports and small series. In more recent years, particularly over the last twenty-five years, GCA has become recognized as one of the most common forms of vasculitis, especially in individuals over the age of 60 years. In addition, polymyalgia rheumatica (PMR), a syndrome linked to GCA and characterized by proximal aching and stiffness, is even more common.[4] Thus, in many populations in the US and Europe, these two conditions are among the most frequent new inflammatory rheumatic diseases that develop in older persons.

GEOGRAPHIC LOCATION AND ETHNICITY

A number of population studies have been carried out determining the incidence rates of GCA in various geographic regions. The results of several selected recent investigations are listed in **Table 1**. The highest incidence rates observed have been reported from Norway. Workers from two neighboring southern Norwegian centers studied the incidence of GCA during time ranges that overlapped. Haugeberg and co-workers[5] searched clinical and biopsy databases to determine the incidence of GCA over a 5-year period, 1992 to 1996, in the population of Vest Agder County, Norway. Fifty-three cases of GCA were diagnosed over this period, 94% of which were biopsy positive. This provided an average annual incidence over the study period of 32.8 cases per year in persons over age 50 years. Gran and Mykelbust[6] asked all physicians in Aust Agder County to refer all patients suspected of having GCA and PMR to the Department of Rheumatology where they worked. They also evaluated suspected cases admitted to other hospital departments. Sixty-six cases of biopsy-verified GCA were identified between 1987 and 1994. These cases produced an annual incidence rate over this time of 29.0 cases per year in persons aged 50 years and older.

TABLE 1
GIANT-CELL ARTERITIS: SELECTED ANNUAL INCIDENCE RATES

Location of Study	Years Included in the Study	Average Annual Incidence Rates*
Norway[5]	1992-1996	32.8
Norway[6]	1987-1994	29.0
Iceland[7]	1984-1990	27
Sweden[8]	1976-1995	22.2
Denmark[9]	1982-1994	20.4
Finland[10]	1984-1988	20.7
Finland[11]	1969-1989	6.9
Olmsted Cty, USA[18]	1950-1999	19.0
Spain[12]	1981-1998	10.2
Israel[13]	1980-1991	10.2
Italy[14]	1980-1988	6.9

*Per 100,000 population aged 50 years and older.

The rates in Iceland have been found to be at a similar level. Baldursson and colleagues[7] ascertained cases by a search of all hospitals in Iceland and a review of all temporal artery biopsies during a seven-year period, 1984 to 1990. The authors identified 133 patients with GCA. The average incidence rate of GCA over this 7-year period was 27/100,000. Petursdottir et al[8] identified all cases of biopsy-verified GCA between 1976 and 1995 in Goteborg, Sweden. Six hundred sixty-five patients had been diagnosed with GCA over this 20-year period. The average annual incidence rate was 22.2 per 100,000 inhabitants over 50 years of age. Elling and co-workers[9] determined the number of patients with GCA between 1982 and 1994 in two regions of Denmark, using national patient registry data. Over this period the average incidence rate was 20.4 new cases each year in persons over 50 years of age.

Two studies from Finland have shown large differences in incidence rates. In one from Western Nyland, reported by Franzen, Sutinen, and von Knorring,[10] 16 patients with biopsy-positive GCA were diagnosed over five years,

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1984-1988, in a population of 44,500 persons. The average incidence rate over the first 44 months of the study period, calculated retrospectively, was 20.7 per year in persons in the population over age 50 years. A 16-month prospective part of the study yielded an increased rate of approximately 28 cases per year per 100,000 persons over age 50 years. The second study, by Rajala and co-workers,[11] was from Tampere, in south central Finland. During the 20-year period from 1969 to 1989, 66 patients with histologically verified GCA were identified. The annual incidence of GCA in persons 50 or older was 4.5 over the period from 1970 to 1979 and 9.2 in the period from 1980 to 1989. The reason for the difference in rates between the two Finnish studies is unknown. The investigation by Franzen et al[10] was performed in a population nearer Sweden and may have contained persons with Swedish ethnicity, whereas those in Tampere may be of different ethnicity.

Population surveys from Mediterranean countries have shown lower incidence rates of GCA. In a recent study by Gonzalez-Gay et al[12] from a region in northwest Spain with a population of approximately 250,000 persons, 161 patients with GCA were diagnosed over 18 years between 1981 and 1998. The average annual incidence rate per 100,000 persons over age 50 was 10.2. In Israel, Sonnenblick and others[13] found 84 patients with GCA in Jerusalem over a 12-year period. The annual incidence rate for this population was also 10.2 per 100,000 persons aged over 50 years. The incidence rate was similar among non-western and western Jews. In northern Italy, Salvarani and colleagues[14] found an incidence rate of 6.9 per 100,000 persons aged 50 and older over a 9-year period.

In the US, incidence studies have been done mainly in Olmsted County, MN, a region in the northern part of the country which has an ethnic background similar to that of mid and northern Europe.[15-17] In a recent as-yet unpublished updated series from the Mayo Clinic, 175 cases of GCA were identified between 1950 and 1999. This produced an average annual incidence of 19.0 per 100,000 in persons 50 years and older.[18] One other center in the US performed a population survey of GCA a number of years ago. Smith and co-workers[19] surveyed the population of Shelby County, Tennessee, for the years 1971 to 1980. Shelby County is in a south central location along the Mississippi River. The average annual incidence was only 1.58 for those over age 50. The incidence was 7 times greater in whites than in blacks. Clinical features in the patients with GCA were similar to those of other populations. The difference from results found in Minnesota could be accounted for only in part by racial distributions. Similar studies in other southern geographic areas are needed to place these latter findings in perspective, but have yet to be done.

In summary, the frequency of GCA in northern Europe has been well documented. There appears to be a decreasing incidence of GCA from northern to more southern countries. This gradient may also be present in the US but has been less well studied. Rates in most Scandinavian countries and in Minnesota, a population of similar ethnic background, are approximately 20 or more per 100,000 persons over the age of 50 years, and those in other parts of Europe are approximately 10 or less.

The different results in the two studies from Finland are intriguing. The different findings could be due to ascertainment or other factors. Rates found by Rajala and co-workers[11] were higher in the second half of the study period. Alternatively, the differences may reflect ethnic variations in different regions of Finland, the higher rates being found in those with a genetic make-up more representative of Sweden, Finland's next-door neighbor, and the lower rates measuring another native ethnic group with genetic characteristics of other central and southern European countries. Incidence rates of GCA in African and Asian countries have not been studied thoroughly, but from information available, the frequency of GCA in these populations appears to be much less than in Caucasians. Reports have shown a relationship between HLA-DR4 and GCA and PMR.[20] HLA types vary in different ethnic groups and HLA-DR4 is less common in Mediterranean and other populations with a low incidence of GCA. Because of the inflammatory nature of GCA and PMR, immunogenetic factors may be important in susceptibility of these conditions. However, it is not known if the varying incidence rates discussed above are related to immunogenic markers. As far as can be determined, clinical features of GCA are similar in the various locations with different incidence rates.

■ YEARLY/SEASONAL VARIATIONS

Nearly all studies have found women to be affected more commonly than men, in a ratio of about 3 to 2. The survey in Spain is the exception where men were noted to be more numerous.[12] Essentially, all surveys that have evaluated incidence over time have shown an increase in the rates over years. However, changes of incidence of PMR, which is linked to GCA, have been less clear. In Olmsted County, the incidence of PMR has varied from year to year but has remained relatively stable over the past three decades.[21] This is surprising in view of the nearly uniform finding of increase in the incidence of GCA and the close link between GCA and PMR. PMR is a less well-defined syndrome in that it has no definitive diagnostic test such as temporal artery biopsy in GCA. It is also possible, for example, that PMR is caused or precipitated by several factors, or may be several disease entities which cannot be separated by clinicians at this time.

In the investigation of GCA in Olmsted County, MN, in addition to an increase in incidence rates over the study period, a cyclic pattern was observed, with peaks every 5 to 7 years **(Figure 1)**. This suggested that an environmental factor(s) might be involved in precipitating the disease.[17] Subsequent to this observation, Gabriel and co-workers[22] prospectively examined temporal artery biopsy tissue from 50 consecutive patients presenting for temporal artery biopsies for the presence of B19 DNA, using the polymerase chain reaction. Amplicons for human beta-globulin, but not for cytomegalovirus, were

produced for all tissue samples. A statistically significant association between histologic evidence of GCA and the presence of B19 DNA in temporal artery tissue was found (P=0.0013). The PCR results for B19 agreed in 29 of 30 samples tested by a second laboratory, the Centers for Disease Control, Atlanta, GA. The finding of B19 in the temporal artery wall in patients with GCA suggested that B19 may play a role in its pathogenesis. Further studies on this virus are needed to fully understand the significance of these results.

Other workers have also noted variations in the incidence of GCA, along with additional evidence of infections or another environmental factor(s) in the development of GCA. Elling, Olsson, and Elling[9] prospectively recorded the incidence rates of GCA and PMR in 13 of 16 counties in Denmark between 1982 and 1993. Pronounced quarterly and annual variations of the incidence of GCA and PMR were found in each of the counties. Cyclic fluctuations were seen simultaneously in several regions irrespective of the incidence rates, with a clustering in five peaks. Distinct peak rates of GCA and PMR occurred in close association with two epidemics of *Mycoplasma pneumoniae* infection. Two incidence peaks were seen, partly related to two epidemics of parvovirus B19 and to one epidemic of *Chlamydia pneumoniae*. The synchronous variations in the incidences of GCA and PMR in the several regions and close concurrence with epidemics of viral infections suggested that GCA and PMR may be triggered by certain viruses or other agents. Petursdottir and colleagues[8] from Goteborg, Sweden, studied changes in incidence of GCA in their population over 20 years. As did others, they noted an increase in rates over the period (P<0.001) and a variability of monthly rates with peaks in late winter and autumn (P=0.04). However, no cyclic occurrence was observed.

URBAN/RURAL INCIDENCE

Two investigations have found higher incidence rates in locations of high population density. This was noted the Danish study above[9] and in a report from Germany.[23] In the latter, Reinhold-Keller and colleagues investigated the prevalence of primary systemic vasculitis in northern and southern Germany. Each catchment area included both urban and rural areas. GCA was the most frequent type of vasculitis found. GCA was also significantly more prevalent in urban than in rural populations. Although a lower ascertainment of GCA in rural regions was not excluded as a cause of the differences, the authors considered it possible that environmental factors may be involved in the development of GCA and should be looked for in future studies.

ASSOCIATIONS WITH INFECTIONS

In addition to the study by Gabriel and coworkers noted above,[22] other investigators have looked for an infectious link to GCA. Duhaut and co-workers[24] performed serologic tests on 350 new patients with GCA or PMR for viruses known to induce multinucleated giant cells in human pathology. These included the parainfluenza viruses, respiratory syncytial virus, measles virus, herpesviruses type 1 and 2, and the Epstein-Barr virus. Positive serologic titers for IgM antibodies against parainfluenza viruses were found in 38% of cases versus 20.9% of controls (P=0.00005). The association was even stronger in the positive-biopsy GCA group. In these patients, 43.3% had positive titers. Only parainfluenza type 1 was associated with GCA regardless of the season or the geographic origin of the cases. Positive rates for all other viruses tested were similar in both cases and controls. The authors concluded that findings indicated that reinfection with parainfluenza type 1 may be associated with the onset of GCA, especially biopsy-positive cases.

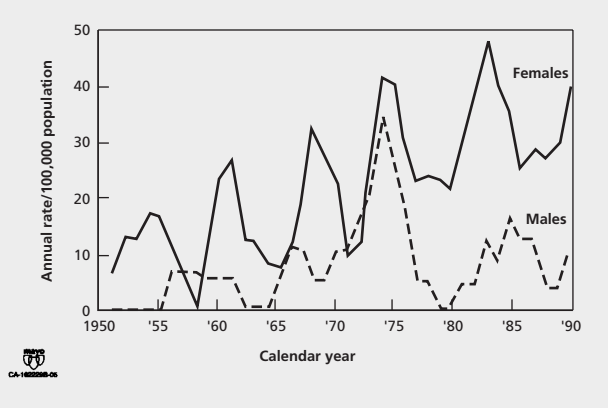

Figure 1. Incidence of giant-cell arteritis in Olmsted County, MN, 1950-1990. Three-year moving average method was used to develop curves. Peak incidence rates appear to occur in females every 5 to 7 years. Data from reference 27.

OUTCOME

The course of GCA is variable, with frequent relapses,[11] but most patients are able to discontinue prednisone after one to two years without recurrence of symptoms, indicating a gradual resolution of vascular inflammation in most patients.[25] Vascular complications, however, may result in the patient's death.[11,26-28] These include strokes due to occlusions of one or more large arteries in the cervical region and rupture of the thoracic aorta as a result of arteritic involvement. In addition, therapy for GCA, especially long-term use of glucocorticoids, is frequently associated with adverse events, such as infections, or hip fractures that may cause disability in older persons. These adverse events related to treatment may contribute importantly to the patient's ultimate outcome.[29] In spite of these events in some patients, the majority of long-term survival studies have shown no excess mortality.[17,30-31] No reliable predictors at the time for disease severity or duration, development of aortic aneurysms, or death have been identified. Hachulla and co-workers[32] found that long-term survival was better in those without initial ocular manifestations and those who were on less than 10 mg of prednisone per day at the end of 6 months of therapy.

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ETHNIC DISPARITY IN THE INCIDENCE OF TEMPORAL ARTERITIS: A 32-YEAR EXPERIENCE AT AN URBAN MEDICAL CENTER

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Background: Temporal arteritis (TA) is the most common primary systemic vasculitis. It occurs in the middle-aged and elderly, seldom occurring below 50 years of age. TA is widely held to be a disease occurring in Caucasians, but few large epidemiology studies have been performed in populations with substantial minority representation. There have been case reports of TA occurring in African-Americans, but TA is reportedly rare in this racial group.

Objective: To investigate the assertion that TA is rare in African-Americans, we examined the demographics of TA at an urban medical center.

Methods: We conducted a retrospective review of positive TA biopsies at the Wilmer Eye Center between 7/68 and 7/00. Demographic and clinical features including ethnicity, gender, age, and erythrocyte sedimentation rate (ESR) at biopsy were recorded.

Results: Ninety cases of biopsy-confirmed TA occurred over the 32-year period. All 90 patients fulfilled the ACR criteria for TA. The 90 cases included 79 biopsies with giant cells (88%) and 11 biopsies without giant cells (12%). The female:male ratio in this group of patients was 3:1. African-Americans comprised only 6% of the overall cohort. The demographic features and ESR of the patients, stratified by ethnicity, are displayed in the table below.

	Caucasian	African-American
Female n = 68 (76%)	63 (93%)	5 (7%)
Male n = 22 (24%)	22 (100%)	0
Mean ESR, mm/hr (range)	73 (12-145)	57 (50-63)
Mean age, years (range)	73 (53-90)	69 (57-81)
Total n = 90	85 (94%)	5 (6%)

The population demographics of the city of Baltimore and the state of Maryland were relatively stable during the period in which these TA biopsies were collected. United States census data (1990) reported that 59% and 25% of the total populations of Baltimore and Maryland, respectively, were African-American.

Conclusion: TA occurs in African-Americans, but the incidence appears to be strikingly lower than in Caucasians. This disparity may be explained partly by biases in referral and differences in access to care, but its magnitude suggests the presence of important genetic and/or environmental risk factors that vary between these two ethnic groups.

Epidemiology of Wegener's granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome

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The primary systemic vasculitides (PSV—Wegener's granulomatosis [WG], Churg-Strauss syndrome [CSS], microscopic polyangiitis [MPA], polyarteritis nodosa [PAN]) are a group of uncommon diseases. The etiology of vasculitis is unknown but is clearly multifactorial; among the influences on disease expression are ethnicity, genes (HLA and others), gender, and environment (infections, toxins, drugs, allergy, smoking, UV light). There are sufficient differences, even with the limited epidemiological data available, to suggest that not all these factors work in the same direction in the various vasculitic syndromes described.

The lack of any clear understanding of the etiology of the systemic vasculitides and universally accepted classification systems hampered accurate epidemiological studies until the 1990s.

CLASSIFICATION OF VASCULITIS

The American College of Rheumatology (ACR), in 1990, proposed criteria for the classification of seven types of vasculitis.[1] The ACR criteria are not perfect. They were established by comparing patients with different types of vasculitis, but not with patients prior to the diagnosis of vasculitis, or with other systemic diseases or even with other connective tissue diseases. The reliability of these criteria when used in patients in whom vasculitis is suspected but not yet diagnosed is poor.[2]

In 1994, consensus definitions were proposed at the Chapel Hill Consensus Conference (CHCC). These provided important and useful definitions of disease but were not considered appropriate for diagnosis or classification of patients as no specific criteria were produced.[3] Microscopic polyangiitis (not included by the ACR study) was defined for the first time. We have shown, for example, that the currently available criteria/definitions often identify different patients.[4] The implication of these developments is that any study must identify the criteria/definitions on which the diagnosis has been based. Furthermore, the ACR classification criteria and the Chapel Hill Consensus definitions did not specifically include ANCA.

EPIDEMIOLOGY

Most studies are hospital-based from tertiary/university referral centers with ill-defined denominator populations and hence referral bias. Patients seen in tertiary hospitals may not be representative of those seen either at district hospitals or in the community, especially in terms of disease severity or age spectrum. Prospective population-based estimates of incidence and prevalence are few.

The epidemiology of PSV has been studied in the Norwich Health Authority (England) with data collected prospectively from 1988. Incidence rates adjusted for age and sex to the 1992 population show an overall annual incidence of primary systemic vasculitis of 19.8 per million. The point prevalence on December 31, 1997 was 144.5 per million. Primary systemic vasculitis was more common in males (23.3 per million) than females (16.4/million). The age- and sex-specific incidence showed a clear increase with age with an overall peak in the 65-to-74-year age group (60.1 per million).[5]

GEOGRAPHICAL FACTORS

A study using the same classification criteria (ACR [1990] and CHCC) in three populations—Norwich; Tromsø (North Norway); and Lugo (North-West Spain), confirmed the older age at onset (compared with previous studies) and showed a similar overall incidence and pattern of vasculitis in terms of age and sex distribution. Microscopic polyangiitis was more common (11.6/million) in Spain compared with Norway (2.7/million), while WG was less common in Spain (4.9/million) compared with Norway (10.5/million). Churg-Strauss syndrome appeared to be more common in Norwich

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(3.1/million) compared with the other two populations.[6] Comparative data from well-defined populations may provide further insights into the role of environmental or genetic factors in the pathogenesis of vasculitis.

There are two studies which reported a particularly high annual incidence of PAN. McMahon and colleagues reported a high frequency of PAN (77/million) in Alaskan Indians.[7] The population was small, and all the cases were positive for hepatitis B surface and e antigen at diagnosis. Detailed hepatitis serology was not available in the other studies. Whether these data reflect geographical and ethnic differences or a high infection rate with hepatitis B is unclear as no other comparable study has been reported. The second population with an apparently high incidence of MPA and PAN is the Kuwaiti national population, where the incidence of classical PAN was 16/million and MPA 24/million. Although incidence figures were only calculated for Kuwaitis, both MPA and PAN occurred in other ethnic groups, suggesting any environmental factor operates irrespective of ethnic origin.[8]

TIME TRENDS

The incidence of Wegener's granulomatosis (WG) may be increasing. Andrews and colleagues (1990) in Leicester (UK) reported an increase in the annual incidence of WG from 0.7/million (1980–1986) to 2.8/million (1987–1989).[9] This was partially attributed to an increase in diagnostic awareness following the introduction of assays for ANCA in 1987. The incidence of Wegener's granulomatosis has, however, increased in Tromsø, Norway, from 5/million to 12.5/million over the last 15 years.[10] We have observed a small but not significant increase during the 10-year period of our study.

INFECTION

The strongest association of infection with vasculitis is PAN with hepatitis B virus infection (HBV); the highest incidence rate for PAN comes from an area endemic for HBV infection. Whether HBV infection results in PAN may be determined by HLA haplotype. In a familial cluster, a father and two sons were infected with HBV through use of a shared razor; however, only the father and one son developed PAN. They shared HLA haplotypes, whereas the other son had a different HLA haplotype.[11]

Tidman and colleagues,[12] in a hospital study of patients with ANCA-associated vasculitis and renal involvement during 1975–1995, noted a periodic fluctuation with peaks every 3 to 4 years, suggesting an infective etiology. This cyclical pattern for ANCA-associated vasculitis has not yet been confirmed.

Wegener's granulomatosis has been linked to parvovirus B19 and *Staphylococcus aureus* infection. The initial studies associating Wegener's granulomatosis and parvovirus B19 infection were case reports, but a detailed study of 42 patients with new-onset disease showed no evidence for this infection.[13] Nasal carriage of *S aureus* has been associated with increased risk of relapse in WG.[14] Over 10 years, we have not been able to demonstrate any periodic fluctuation, nor have we been able to associate onset with mycoplasma, parvovirus, chlamydia, or influenza.

ENVIRONMENTAL FACTORS

A number of other trigger factors have also been reported in association with systemic vasculitis, including silica, solvents, allergy, and vaccination.

A case-control study undertaken in Norfolk showed an association between occupations with high exposure to silica and PSV. In particular, pANCA/MPO-positive patients showed an association rather than cANCA/PR3. It is notable that the incidence of MPA doubled after the Kobe earthquake, when exposure to silica would have been high.[15] Three case-control studies also support the association of silica with ANCA-positive glomerulonephritis, MPA, and WG.[16-18] Our case-control study also found a significant association of occupational exposure to organic solvents with WG and cANCA-positive vasculitis.

We have found an association between farming and PSV (adjusted odds ratios between 2.2 and 6.3). Interestingly, a significant association was not seen for CSS. It was not possible to identify a particular causal exposure, but exposure to livestock showed a stronger association than crop exposures. In particular, exposure to cows, sheep, and chickens showed an association with PSV overall. cANCA and pANCA showed similar associations to farming as a whole, predominantly livestock exposures. Duna and colleagues[19] studied self-reported exposure to heat, fumes, and particulates. There was a higher incidence of exposure in WG patients compared with normal control subjects, but no difference between WG patients and patients with pulmonary disease. They found no association between farming and WG, which may be explained by selection bias of cases.

Clusters of Wegener's granulomatosis occurring in families have been described (reviewed in reference 20). In most clusters, no more than two people have been affected, usually one parent and a child or two siblings. Distant family members are rarely reported. The occurrence of clusters in first-degree relatives and not in more distant family members suggests that environmental triggers play an important role in the etiology, as parents and children or siblings share their environment as well as genetic background.

SEASONALITY

Raynauld and colleagues in 1993 reported a higher rate of onset in winter (29.8%) compared with the summer (14.3%).[21] This trend was also supported by a study of ANCA-associated glomerulonephritis and systemic vasculitis, which showed a higher onset in winter.[22] We have been unable to demonstrate any significant seasonal differences.

DRUGS

Many drugs have been associated with vasculitis; in the majority of cases these have been anecdotal. Propylthiouracil and hydralazine are drugs known to be

associated with vasculitis and propylthiouracil, in particular with high titers of pANCA/MPO.[23]

Wechsler and colleagues[24] reported eight patients with glucocorticosteroid-dependent asthma receiving the sulphidopeptide-leukotriene antagonist zafirlukast who developed CSS associated with corticosteroid withdrawal. Two patients probably had preexisting CSS with asthma, neuropathy, and infiltrates. In the other patients, zafirlukast improved asthma control sufficiently to permit reduction in glucocorticoid dose. CSS became apparent within days or months of the dose reduction. Although allergic vasculitis due to zafirlukast is possible, it is more likely that reduction of steroid dose unmasked underlying CSS.

CONCLUSIONS

The systemic vasculitides are a group of important inflammatory conditions resulting in inflammation and necrosis of blood vessel walls. They are associated with significant morbidity as well as mortality, particularly microscopic polyangiitis. They are commoner than previously believed, with an annual incidence of primary systemic vasculitis of 20/million/year. The primary vasculitides are also seen in a much older population than was previously believed due to the development of vasculitis registers in district hospitals such as Norwich. Classification criteria and disease definitions are now well established, which has led to conformity between different centers, allowing geographical comparisons. The numbers of studies are still relatively small, and further understanding of these diseases, particularly in the Indian subcontinent and the Far East, may be important in areas where other infections are common, particularly TB.

Studies of epidemiology have suggested some important avenues for research in terms of environmental factors, particularly the role of silica and potential infections associated with farming. Data, however, are still essentially descriptive at this stage, but further epidemiological studies will hopefully shed further light on etiopathogenesis.

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Epidemiology of Henoch-Schönlein purpura

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Epidemiology is the study of diseases and the factors associated with the occurrence of diseases in defined populations. The epidemiology of most diseases can be viewed on two levels. Traditional macro-epidemiology examines the influence of age, gender, ethnic background, geography, environmental factors, and other variables on the susceptibility to a given disease. Micro-epidemiology examines the genetic and molecular factors that render some individuals or populations susceptible to a disease and others protected. This paper will review the macro-epidemiology of Henoch-Schönlein purpura (HSP) and discuss recent advances in understanding the micro-epidemiology of HSP.

MACRO-EPIDEMIOLOGY

HSP is an acute vasculitis that primarily affects children. The dominant clinical manifestations include purpura, arthritis, abdominal pain, gastrointestinal bleeding, and nephritis. The clinical features of HSP are a consequence of widespread leukocytoclastic vasculitis owing to IgA deposition in vessel walls. IgA deposition in the renal mesangium causes nephritis in some patients.

HSP is the most common acute vasculitis affecting children, with an incidence of approximately 10 cases per 100,000 children per year.[1-4] Most studies on the incidence of HSP have been performed in Europe and the Middle East. There is little available information comparing the incidence of HSP in other parts of the world or in populations of widely disparate ethnic origins. HSP has been reported in patients as young as 6 months of age to as old as 86 years, but the vast majority of patients with HSP are young children. The mean age of the patients in most large series is 6 years.[4-6] Approximately 75% of children are less than 8 years of age, and 90% are less than 10 years of age. Most studies report slightly more boys affected (60%) than girls (40%).

HSP occurs throughout the year, but a number of studies have noted seasonal skewing, with most patients presenting from fall through spring, and a paucity of cases during the summer months.[4-6] Clusters or epidemics of HSP are rare. Farley et al[7] reported a cluster of 16 cases of HSP, including 2 pairs of siblings during a 7-month period in Connecticut. However, other large epidemiologic studies have found no evidence of geographic or temporal clustering of cases.[1,4] Moreover, the occurrence of multiple cases within a family is very uncommon.

Schönlein was the first to observe that respiratory infections commonly precede the onset of symptoms, an observation that has subsequently been made by many authors. Given the epidemiology of HSP (a disease affecting young children with a peak occurrence during the fall and winter months), it is not surprising that a large proportion of children have a history of upper respiratory infection. Nevertheless, there have been very few controlled studies examining the incidence of infections with specific pathogens in children with HSP compared to control children.

Of all the pathogens linked to HSP, group A beta-hemolytic streptococcus (GABS) has been the most extensively studied. Gairdner[8] first proposed that HSP was associated with GABS infection in 1948. In Gairdner's study, 50% of patients but only 10% of controls had a positive throat culture for GABS. Subsequent studies, many without controls, found positive throat cultures for GABS in 10-30% of patients[1-3,5] and increased antistreptolysin O (ASO) titers in 20-50% of patients.[1,2,5] Al-Sheyyab et al[9] reported a statistically significantly higher proportion of children with HSP had increased ASO titers compared to controls. Other studies, however, have found no increased incidence of concomitant or preceding GABS infection in children with HSP compared to control children.[10,11] Taken together, these results indicate that a substantial minority of children with HSP have concurrent or recent GABS infection, but most cases have no direct link to GABS infection.

Anecdotal reports have implicated virtually every microbial pathogen in the etiology of HSP. However, very few studies have compared the incidence of infection with a specific pathogen in HSP versus controls. For example, there are a number of reports of parvovirus B19 infection in patients with HSP, but a recent study demonstrated the incidence of acute parvovirus B19 infection was no different in children with HSP compared to control children.[12] In addition to infections, HSP has been associated with a wide variety of drugs and other agents.[4-6] Nevertheless, none of these agents have been proved to be associated with HSP in controlled studies. Thus, despite extensive efforts and scores of anecdotal reports, there appears to be no single pathogen or environmental agent that has emerged as a dominant precipitating cause of HSP.

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There are important differences in the epidemiology and the clinical features of HSP in adults compared to children. The incidence of HSP in adults is about one-tenth that of children, with available data indicating 13-14 cases per 1,000,000 adults per year.[13,14] In several large series, the mean age of adults with HSP was approximately 50 years. Men and women are affected with equal frequency. There is little seasonal variability, and preceding infections are less common in adults compared to children. HSP is often more severe in adults. Nephritis occurs in 50-80% of adults, but only 20-40% of children. Ten to twenty percent of adults with HSP nephritis develop end-stage renal disease compared to 1% of children.[15,16]

MICRO-EPIDEMIOLOGY

Although the etiology is unknown, it is clear that IgA plays a pivotal role in the pathogenesis of HSP. HSP is associated with a variety of abnormalities involving IgA, including increased serum IgA concentrations, IgA-containing circulating immune complexes, and IgA deposition in vessel walls and renal mesangium. There are two subclasses of IgA—IgA1 and IgA2. IgA1 accounts for 80-90% of serum IgA, while secretory IgA is composed of roughly equal proportions of IgA1 and IgA2. It is noteworthy that HSP is associated with abnormalities involving only IgA1, but not IgA2. The reasons for the exclusive involvement of IgA1 in the immunopathogenesis of HSP are beginning to emerge.

One important difference between IgA1 and IgA2 involves the hinge region of the heavy chain, and the glycosylation sites therein. IgA1 contains a proline-rich hinge region between the CH1 and CH2 domains of the heavy chain. It is composed of 18 amino acids, of which 5 are O-linked glycosylation sites. The basic structure is *N*-acetylgalactosamine (GalNAc) O-linked to serine or threonine. The oligosaccharide chain is usually extended by the addition of galactose (Gal) in β1,3 linkage with GalNAc, and with one or two sialic acid residues in α2,3 linkage with Gal or α2,6 linkage with GalNAc. IgA2 molecules do not contain a heavy-chain hinge region and, thus, no O-linked oligosaccharides.

In order to understand the exclusive involvement to IgA1 in the pathogenesis of HSP, attention has focused on the hinge region glycosylation of IgA1 in HSP and also in IgA nephropathy (IgAN). The latter disorder shares a number of clinical and immunologic features in common with HSP. Importantly, the immunopathogenesis of IgAN also involves IgA1 exclusively. Although most of the work in this area has involved patients with IgAN, there is clear relevance to the pathogenesis of HSP.

Using a variety of techniques, a number of investigators have found that the hinge region O-linked glycans of IgA1 in patients with IgAN are deficient in galactose,[17] sialic acid,[18] or both.[19] Despite extensive work in patients with IgAN, there have been only two studies examining IgA1 glycosylation in patients with HSP. Saulsbury[20] reported that the hinge region of IgA1 in children with HSP was deficient in sialic acid, but the content of Gal and GalNAc were normal. Allen et al[21] reported diminished hinge region Gal content in IgA1 from HSP patients with nephritis, but no difference in IgA1 glycosylation in HSP patients with no nephritis compared to controls.

The mechanisms of aberrant glycosylation of IgA1 in HSP and IgAN remain unclear. Nevertheless, aberrant glycosylation may have important consequences for the IgA1 molecule, and may explain many of the immunologic and histologic features of HSP. IgA1 molecules that are deficient in sialic acid or Gal have a tendency to aggregate and form macromolecular complexes.[18,22] In addition, sialic acid and Gal deficient hinge regions interact with IgG antiglycan antibodies to form IgA-IgG complexes.[22] Lastly, aberrantly glycosylated IgA1 has a propensity to deposit in the kidney.[23,24]

In summary, the macro-epidemiology of HSP has been well documented over the past 200 years. Nevertheless, macro-epidemiology has not provided definitive information concerning the etiology of HSP. The micro-epidemiology of HSP is far less clear, but understanding of this aspect of the epidemiology will ultimately shed light on the etiology of HSP.

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18-010

HENOCH-SCHÖNLEIN PURPURA AND CUTANEOUS LEUKOCYTOCLASTIC ANGIITIS EXHIBIT DIFFERENT HLA-DRB1 ASSOCIATIONS

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Objective: To examine the HLA-DRB1 genotype of patients with cutaneous leukocytoclastic angiitis (CLA), a small-sized blood vessel vasculitis limited to skin, and determine if differences exist with Henoch-Schönlein purpura (HSP), a small-sized blood vessel vasculitis with cutaneous and systemic complications.

Methods: A retrospective study was performed on an unselected population of patients from Northwest Spain with primary cutaneous vasculitis classified according to proposed criteria (Michel et al, J Rheumatol 1992; 19:721-8). Patients who fulfilled classification criteria for hypersensitivity vasculitis were included in this study if they had a biopsy-proven leukocytoclastic vasculitis limited to skin and, due to this, they also met the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis definitions for CLA. Patients were included in this study if they had at least 2 years of follow-up. Ninety-six Caucasian patients (58 HSP and 38 CLA) were studied. Patients and ethnically matched controls (n=145) were HLA-DRB1 genotyped from DNA using molecular-based methods.

Results: No HLA-DRB1 genotype differences between patients with CLA and controls were seen. As previously described, HLA-DRB1*01 was increased and HLA-DRB1*07 reduced in HSP patients compared to controls. When HLA-DRB1 genotypes of patients with CLA and HSP were compared, a significant increase of HLA-DRB1* 15/16 and especially of HLA-DRB1*07 was observed in the group of patients fulfilling definitions for CLA compared to those with HSP.

Conclusions: HSP and CLA exhibit different HLA-DRB1 genotype associations.

Cytokines in giant-cell arteritis

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Giant-cell arteritis (GCA) in its typical form is a granulomatous disease that, for reasons yet unknown, targets the wall of medium-size and large arteries. Inflammatory infiltrates, composed primarily of T lymphocytes and macrophages, accumulate in the vascular wall. The tissue injury initiated by these inflammatory cells leads to the clinical manifestations of arteritis—most often ischemia and necrosis caused by vascular occlusion. In almost all cases, the arteritic lesions are combined with an intense syndrome of systemic inflammation. A definitive assignment for the site of the systemic inflammation has not been made. The existence of a related syndrome of systemic inflammatory activity in the absence of frank vasculitis, clinically identified as polymyalgia rheumatica (PMR), strongly suggests that GCA and PMR have an extravascular site of disease where inflammatory reactions unfold, leading to malaise, anemia, weight loss, night sweats, highly elevated acute-phase responses, and myalgias.

Cells in inflammatory infiltrates communicate by releasing hormone-like mediators, often categorized under the heading of cytokines. Resident cells in the tissue attacked by inflammation respond with the production of mediators, some of which are also classified as cytokines. Cytokines are directly involved in accomplishing the primary goal of inflammation, to eliminate the stimulus of tissue injury, cordon off the lesion to prevent spreading, remove tissue debris, and remodel the lesion with newly formed cellular and matrix components. Considering the complexity of inflammatory reactions, the need to orchestrate the interaction between an array of cells, and the diversity of tasks, it is no surprise that the list of cytokines recognized and implicated in inflammatory diseases is ever growing. Most knowledge is of interleukin (IL)-1, tumor necrosis factor (TNF)-α and IL-6. Some of the cytokines on the list have been studied in detail in GCA and PMR **(Table 1)**. These studies have provided valuable insights, and there is evidence that cytokines could be potentially helpful in the diagnosis and management of these diseases.

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TISSUE CYTOKINES IN GCA AND PMR—CLUES TO PATHOGENIC EVENTS

Macrophages are a major component of the cellular infiltrates in arteritic lesions, and they produce an array of cytokines, chemokines, growth factors, enzymes, and oxygen radicals. The most interesting aspects of macrophage biology in GCA have derived from the observation that a close correlation exists between the locale and the functional commitment of macrophages.[1] Vessel wall macrophages synthesize IL-1 and IL-6, often in conjunction.[2] IL-1β and IL-6 are released by macrophages in the adventitia; they rarely are produced in macrophages in the media or the intima. In the adventitia, IL-1/IL-6–producing macrophages partner with CD4 T cells that have all the features of recently stimulated lymphocytes.[3] T cells in the vascular infiltrate have undergone clonal expansion, suggesting antigen-driven responses.[4,5] The precise role of IL-1β and IL-6 in the granulomatous reaction is not entirely understood. Perivascular inflammation in mice with a genetic defect in the IL-1R antagonist gene suggests a direct amplifying contribution of IL-1 to vascular inflammation.[6] In GCA, a contribution of IL-1/IL-6–releasing macrophages in antigen presentation and T-cell activation is more likely. GCA is an HLA class II–associated disease, which alludes to a selective binding and presentation of arteritic antigens.[7] All available data indicate that this key event in the disease process occurs in the adventitia of the affected blood vessel, the site where IL-1/IL-6–producing macrophages accumulate.[8,9] Questions that need to be answered relate to the signals that adventitial macrophages receive in their microenvironment, the nature of the antigens they present on their surface, and the molecular interactions between adventitial macrophages and T cells.

Induction of IL-1β and IL-6 appears to be an early event in the disease process. This can be deducted from studies that identified IL-1β and IL-6 transcripts in temporal artery specimens from patients with PMR, arteries that lacked microscopically detectable infiltrates and were classified as negative for GCA by a pathologist.[10] Critical progress in understanding the pathogenesis of PMR and GCA could come from unraveling whether macrophages are triggered to produce IL-1 and IL-6 after they have entered the vessel wall through vasa vasorum, or whether they respond to a signal delivered in the periphery and then infiltrate into the arterial wall as a consequence.

Tissue production of IL-1 and IL-6 is highly sensitive to corticosteroid therapy. This has been useful in dissecting

TABLE 1
TISSUE CYTOKINES IN GCA

Cytokine	Possible function	Consequence/ outcome
IL-1β	Endothelial cell activation	Adhesiveness and cell recruitment
	Smooth muscle cell activation	Phenotypic/ functional switch
	Dendritic cell activation/ T-cell co-stimulation	Increased T-cell reactivity
TGF-β	Chemotaxis	Cell recruitment
	Fibroblast activation	Intimal hyperplasia
	Matrix synthesis	Intimal hyperplasia
IL-6	?	?
IFN-γ	Macrophage activation	
	–monokines and chemokines	Amplification of inflammation
	–metalloproteinases	Digestion of elastic membranes
	–oxygen radicals	Smooth muscle cell injury
	–growth factors	Intimal hyperplasia
	–angiogenesis factors	Neoangiogenesis

the anti-inflammatory activities of dexamethasone and acetyl salicylic acid in GCA.[11,12] Interestingly, the functional activity of adventitial macrophages cannot be abrogated by corticosteroid treatment. Macrophages in the adventitia persist and continue to synthesize TGF-β. The contribution of this cytokine to GCA is unresolved, but the chemotactic activities of TGF-β may contribute to the chronicity of the disease. TGF-β could also have a central role in the events leading to the mobilization, migration, and hyperproliferation of myofibroblasts and the deposition of matrix in the formation of lumen-occlusive intimal hyperplasia.

A key cytokine in the arterial wall is interferon (IFN)-γ.[3] It derives from CD4 T cells in the adventitia of affected blood vessels. It is an indicator of an adaptive immune response, reemphasizing the roles of antigen recognition and T-cell responsiveness as critical pathogenic steps. In model systems of GCA, depletion of IFN-γ–producing T cells is the only predictable intervention to abrogate vasculitis.[13] IFN-γ is the most potent activator of macrophages, and it almost certainly functions in the arterial wall by orchestrating the differentiation of macrophages into effector cells with tissue-destructive potential. Macrophages in the media become a source of oxidative stress with lipid peroxidation products attacking smooth muscle cells.[14-16] Distinct macrophage populations produce growth factors, such as platelet-derived growth factor (PDGF), and angiogenesis factors, such as vascular endothelial growth factor (VEGF).[17,18] These growth and angiogenesis factors are instrumental in driving intimal hyperplasia, the ultimate process leading to vaso-occlusion and ischemia. Tissue concentrations of IFN-γ and growth factor transcripts vary considerably between different patients, but they correlate with each other and the degree of intimal hyperplasia. This variability is biologically relevant, as emphasized by the correlation between clinical phenotype of GCA and the tissue concentrations of key cytokine transcripts, in particular IFN-γ **(Table 2)**. The central role of IFN-γ in these events makes this cytokine a preferred target for therapy as well as for distinguishing different types of vascular reactions.[19]

■ CIRCULATING CYTOKINES IN GCA AND PMR—TOOLS FOR DISSECTING DISEASE HETEROGENEITY

Circulating cytokines in GCA and PMR attract attention for two reasons. They are easily accessible, making them preferred tools in clinical practice, and they provide information about the systemic component of GCA and PMR, a component that is, at least in part, independent of the vascular component. Acute-phase reactants and downstream consequences of acute-phase responses have long been used as diagnostic clues for GCA/PMR, usually by measuring erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) levels. Acute-phase proteins are released upon cytokine signaling, mostly from hepatocytes. They are believed to play a role in tissue defense, tissue repair, and, when produced excessively, tissue damage. A critical cytokine in acute-phase induction is IL-6, formerly known as hepatocyte stimulating factor. IL-6 has wide-ranging biological activities, crudely divided into those regulating the hematopoietic system and those modulating innate immunity.[20] IL-6 stimulates B lymphocytes and drives proliferation of hematopoietic and megakaryocytic progenitors. Its action on non-immune cells is tied to activation of the hypothalamic-pituitary-adrenal axis. It is pyrogenic and is the most potent inducer of hepatic acute-phase proteins. Possibly important in the context of GCA and PMR is the ability of IL-6 to downregulate monokine production, such as TNF-α and IL-1β, and to suppress chemokine secretion. In essence, it may be regarded as a physiologic anti-inflammatory cytokine with potentially beneficial effects.

More than any other cytokine, IL-6 functions like a hormone and is released into the circulation.[21] Its cellular source in GCA and PMR is unknown. It could be speculated that it derives from vasculitic infiltrates, yet IL-6 levels are highly elevated in patients with PMR who have minimal arterial wall lesions, suggesting an alternative source. Given the marked changes in bone marrow function in PMR/GCA, this primary lymphoid organ is a prime suspect as the site of the intense systemic inflammatory reaction. Patients with GCA/PMR often have signs of suppressed red blood cell production combined with accelerated turnover of megakaryocytes. The precise mechanisms are unclear, but a recent study by Orphanos et al[22] has described a switch in the cytokine gene expression profile of marrow stromal cells of patients with GCA. Specifically, marrow samples of GCA patients expressed reduced amounts of stem cell factor, TGF-β, and TNF-α

and instead contained IL-1α and IFN-γ.

Circulating macrophages in patients with untreated GCA/PMR are constitutively activated, and 60% to 80% of them produce IL-1β and IL-6.[2] This intense stimulation of the innate immune system could result from a number of stimuli. Compounds triggering pattern recognition receptors as microbial products, stress proteins, or bacterial or viral DNA/RNA, could all rapidly induce macrophage activation. Alternatively, a powerful activator of monocytes/macrophages is IFN-γ, a specific product of the immune system responding to antigenic challenge. While inflammation of the arterial wall is T-cell dependent, it is not known whether circulating cytokines in GCA are a reflection of an innate immune response or are the result of T-cell recognition of a defined antigen.

IL-6 is highly elevated in patients with GCA and PMR.[23] Serum levels rapidly respond to immunosuppression with steroids and closely correlate to clinical symptoms, particularly those of myalgias and stiffness. The high correlation between IL-6 and clinical presentation has encouraged studies using the marker to dissect the clinical heterogeneity of PMR and GCA **(Table 3)**. In a prospective study of patients with PMR, IL-6 plasma concentrations were helpful in distinguishing patients in terms of steroid requirements and prognosis.[23] Pretreatment IL-6 values of <10 pg/ml were found in patients with a benign disease course characterized by lack of disease recurrence and steroid treatment <1 year. If pretreatment levels of IL-6 in the circulation were >10 pg/ml, patients fell into two categories. Those with normalization of IL-6 concentrations upon initiation of steroid therapy required one to two years of treatment and had infrequent clinical flares during tapering. Patients with high pretreatment IL-6 levels and continuous production of IL-6 despite corticosteroid therapy were classified as non-responders. They required increasing doses of prednisone to control clinical symptoms, had frequent exacerbations of clinical symptoms with dose reduction, and included patients who progressed to full-blown vasculitis. Studies in larger patient cohorts are necessary to understand the potential of IL-6 in differentiating clinically meaningful subsets of PMR. As we strive for patient-targeted therapy, blood cytokines are the most promising tools in assigning patients to diagnostic and therapeutic categories.

TABLE 2
TEMPORAL ARTERY CYTOKINE PATTERNS AND DISEASE HETEROGENEITY

Disease phenotype	IL-2	IFN-γ	IL-1β	PDGF	VEGF
PMR	++*	–	+	–	?
Aortic arch syndrome	++	+	+	?	?
Fever and wasting	++	+	+	–	–
Jaw claudication, visual loss	+	+++	+++	+++	+++

* –, transcripts not detected by PCR;
+ - +++, transcripts present at different levels.

■ CIRCULATING CYTOKINES IN GCA AND PMR—BIOLOGICAL MARKERS OF DISEASE ACTIVITY?

The important practical issue of classifying GCA and PMR patients into subsets with distinct therapeutic needs is closely related to monitoring disease activity during immunosuppression. Empirically, corticosteroids have been superior to any other immunosuppressive drug in treating this inflammatory vasculopathy. Mechanistic studies in vivo have indicated that corticosteroids target the NF-κB pathway and the genes dependent on this transcription factor. These studies also revealed that corticosteroid therapy only inhibits some mediators while others are relatively resistant.[12] In fact, vascular infiltrates persist in an animal model of GCA as well as in patients despite ongoing corticosteroid therapy. These findings have suggested an interesting discrepancy between the success in improving clinical symptoms and the failure of eliminating vascular lesions. This discrepancy provides an explanation for the chronicity of the disease with a need for prolonged immunosuppression although patients improve dramatically within a few days. Impressive clinical improvement despite persistent vasculitis also re-emphasizes that the disease has two components. These two components may vary profoundly in their sensitivity towards the immunosuppressive effects of corticosteroids.

IL-6 is rapidly responsive to corticosteroids, in holding with the role of NF-κB in the regulation of the IL-6 gene. The upstream positioning of IL-6 in the cascade of acute-phase responses would encourage use of this marker to carefully monitor activity of the disease process. Available data suggest that IL-6 might be an ideal candidate to titrate steroid requirements. In a cohort of patients with biopsy-proven GCA, serum IL-6 levels were superior in detecting vasculitis when compared with traditional laboratory parameters.[25] IL-6 was above normal in 92% of all untreated patients, whereas ESR identified only 74% of the patients. All patients were treated with glucocorticoids using a predetermined protocol of steroid tapering and all responded to initiation of therapy with normalization of the ESR. This excellent response rate did not apply to concentrations of circulating IL-6. In only 46% of the patients could 60 mg prednisone per day suppress IL-6 into the normal range. Upon follow-up of the patients, ESR results were only partially helpful in making decisions whether the patient was sufficiently treated or suffered from a disease flare. IL-6 detected almost 90% of disease reactivation. More importantly, IL-6 remained moderately elevated in patients considered clinically to be non-active or in remission. IL-6 outperformed CRP and ESR in demonstrating ongoing disease activity. Indeed, the marker seems to be so sensitive that a new therapeutic issue in managing GCA has arisen. Should the patient be treated until all clinical signs of inflammation are controlled, or should we attempt to reach total suppression of the disease process, including the release of pro-inflammatory cytokines? What is the risk of a patient experiencing clinically undetectable but biochemically detectable

TABLE 3
PERIPHERAL BLOOD IL-6 LEVELS AND DISEASE SEVERITY

Disease severity	Pre-treatment	Treatment	Flares	Post-treatment
Mild PMR	<10 pg/ml	Normal	No flares	?
Moderate PMR	>10 pg/ml	Normal	Increase in IL-6	?
Resistant PMR	>10 pg/ml	>10 pg/ml	Increase in IL-6	?
GCA	>10 pg/ml	>10 pg/ml	Increase in IL-6	Elevated

disease? What is the site of disease? Results in the animal model of GCA would suggest that vascular infiltrates persist. Are they the source of IL-6 and do they pose a risk to the patient? Does the risk of side effects from more aggressive therapy outweigh the benefit for the patient? Appropriately designed therapeutic trials will be able to give answers to some of these questions, but the community of physicians must be prepared to follow patients for more than a decade because late disease manifestations may not be detectable prior to that time.

SUMMARY

Cytokines are small proteins that serve as chemical messengers between cells, regulating cell growth and differentiation, tissue repair and remodeling, and many aspects of the immune response. Cytokines are instrumental in determining the nature, magnitude, and duration of inflammatory reactions and, as such, represent ideal targets for interfering with pathogenic processes. In GCA and PMR, cytokines are encountered in two locations, the inflammatory infiltrates accumulating in the arterial wall and in the circulation. IL-6, a cytokine involved in stimulating acute-phase responses, is located upstream of many of the laboratory abnormalities considered helpful in diagnosing and managing GCA/PMR, including elevated ESR and CRP. IL-6 has the potential to be helpful in predicting disease severity and may allow for a tailoring of immunosuppressive therapy. There is evidence suggesting that IL-6 outperforms other chemical markers in detecting disease activity and could, therefore, have a role in monitoring treatment. Interesting pathogenic clues have been derived from studies of cytokines produced in the vascular lesions. IFN-γ has emerged as a key regulator in determining the nature and direction of the inflammatory response. IFN-γ appears to be critically involved in modulating the process of intimal hyperplasia, the most destructive consequence of vasculitis, and, as such, emerges as a prime target for novel therapeutic approaches.

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Utility of imaging studies in assessment of vascular inflammation

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Imaging studies used in vasculitis can be divided into isotopic, ultrasonographic, and radiographic studies. The most important role of nuclear medicine in systemic vasculitis is the use of inflammation-specific agents to monitor the inflammatory activity of the disease.[1] The agents in clinical use today to target inflammation are leukocytes, labeled with technetium (Tc)-99m or with indium (In)-111, gallium-67 and 18-fluorodeoxyglucose (FDG). Ultrasound examinations allow the noninvasive diagnosis of temporal arteritis.

Radiographic studies (classic and magnetic resonance angiography) are used in those forms of vasculitis in which it is difficult to obtain histologic proof, due to difficulties in performing biopsies on affected organs. Examples of these diseases are Takayasu's arteritis, classical polyarteritis nodosa (PAN) and isolated cerebral vasculitis. Pulmonary vasculitic involvement, eg, in Wegener's granulomatosis, can be seen on a plain x-ray of the lungs or on the much more sensitive high-resolution computed tomography (CT). (Para)nasal involvement in Wegener's disease patients or periaortic inflammation in periaortitis are also best appreciated on CT scan.

ISOTOPIC STUDIES

Labeled leukocytes

The effective ingredient in a labeled leukocyte preparation is the neutrophil, since lymphocytes are too sensitive to ionizing radiation, which prevents them from recirculating normally through the lymphoid system within hours of labeling.[2] This limits the use of labeled leukocytes in vasculitis, in which the chronicity of inflammation and its neutrophilic content may vary widely. The normal distribution of Tc-99m and In-111 labeled leukocytes is the reticulo-endothelial system, but Tc-99m can also be seen in the urinary tract, gall bladder, and gut.

Isotopic studies with labeled leukocytes in small-vessel vasculitis were reported for patients with Wegener's granulomatosis and microscopic polyangiitis. Splenic photopenia on In-111–labeled leukocyte scintigraphy was described in a Wegener's granulomatosis patient due to splenic necrotizing vasculitis with granuloma formation.[3] In a retrospective study of 12 patients with systemic vasculitis (six each with Wegener's granulomatosis and microscopic polyangiitis), all with renal disease, Jonker et al observed increased diffuse lung radioactivity soon after the injection of In-111–labeled granulocytes or Tc-99m–labeled leukocytes in all patients with Wegener's granulomatosis and in three with microscopic polyangiitis. The majority of patients with systemic vasculitis had scintigraphic evidence of abnormal splenic function (2 with splenic defects, 7 with an increased labeled cell uptake). Focal nasal uptake was seen in Wegener's granulomatosis patients, but renal disease was scintigraphically apparent in only one patient.[4]

In a large retrospective study on 50 patients with systemic vasculitis (Wegener's granulomatosis in 32, microscopic polyangiitis in 12, Churg-Strauss syndrome in 4, and temporal arteritis in 2), leukocyte imaging was useful for detecting unsuspected sites of disease and monitoring disease activity. Scintigraphy was superior to conventional radiography or CT scanning for detecting and monitoring vasculitic involvement of the respiratory tract. Nasal uptake on leukocyte scans could differentiate between Wegener's granulomatosis and microscopic polyangiitis. Also in this study, the scans were not sensitive in detecting renal vasculitis.[5]

In-111–labeled leukocytes were also used in large-vessel vasculitis. Fink et al described three patients with nonspecific features, including unexplained fever due to aortitis, which was diagnosed by this isotopic technique. The final diagnoses were periaortitis in Wegener's granulomatosis, aortic dissection in giant-cell arteritis, and streptococcal aortitis with impending rupture.[6] In Takayasu's arteritis, In-111 mixed leukocyte scans had a low sensitivity for active disease (25% only, increased vessel uptake in two of eight scans). Possible explanations are the size of the vessels involved, the small volume of the cellular infiltrate at any one site of inflammation, and the lymphocytic predominance in the cellular infiltrate.[7]

Gallium-67 scintigraphy

Gallium-67 citrate binds to the transferrin receptor expressed on the surface of activated macrophages.[8] In 1988, Yuasa et al reported that granulomatous involvement of the lungs in a Wegener's granulomatosis patient could be detected on gallium scintigraphy.[9] Other case reports de-

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scribe gallium uptake in cutaneous lesions of periarteritis nodosa.[10,11] In a large study of 46 consecutive infants and children with Kawasaki disease, clinically suspected myocarditis could be demonstrated in 63% using imaging with gallium-67 citrate by planar imaging and even in 80% by single photon emission CT imaging.[12]

Gallium scintigraphy was performed in 4 patients with Takayasu's arteritis. Vascular uptake was noted in 3 patients before therapy. After total lymphoid irradiation, gallium scans returned to normal.[13] Significant gallium-67 uptake in the distribution of the aortic arch and great vessels was reported in several case reports.[14-16]

Généreau et al evaluated temporal gallium-67 uptake in a prospective study on 24 patients with temporal arteritis, which was biopsy-proven in 19 cases. Compared to 18 elderly controls undergoing gallium scans for various inflammatory diseases, the gallium uptake in the temporal region was significantly higher in biopsy-proven and biopsy-negative temporal arteritis patients. The specificity of a gallium-uptake ratio >0.4 (compared to a parietal region of interest) was 94% and its predictive positive value was 90%, while its sensitivity reached only 38%. Gallium uptake ceased during remission (six months after diagnosis, not with short-duration steroid treatment, <38 days). The authors concluded that gallium scintigraphy may contribute to the diagnosis in temporal artery biopsy-negative patients.[17]

FDG-positron emission tomography (PET)

FDG is a glucose analogue which is transported across the capillary and sarcolemmal membranes in proportion to the rate of glucose uptake. Increased FDG-uptake can be observed in cells with high metabolic requirements, such as inflammatory and tumor cells.

In 1996, we undertook a prospective study in order to compare FDG-PET scintigraphy with gallium-67 scanning in the workup of patients with fever of unknown origin.[18] In some of these patients, we saw a very profound FDG-uptake in the large thoracic vessels. These patients later on turned out to suffer from giant-cell arteritis. In view of these findings, we started a second prospective study in which all consecutive patients who presented to our department with a clinical picture compatible with giant-cell arteritis and/or polymyalgia rheumatica underwent a temporal artery biopsy and FDG-PET scintigraphy before eventual therapy with steroids was started. Owing to high uptake in the brain, the small diameter of the vessel, and the relatively high background of the skin, direct evaluation of the temporal arteries is not possible on whole-body PET investigation. A preliminary series of 5 patients with polymyalgia rheumatica, 6 patients with giant-cell arteritis, and 23 age-matched control patients was published in 1999.[19] Vascular FDG-uptake in the thoracic vessels and in the upper legs was seen significantly more in the temporal arteritis and polymyalgia patients than in the controls. Thoracic vascular FDG-uptake especially was very specific for temporal arteritis and/or polymyalgia rheumatica since it was encountered in only 1 control patient compared to 8/11 patients. In 4 patients, FDG-PET scan was repeated under steroid treatment, at a time when all symptoms had disappeared and inflammatory parameters had normalized. Vascular FDG-uptake had clearly decreased at that time.[19] A larger series on the use of FDG-PET scintigraphy in patients with giant cell arteritis (n = 25) and polymyalgia rheumatica (n = 13) was published in 2000.[20] Thoracic vascular FDG-uptake had a sensitivity for the diagnosis of giant-cell arteritis or polymyalgia rheumatica of 56%, a specificity of 98%, and a positive predictive value of 93%. Vascular FDG-uptake in the legs had a slightly higher sensitivity of 64% but a lower specificity of 77%. The highest yields were obtained in patients with predominant systemic symptoms, such as fever, weight loss, or general malaise.[20] These studies confirmed former clinical reports that giant-cell arteritis affects not only the temporal arteries, but also the aortic arch, the abdominal aorta, the subclavian and even the femoral arteries. Recently, other groups reported similar FDG-PET findings in single patients.[21-23] Rare false-positive FDG-PET scans may be due to severe atherosclerosis,[24] although we could not find a correlation with clinical atherosclerosis in our controls with false-positive upper leg vascular uptake.[19]

In April 2000, we included our first patient with giant-cell arteritis/polymyalgia rheumatica into a new prospective study with the intention to see if vascular FDG-uptake in these disorders has any influence on the relapse rate. All patients got a FDG-PET scintigraphy before treatment with steroids was started, at 3 months (if the initial PET scan showed vascular FDG-uptake) and at 6 months (whenever the PET scan performed at 3 months was still positive). Patients with isolated polymyalgia rheumatica were treated with methylprednisolone 12 mg/day, which was then gradually tapered and stopped after 6 months of therapy. Patients with temporal arteritis received 32 mg of prednisolone/day as an initial treatment, which was then also gradually diminished and stopped after 1 year of treatment. At this moment (December 2001), 20 patients with isolated polymyalgia and 20 patients with giant-cell arteritis were enrolled. In the 20 patients with isolated polymyalgia (temporal artery biopsy performed in 18, always negative), vascular FDG-uptake was visible in only 3 (15%). Eighteen out of 20 (90%) showed intense FDG-uptake in their shoulders and hips. Today, 13 patients finished their 6 months of steroid treatment, but 10 of them relapsed, most frequently when taking 2 mg methylprenisolone/day or a few weeks or months after stopping treatment. Since only a few patients showed vascular FDG-uptake and almost all showed (peri)articular FDG-uptake, we cannot make any statement about the possible predictive role of vascular FDG-uptake in isolated polymyalgia at this moment. It is clear, however, that a 6-month treatment for isolated polymyalgia is too short, since most patients relapsed.

In patients with giant-cell arteritis (which was biopsy-proven in 18 out of 20), intense FDG-uptake in the large vessels was seen in 16 (80%). Fourteen of these 16 already underwent a second FDG-PET scan after 3 months of steroid treatment (while they were taking 16 mg methylprednisolone/day). FDG-uptake had disappeared in 7 and had diminished but was still detectable in another 7. Six

patients thus far of those with residual uptake at 3 months underwent a third FDG-PET scan at 6 months of treatment (taking 8 mg methylprednisolone). There was no clear further decrease in FDG-uptake at 6 months compared to 3 months of steroid treatment. All these patients are still taking methylprednisolone at this moment (December 2001), but it will be interesting to see if they will have more relapses of their vasculitis, compared to those whose FDG-PET scan normalized. A typical sequence of FDG-PET scintigraphy before the start of steroid treatment, at 3 months, and at 6 months is shown in **Figure 1**.

Large-vessel inflammation in Takayasu's arteritis could also be demonstrated using FDG-PET scintigraphy.[25-27] Meller et al had 3 patients with Takayasu's arteritis in their series of FUO patients. The thoracic aorta was positive on transaxial FDG tomography in these 3 patients, while gallium-67 scintigraphy was negative in 2. In one patient, FDG scintigraphy was repeated following 3 months of glucocorticoid therapy; it had normalized by that time.[26]

During the past 2 years, we had the opportunity to study several patients with idiopathic periaortitis, both at thoracic and abdominal level, with FDG-PET scintigraphy. There was always a high FDG-uptake in the inflamed aortic tissue, which normalized during steroid therapy.[28,29]

Few patients with medium-sized vasculitis (Churg-Strauss syndrome, PAN) underwent FDG-PET scintigraphy. No vascular FDG-uptake was detected.[27] In small-vessel vasculitis, internal organ involvement can be visualized with FDG-PET scintigraphy, especially in the lungs and the nose (in case of Wegener's granulomatosis).[27,30]

■ ULTRASONOGRAPHIC STUDIES

In a prospective study on 30 patients with temporal arteritis (biopsy-confirmed disease in 21), Schmidt et al reported that color duplex ultrasonography showed a dark halo around the lumen of the temporal arteries in 22 patients (73%). These halos disappeared after a mean of 16 days of treatment with corticoids. No halos were identified in 37 patients with isolated polymyalgia rheumatica nor in 45 control patients, which makes it a very specific sign. Stenoses or occlusions of temporal artery segments were found in 24 patients (80%) compared to only 6 patients (7%) with polymyalgia or other diseases. Twenty-eight temporal arteritis patients (93%) had stenoses, occlusions, or a halo.[31] Lauwerys et al, in contrast, reported thickening of the vessel wall in only 2 out of 11 temporal arteritis patients, but they found a significantly lower peak systolic velocity compared to the velocities measured in 21 polymyalgia patients and 32 controls. Follow-up with color Doppler sonography in 6 patients with giant-cell arteritis under treatment produced evidence of a significant increase in the mean peak systolic velocity.[32]

We are using color duplex ultrasonography in a prospective way to study the temporal arteries of all patients suspected of having giant-cell arteritis. Until now, 95 patients were studied, of whom 19 indeed had temporal arteritis, as evidenced by temporal artery biopsy. The typical halo, described by Schmidt et al, was found in 14 of these 19 patients and in only 3 of the 76 patients with isolated polymyalgia or with other diseases. Therefore, sensitivity of the halo sign for giant-cell arteritis was 74%, its specificity 96%, its positive predictive value 82%, and its negative predictive value 94% (Guy Beyens et al, unpublished data).

■ RADIOGRAPHIC STUDIES

Angiography

Visceral arterial aneurysms were already noted in the original description of PAN by Kussmaul and Maier in 1866[33] and were first demonstrated angiographically by Fleming and Stern in 1965.[34] The angiographic findings in classical PAN include aneurysms, irregular beading, stenoses and/or occlusions of medium-sized vessels, usually best seen in the renal and hepatic vascular territories. The presence of aneurysms is generally associated with more severe disease and hypertension.[35] When typical arterial changes are present in the right clinical context, the diagnosis of PAN can usually be made, although slight dilatations have also been encountered in Wegener's granulomatosis, Churg-Strauss syndrome, and vasculitis look-alikes such as infective endocarditis.[36]

In Takayasu's arteritis, angiography is now being replaced by magnetic resonance imaging (MRI) and magnetic resonance angiography (see below). Indeed, angiography can only visualize alterations of the vessel lumen, eg, stenoses, occlusions, or aneurysm formation. This is at a stage when the disease has already progressed considerably, while MRI can detect earlier alterations in the vessel wall. Angiographically, stenoses and occlusions are found in the thoracic and abdominal aorta (with aneurysm formation as well), the subclavian and renal arteries, and also in the pulmonary arteries. Embolization of hypertrophied bronchial arteries, which can develop as a result of pulmonary infarction, can be lifesaving in case of severe hemoptysis.[37]

Primary angiitis of the central nervous system is usually suspected clinically and recognized by angiography, but a definitive diagnosis still requires tissue documentation of the presence of a true vasculitis.[38] CT scan and MRI of the brain will frequently show alterations, but these are very unspecific for vasculitis. A brain biopsy is of course very invasive, and rather frequently one has to be satisfied with a compatible angiography. Sensitivity of a high-probability angiogram is less than 40% in histologically confirmed cases (and 100% in reports not supported by histology), whereas its specificity lies around 20% since atherosclerosis, vasospasm, and infection can give very similar images.[39]

Magnetic resonance imaging

Conventional x-ray angiography has played a prominent role in the evaluation of large- and medium-sized vessel vasculitis. Recently, noninvasive magnetic resonance methods have been introduced for the evaluation of large-vessel vasculitis. Magnetic resonance provides high-resolution anatomic information, including lumen configuration and vascular wall thickening, and physiologic data, such as measurements of the degree of wall en-

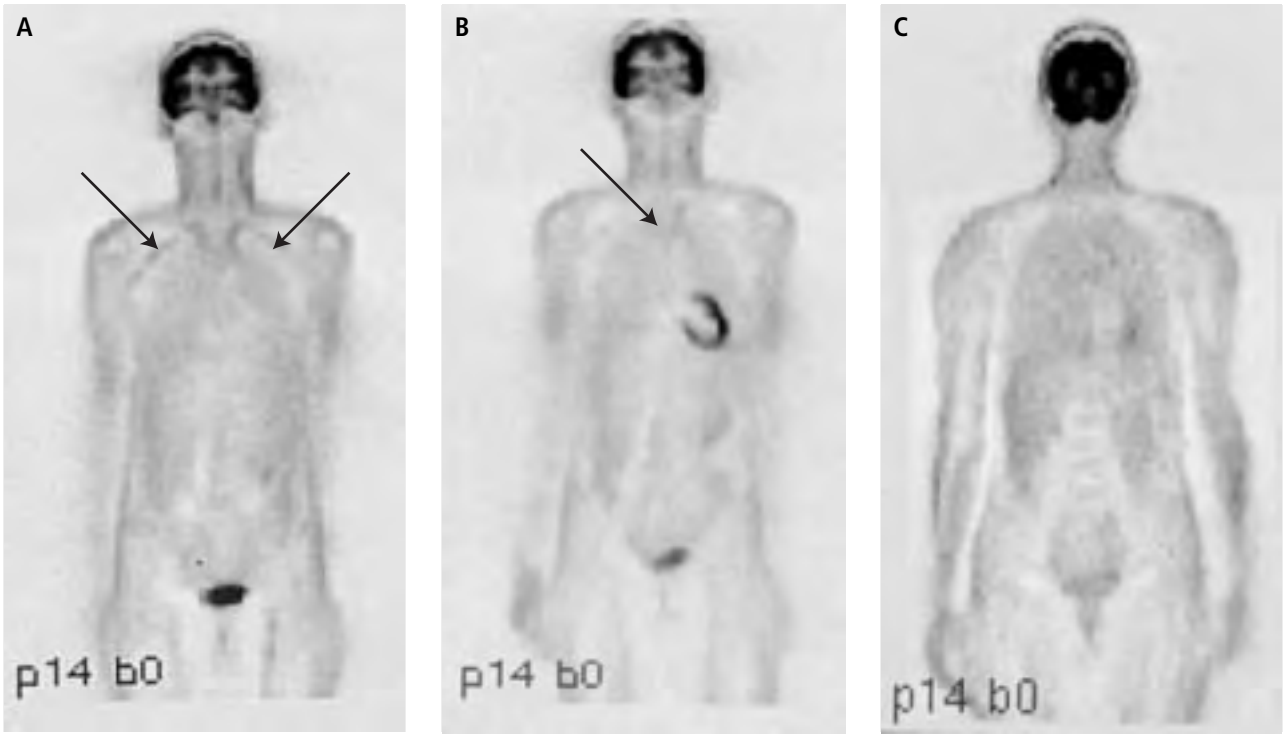

Figure 1. (A) Increased FDG-uptake at both subclavian arteries (arrows) in a patient with GCA, before therapy with steroids was started. (B) Same patient, 3 months later during steroid treatment (methylprednisolone 16 mg/day). Almost complete disappearance of vascular FDG-uptake (arrow). (C) Same patient, at six months of steroid therapy (methylprednisolone, 8 mg/day). There is no FDG uptake visible any more at the large thoracic arteries.

hancement and the presence of edema.[40] Using breath-hold three-dimensional magnetic resonance angiography, Yamada et al demonstrated a 100% sensitivity and specificity for the diagnosis of Takayasu's arteritis in a prospective study on 30 people suspected of having the disease.[41] Choe et al suggested that strong enhancement on MRI of the thickened aortic and carotid artery walls, equal to or greater than myocardial tissue signal, correlates with active inflammation in Takayasu's arteritis.[42] Vascular wall thickening is an important finding in the acute phase of Takayasu's arteritis, subsiding after appropriate therapy.[43] Mural edema is a characteristic pattern of active and progressive Takayasu's arteritis, but it is absent in the chronically active state.[44]

CONCLUSIONS

Most forms of vasculitis can be readily diagnosed through a combination of clinical symptoms, biochemical parameters, and radiographic and/or bioptic findings. Probably the most difficult diagnosis in the field of the vasculitides remains primary angiitis of the central nervous system, since angiography is not very specific and brain biopsy is very invasive. For those patients with very aspecific complaints (eg, only fever or weight loss) who suffer from large-vessel vasculitis, FDG-PET scan can reveal giant-cell arteritis or periaortitis. Large thoracic vessel FDG-uptake is a very specific sign for giant-cell arteritis (nearly 100%), and its sensitivity approaches 80%. In isolated polymyalgia rheumatica, synovial FDG-uptake in the shoulders and hips is the most frequent finding, but this picture cannot differentiate from other (peri)articular inflammatory disorders.

In the follow-up of ANCA-related vasculitides, serial ANCA-titer determinations are useful as a measurement of disease activity. Flare-ups of giant-cell arteritis or polymyalgia are characteristically accompanied by increases in sedimentation rates and CRP levels. These inflammatory parameters normalize very rapidly upon the start of steroid treatment and hence cannot be used really to guide therapy in order to prevent relapses. Perhaps FDG-PET scan, which can remain pathologic even after 6 months of steroid therapy, may be a more helpful determinator of treatment duration or dosage.

In the diagnosis of Takayasu's arteritis, nuclear magnetic resonance angiography has replaced the more invasive classic angiography. In the assessment of the activity of the disease, MRI of the aortic wall (its thickness and presence of edema) has proven to be valuable.

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Measuring disease activity and outcomes in clinical studies

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Untreated systemic vasculitis has an appalling prognosis. The pathogenesis is still being addressed, and we have no clear etiological agents defined in most cases of these uncommon but not rare diseases.[1,2] Our lack of understanding of pathogenesis has led to the widespread use of classification systems, which describe groups of patients with different forms of vasculitis in predominantly pathological and clinical terms using vessel size as the dominant classification.[3-5] These classification criteria are widely applied as diagnostic criteria, which is inappropriate since they do not serve this purpose well.[6] In many ways, however, this distinction between different forms of vasculitis has served as the empirical basis for different forms of therapy.[7] Large-vessel vasculitis is predominantly managed with steroid therapy alone, with the use of immunosuppressive drugs such as azathioprine or methotrexate only if there is resistance to steroids. By contrast, the small-vessel vasculitides, such as Wegener's granulomatosis involving the kidney, or microscopic polyangiitis with kidney and lung involvement, are primarily managed by the use of cyclophosphamide and steroids. Therefore, at the classification or diagnostic stage we are already making a distinction between diseases that we treat more or less aggressively. As a further refinement of this process, within each disease it is becoming increasingly apparent that there is a need to distinguish the pattern of involvement in each individual case and the current level of disease activity, which might require different levels of immunosuppressive treatment at any point in time.[8,9] This strategy leads to a more logical framework for addressing the need for appropriate immunosuppression. The concept that vasculitic diseases are a one-shot illness is no longer valid. We recognize that modern management has transformed the outcome for survival to a very high survival probability. However, survival is characterized by frequent episodes of reactivation of the original disease (in up to 50% of cases in some series) or persistent grumbling low-grade disease.[10,11] In addition, the chronic effects of disease scarring and drug toxicity take their toll and result in some cases in significant organ damage.[12] Accurate assessment of disease activity is therefore an essential part of the management of these complex diseases. We need to discriminate between appropriate levels of immunosuppression and also distinguish active disease from chronic scarring for therapeutic strategies to be more appropriate. Disease assessment may also offer the opportunity to predict future outcome and is increasingly of use in determining how aggressive to be at disease onset.[8] Therefore we must consider not only current levels of disease activity in their immediate context but also what they may be telling us of the likely future outcome. These are difficult tasks to expect any individual assessment to provide.

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PATHOLOGICAL AND SEROLOGICAL ASSESSMENT IN SYSTEMIC VASCULITIS

Histological evaluation is an important diagnostic step in systemic vasculitis. A recent study has suggested that the presence of tubular inflammation in kidney biopsies of patients with renal vasculitis is predictive of outcome[13]; in Henoch-Schönlein purpura, the presence of crescents, interstitial fibrosis, and of dense subendothelial deposits are all predictors of chronic renal failure.[14] Unfortunately, biopsies from other affected organs have not been shown to provide prognostic information. The role of repeated biopsies to assess disease activity or prognosis is limited by the morbidity of the procedure. In practical terms, it is difficult to justify serial biopsies to evaluate progress. Serological markers would be of importance in this regard; unfortunately, the ESR is very unpredictable and may be influenced by a number of factors including infection or chronic inflammation. Similarly, the C-reactive protein is a poor discriminator between infection and active vasculitis. Recent studies have suggested that procalcitonin levels may be a better discriminant between disease activity and infection in the setting of acute vasculitis.[15,16] The role of anti–neutrophil cytoplasmic antibody (ANCA) titers in measuring disease activity is still controversial. The ANCA pattern has been shown to predict the outcome in microscopic polyangiitis,[17] where the C-ANCA pattern has a higher risk of mortality associated with it than the P-ANCA pattern (3.78:1). However, this differential outcome has not been used as the basis of any published therapeutic studies so far. Although serial testing of ANCA has been correlated with disease activity,[18] there is still a con-

siderable overlap between normal variation in ANCA levels in these patients and disease activity, so that 29-43% of the ANCA rises may be in the absence of clinical disease activity.[19] Russell et al (2001) have suggested that antibodies to pro forms of ANCA are more closely linked to clinical disease activity.[20] It is possible that these ANCA rises are occurring on the basis of subclinical disease, but it would be difficult to justify therapy on the basis of rises in ANCA titer alone.

CLINICAL ASSESSMENT OF DISEASE ACTIVITY IN SYSTEMIC VASCULITIS

Clinical evaluation remains the gold standard for disease assessment in systemic vasculitis. It is perhaps the most natural system for clinicians managing these patients and has formed the basis for accurate assessment tools of disease activity, which have prognostic as well as practical importance on a day-to-day basis. Overall, however, each tool may contribute towards the evaluation of patients with vasculitis, and it is important to consider the evidence provided by a combination of clinical assessment, serologic assessment, and pathologic or radiologic assessment where appropriate. Together they allow better definition of vague terms such as relapse or remission, which can then be qualified in more objective evaluations.

Evaluation of disease for the purpose of clinical trials has been largely based on the use of clinical tools, both as measures of active disease and as prognostic discriminators. The five factor score has been used as a stratification process whereby patients with poor prognosis (ie, those with a score of 1 or greater) are scheduled to receive aggressive therapy, including cyclophosphamide, whereas patients with a good prognosis (ie, those with none of the 5 factors) are scheduled to receive steroids alone.[8,9,21] There has been more widespread use of the Birmingham Vasculitis Assessment Score (BVAS) in measuring disease activity during the course of the disease in clinical trials.[22] The European Vasculitis Group (EUVAS) have modified and used BVAS extensively in many of their clinical studies,[23-25] and the Wegener's Granulomatosis Etanercept Trial (WGET) investigators have developed a specific version of BVAS for use in a trial of etanercept therapy in Wegener's granulomatosis.[26] BVAS consists of a checklist of items that are predominantly based on clinical history and examination but supported by some laboratory investigation, such as serum creatinine and the presence or absence of blood or protein in the urine. BVAS has been applied to a variety of forms of vasculitis and shown to have biological validity and is highly reproducible. It aims to be objective by avoiding rating of abnormalities. It addresses the question of disease activity in the context of vasculitis and is designed to measure only those features that currently represent active vasculitic disease requiring therapeutic intervention. In other words, it attempts to record objectively the usual clinical decision-making process that forms the everyday practice of clinicians dealing with vasculitis. If an item is recorded on BVAS, the clinician recording it should be doing it with the conviction that the abnormality requires active therapeutic intervention. The distinction between what is new or worsening vasculitis activity as compared to a new event that does not represent active vasculitis (such as infection or side effects from treatment) is an important one and lies at the heart of the BVAS system. It is therefore heavily dependent on clinician expertise and judgement. In practice, these distinctions have to be made "on the spot" so that decisions on therapy can follow immediately. Therefore, the BVAS represents an intention-to-treat–based system of clinical assessment that is meant to be of direct practical value in the management of these patients.

The disease extent index (DEI) is applicable to Wegener's granulomatosis and has a high correlation with BVAS, but also includes an element of damage, therefore giving a cumulative assessment of disease.[27] The vasculitis activity index (VAI) is an analogue-scale measurement of organ activity in each of nine organ systems, and also includes indirect measures of activity, such as the sedimentation rate.[28] However, this does not allow for the detailed descriptions offered by either BVAS or DEI. It also suffers from the potential criticism of observer bias.

CLINICAL ASSESSMENT OF DISEASE DAMAGE IN SYSTEMIC VASCULITIS

Assessment of damage is important to distinguish from active disease. It may contribute significantly to the patient's overall state of health yet require very different management from that for activity. The vasculitis damage index (VDI) is an objective item list based on 11 organ systems, incorporating damage attributable to the disease as well as to its treatment.[29] Using the VDI, damage is detected surprisingly early, relating to the initial presenting episode.[12] In a cohort of 120 patients, one-third had already sustained damage before presentation to hospital. By six months, most patients had 2 to 4 damage items; only 5% had no damage items, while some patients had already accumulated up to 8 items. Damage was not restricted to a single organ system, since two-thirds of patients had two or more systems involved—a minority as many as six. This rate of damage accumulation was not maintained subsequently, and damage is not necessarily progressive in patients followed for up to 5 years.[30] This has implications for therapy, highlighting the need to control disease activity rapidly at presentation in the attempt to prevent early development of scarring. Damage is an important surrogate measure of outcome and is of predictive value in studies of systemic vasculitis. Therefore, the damage index may provide an important evaluation method for determining the success or failure of therapies in vasculitis. The systemic necrotizing vasculitis damage index (SNVDI) has been developed for specific use in polyarteritis and Churg-Strauss syndrome.[31] It is very similar to the VDI. Some aspects of damage are measured by the DEI, since it is an attempt to describe the overall spread of vasculitis throughout different organ systems in the individual patient.

PROGNOSIS IN VASCULITIS

Determining future outcome on the basis of current information would be of great value in systemic vasculitis. The DEI has been used to predict treatment responses in ANCA-positive patients with Wegener's granulomatosis where patients with low DEI levels (9 or less) favor better treatment response from pulse high-dose cyclophosphamide, whereas high DEI levels (above 9) benefit from

continuous oral standard-dose cyclophosphamide.[32] The ANCA pattern may be helpful in ANCA-positive microscopic polyangiitis, where mortality risk is higher (3.78-fold higher) for patients who have C-ANCA rather than P-ANCA.[19] BVAS and five factor score are of help in predicting mortality[9,22]; essentially, the higher the score, the greater the mortality risk. The VDI and its sub-scores (especially the critical damage index) are predictive of mortality. Comparing the VDI scores of a subgroup of patients who subsequently died with those who survived for at least a 5-year follow-up period, at the last available examination the fatal cases scored positive for significantly more items than the survivors, and this damage involved significantly more organ systems. It is also relevant that the final examination was at a mean of 2.6 years in the severe group but at 5 or more years in the others. In fatal disease, more items of damage, involving more organ systems, are accumulated at a faster rate than in non-fatal cases.[30] Patients who had a VDI score of greater than 5 carried a 6-fold increased risk of mortality. A system score of more than 3 nearly doubled that risk, while involvement of more than one item of critical organ damage carried a relative risk of 17.

CONCLUSIONS

The histologic, clinical, and serologic tools available in systemic vasculitis allow us to begin the task of stratifying patients according to outcome category as well as defining targets for improvement with different immunosuppressive regimens. Both of these aspects are essential in clinical trials of systemic vasculitis. Until we have pathophysiologically based evaluations, our clinical methods supported by laboratory tests remain the gold standard for management of these diseases and therefore also the gold standard for measurement tools in clinical studies.

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19-022

INCREASES OF PR3 AND MPO GENE TRANSCRIPTION IN CIRCULATING LEUKOCYTE OF ANCA-ASSOCIATED DISEASE CORRELATE WITH DISEASE ACTIVITY

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Proteinase (PR3) and myeloperoxidase (MPO) are the primary autoantigens of anti-neutrophil cytoplasmic autoantibodies (ANCA). PR3 has been reported to be present on the surface of polymorphonuclear neutrophils and monocytes, and to exist in the circulation of patients with active ANCA vasculitis and glomerulonephritis (ANCA-GN). We investigated circulating leukocytes from patients with ANCA-GN for changes in levels of PR3 and MPO mRNA transcripts.

Leukocytes were isolated from 46 blood samples of patients with ANCA-GN (PR3-ANCA n=29; MPO-ANCA n=17); 24 samples from healthy donors served as normal controls, and 25 samples from end-stage renal disease (ESRD) and 17 samples from systemic lupus erythematosus (SLE) patients as disease controls. The mRNA levels of PR3 and MPO were measured by TaqMan quantitative PCR. The data are expressed as relative fold-change, as compared to a healthy control reference sample.

The relative levels of PR3 mRNA increased significantly to 20.5 ± 28.7-fold ($P < 0.05$) in patients with active ANCA-GN, above that in healthy donors (0.6 ± 0.6-fold) (active+: 2.9 ± 2.3, $P < 0.05$; active++: 16.7 ± 27.3, $P < 0.05$; active+++: 37.3 ± 32.4, $P < 0.05$). PR3 mRNA did not increase in ANCA patients in remission (3.8 ± 4.7, $P > 0.05$), ESRD (1.6 ± 3.2, $P > 0.05$) or SLE patients (1.0 ± 1.4, $P > 0.05$). MPO mRNA levels markedly increased (13.9 ± 21.8, $P < 0.05$) compared to healthy donors (0.9 ± 0.8) (active+: 3.9 ± 4.6, $P > 0.05$; active++: 12.8 ± 22.1, $P < 0.05$; active+++: 33.9 ± 27.0, $P < 0.05$), but not in ANCA patients in remission (2.8 ± 2.5, $P > 0.05$), ESRD (2.1 ± 3.6, $P > 0.05$) or SLE (1.8 ± 2.4, $P > 0.05$). There was a positive correlation between the increase in PR3 mRNA and the increase in MPO mRNA ($R^2=0.82$, $P < 0.0001$), regardless of whether the patient was PR3- or MPO-ANCA-positive.

In conclusion, these data indicate that ANCA-GN is associated with an increase of PR3 and MPO mRNA expression by peripheral blood leukocytes. PR3 and MPO mRNA levels may be a marker for disease activity in ANCA-GN.

20-119

CARDIAC INVOLVEMENT IN WEGENER'S GRANULOMATOSIS: ECHOCARDIOGRAPHIC FEATURES AND CLINICAL OUTCOMES

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Background: The spectrum of extra-cardiac disease in Wegener's granulomatosis (WG) has been well described. However, cardiac involvement in WG has not been systematically studied and remains poorly understood.

Methods: Review of echocardiographic and clinical data of 85 consecutive patients with proven WG over 21 years. Cardiac abnormalities at echocardiography were analyzed at the time of the diagnosis of WG. Echocardiographic lesions were attributed to WG when no other coexisting disease could explain them, or when objective data such as resolution of the lesion with immunosuppressive therapy was documented. Follow-up data were obtained by reviewing the patients' last recorded visit or correspondence with Mayo Clinic.

Results: Echocardiographic abnormalities were found in 73 patients (85.8%). In 26 patients (31%), lesions appeared directly related to WG. Of these, regional wall motion abnormalities (RWMA) were the most frequent abnormalities found in 16 patients (61.5%); the ventricular septum was most consistently involved (50%), followed by the inferior (42%), apical (34%), anterior (30%), and lateral wall (26%). Left ventricular (LV) systolic dysfunction with a mean ejection fraction of 46% ± 14% was found in 13 (50%) and pericardial effusion in 5 (19%) patients. Other findings included acute aortic regurgitation, LV aneurysm, intracavitary thrombus, and a large mass in the LV outflow tract. There was a 42% increased mortality in patients with WG cardiac involvement at echocardiography compared to patients with abnormal echocardiograms from other causes (RR 2.9). Cox regression analyses showed that cardiac WG was a univariate predictor of poor survival.

Conclusion: We found a high frequency of echocardiographic abnormalities in WG. These lesions seem to be directly related to WG and associated with increased mortality. Because cardiac involvement of WG can be clinically silent, associated with significant morbidity and portend worse prognosis, echocardiography screening in active WG may be of clinical value.

21-041

ENDOTHELIAL MICROPARTICLES: JUST BLOOD "DUST," OR A "MUST" FOR THE DIAGNOSIS AND MONITORING OF DISEASE ACTIVITY IN CHILDHOOD VASCULITIDES?

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Introduction: Microparticles (MPs) are released from endothelial cells in response to a variety of injurious stimuli and recently have been shown to be increased in multiple sclerosis and antiphospholipid syndrome.

Aims: This study examined endothelial and platelet MP profiles in children with systemic vasculitis (SV) to test the hypothesis that endothelial MPs may provide a tool for the diagnosis and monitoring of disease activity.

Patients: 12 children with active SV (9 with polyarteritis, 2 with Kawasaki disease, and 1 with hypersensitivity vasculitis); 8 children with inactive SV; 8 disease control children without SV; and a control group of 28 healthy subjects comprising 11 healthy children and 17 young adults were studied. Additionally, paired samples from 4 children with SV pre and post induction of remission were examined.

Methods: Plasma was centrifuged at 13,000G for 60 min-

utes, and the pellet resuspended and prepared for flow cytometry. MPs were defined as particles less than 2 microns in diameter and with surface binding of annexin-V. Fluorescent conjugated monoclonal antibodies to several endothelial markers and platelet markers were used to identify and quantify MPs.

Results: Plasma from patients with active vasculitis contained a 12.4-fold elevation of E-selectin positive endothelial MPs compared with patients in remission (p=0.001), a 5.7-fold elevation compared with controls (p=0.000) and a 7.9-fold elevation compared with disease controls (p=0.001). A similar result was obtained for MPs expressing the endothelial marker CD105. No difference was observed for MPs of platelet origin between the groups. 4/4 patients with active vasculitis demonstrated high levels of endothelial MPs which fell to normal following induction of remission (a 10.5-fold decrease for CD105 MPs and an 8.7-fold decrease for E-selectin MPs).

Conclusion: Endothelial MPs may provide a "window" to the activated endothelium, and these preliminary data suggest that they may be useful diagnostically and for the monitoring of disease activity in SV of childhood.

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Diagnostic strategies in vasculitis affecting the central nervous system

LEONARD H. CALABRESE, DO

Vasculitis affecting the central nervous system (CNS) represents a heterogeneous group of inflammatory diseases arising from a variety of neurologic insults including autoimmune diseases, infection, drug exposure, radiation, and malignancies. It can be broadly classified into two major categories, namely, primary, referring to those patients with vasculitis isolated primarily to the brain, cord, and their leptomeninges occurring in the absence of recognizable triggers or conditions, and secondary, referring to where such associated conditions or co-factors are apparent.

Unlike most other clinical areas of vasculitis, the diagnosis, treatment, and investigation of CNS syndromes is hampered by a variety of factors dissimilar to those encountered in vasculitis affecting many other end organs. These include the inability to readily obtain tissue for biopsy confirmation and the extremely limited amounts obtained even when successful biopsy is attempted. In addition, most diagnostic strategies center on non-invasive neuroimaging techniques of undefined sensitivity and specificity. Further complicating the management of this disease are difficulties surrounding assessment of disease activity. In CNS vasculitis, even when the diagnosis appears secure, the assessment of disease activity is complex and limited by the lack of dynamic change observed in CNS dysfunction due to ischemic injury and the uncertainties surrounding the clinical significance of serial neuroimaging investigations. Despite these limitations, progress has been made in the clinical approach to CNS angiitis, which will be summarized in this report.

DIAGNOSTIC MODALITIES

Test operating characteristics

The use and interpretation of any diagnostic test, ranging from findings obtained on physical examination to specific laboratory tests or radiologic investigations or even biopsy procedures are influenced by several factors. These include test sensitivity, specificity, and, most importantly, the clinician's estimate of the likelihood of disease at the time of testing, or pre-test probability. While a discussion of these variables is beyond the scope of this presentation, they have been the subject of several reviews.[1,2] Several summary points are important to keep in mind when discussing diagnostic strategies.

Test sensitivity is the operating characteristic most readily calculated and is expressed as the frequency of a positive test in the presence of a given disorder. Derivation of test sensitivity implies that a gold standard of diagnosis exists for a given disease and has been validated. Such gold standards are more readily available for certain forms of vasculitis, such as renal or pulmonary disease, which are more accessible to detailed and routine pathologic analysis than vasculitis of the CNS. In CNS vasculitis, clinicians are often forced to rely on indirect tests such as the cerebral angiogram, which is of poorly defined specificity.[3] In general, tests of high sensitivity are most valuable at ruling out the presence of disease. This has been referred to by the acronym SNOUT[1] (high sensitivity rules OUT the diagnosis).

Data on specificity is often more difficult to obtain and must be derived from analysis of test results on a well-characterized population of patients without the disease in question. Preferably, such nondiseased "controls" include patients with conditions that closely mimic the disease in question. For example, relevant data on specificity for ANCA testing come not from healthy subjects but rather from patient populations with relevant mimics such as chronic granulomatous diseases, diffuse pulmonary diseases, and other forms of glomerulonephritis.[4] In general, tests of high specificity are of their greatest diagnostic value in ruling in a given diagnosis and may be remembered by the SPIN rule[1] (ie, high specificity rules IN the diagnosis).

More than the mere awareness of test sensitivity and specificity, we clinicians are interested in how the results of a given test changes our minds, from what we thought the likelihood of a given disease was before we order the test (pre-test probability) to what we think afterward (post-test probability). Post-test probability can be calculated through a variety of techniques, all factoring in knowledge of sensitivity, specificity, and assessment of pre-test probability. Methods of calculation include the direct use of Bayes' theorem[2] or more conveniently through nor-

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mograms derived from Bayes' theorem.[1] The more recent popularization of likelihood ratios[1] has allowed us to utilize Bayes' theorem to accommodate multiple levels of probability for tests with other than bivariate results.

Each variation of Bayes' theorem starts with assessment of pre-test probability, which is derived from a synthesis of the clinician's findings (ie, clinical exam, history, available lab, awareness of prevalence and epidemiologic features of a given disease, and experience).[2] Pre-test probability can vary greatly depending on the skill and seasoning of the clinician, and thus some tests may be of far more value in the hands of certain clinicians.

After considering the operating characteristics of a given test and factors influencing pre-test probability, several additional questions must be answered before deciding on a given diagnostic strategy. These include:

Is the diagnostic test available, affordable, precise, and accurate in our hands?

Is the pre-test probability we have derived clinically sensible?

Will the post-test probability we arrive at influence our clinical decision-making and help our patient?

With these issues in mind, we will briefly consider major categories of diagnostic testing in CNS vasculitis.

Laboratory tests

There is no laboratory test of sufficient sensitivity or specificity to rule out or diagnose any form of primary or secondary CNS vasculitis. Since in most instances the diagnosis of CNS vasculitis hinges on a combination of either a positive biopsy or a high-probability vascular imaging study such as angiography while excluding all those conditions capable of mimicking findings, laboratory testing is largely relegated to detecting the myriad of mimicking conditions.[4] These, in general, include infections, malignancies, hypercoagulable and embolic states, and other inflammatory diseases. Lumbar puncture is an invariant part of the work-up for CNS vasculitis based on its value for ruling out infectious and malignant mimickers. Unfortunately, markers sensitive in other conditions such as the presence of elevated acute-phase reactants in systemic necrotizing vasculitis are frequently normal in CNS vasculitis, leaving the clinician unable to rule out CNS vasculitis with any single or combination of laboratory tests.

Neuroimaging

The development of progressively more-sophisticated neuroimaging techniques (ie, computerized tomography, magnetic resonance imaging, single-photon-emission tomography, etc.) have greatly improved our ability to diagnose unexplained CNS ischemic syndromes. While there are no such tests with specificity high enough to secure a diagnosis of CNS vasculitis, these modalities may be extremely useful when applied in stepwise fashion while keeping in mind their limitations. In general, MRI is more sensitive than CT and should be the initial study of choice when approaching a patient with unexplained ischemia except when cerebral hemorrhage is suspected. In terms of test sensitivity, the data vary depending upon the series examining the question and what is the gold standard utilized for the final diagnosis of CNS vasculitis. In the recent series of Pomper et al[6] where all patients were angiographically defined, the MRI had a sensitivity of 100%, whereas in the similar angiographically documented series of Hajj-Ali et al[7] the sensitivity was 77%. In our experience with biopsy-proven cases, the sensitivity of MRI approaches 100%. Findings on MRI examination are variable, but the most specific findings are found on serial examination where multiple foci of ischemia are detected in varying anatomic locations and distributed over time.[5,9] Both gray and white matter can be affected in supratentorial and infratentorial distributions. Modifications of MRI technology such as the inclusion of diffusion and FLAIR sequences will probably increase sensitivity but not specificity of the technique. Combining neuroimaging with lumbar puncture appears to increase the overall sensitivity, and thus a normal MRI and lumbar puncture have a high negative predicative value (ie, SNOUT) and should serve to rule out the disorder except in rare cases.

In patients with the granulomatous variant of primary angiitis of the CNS (PACNS), enhancement of the leptomeninges is occasionally observed and may serve to increase the sensitivity of biopsy. Less well appreciated is the fact that 15% of PACNS patients may present with mass-like lesions and thus should be approached as suspected infection or tumor.[5] More specialized studies such as SPECT and PET scanning may increase sensitivity but are in no way specific for the diseases and should not be used to secure the diagnosis.

Angiography

In the evaluation process of CNS vasculitis, cerebral angiography is both the most powerful and poorly understood diagnostic modality. Its power is derived from the fact that it is frequently and justifiably used as a gold standard for diagnosis. Its limitations derive from its lack of quantitative and qualitative codification and the fact that most clinicians and neuroradiologists fail to appreciate its low level of specificity. A comprehensive classification of cerebral arteritis from the angiographic perspective was published nearly three decades ago by Ferris and Levine,[8] and little has been added in terms of furthering the technique's diagnostic accuracy beyond these qualitative descriptions or patterns that neuroradiologists apply to their reading. More recent reviews[5,7,9] continue to emphasize patterns characteristic of vasculitis, including alternating areas of stenosis and ectasia (ie, beading). These types of changes may be seen in multiple vessels in multiple vascular beds but also may be limited to a single vessel. Other angiographic abnormalities described in CNS vasculitis include absence or cut off of one or more vessels and may be entirely normal in up to 40% of biopsy-proven cases.[10]

Our informal assessment and experience with this technique suggest that, in terms of diagnostic specificity, the highest level is associated with beading in multiple vessels in multiple vascular beds (ie, high probability), whereas similar findings in a single vessel or bed is intermediate in terms of specificity. All other findings includ-

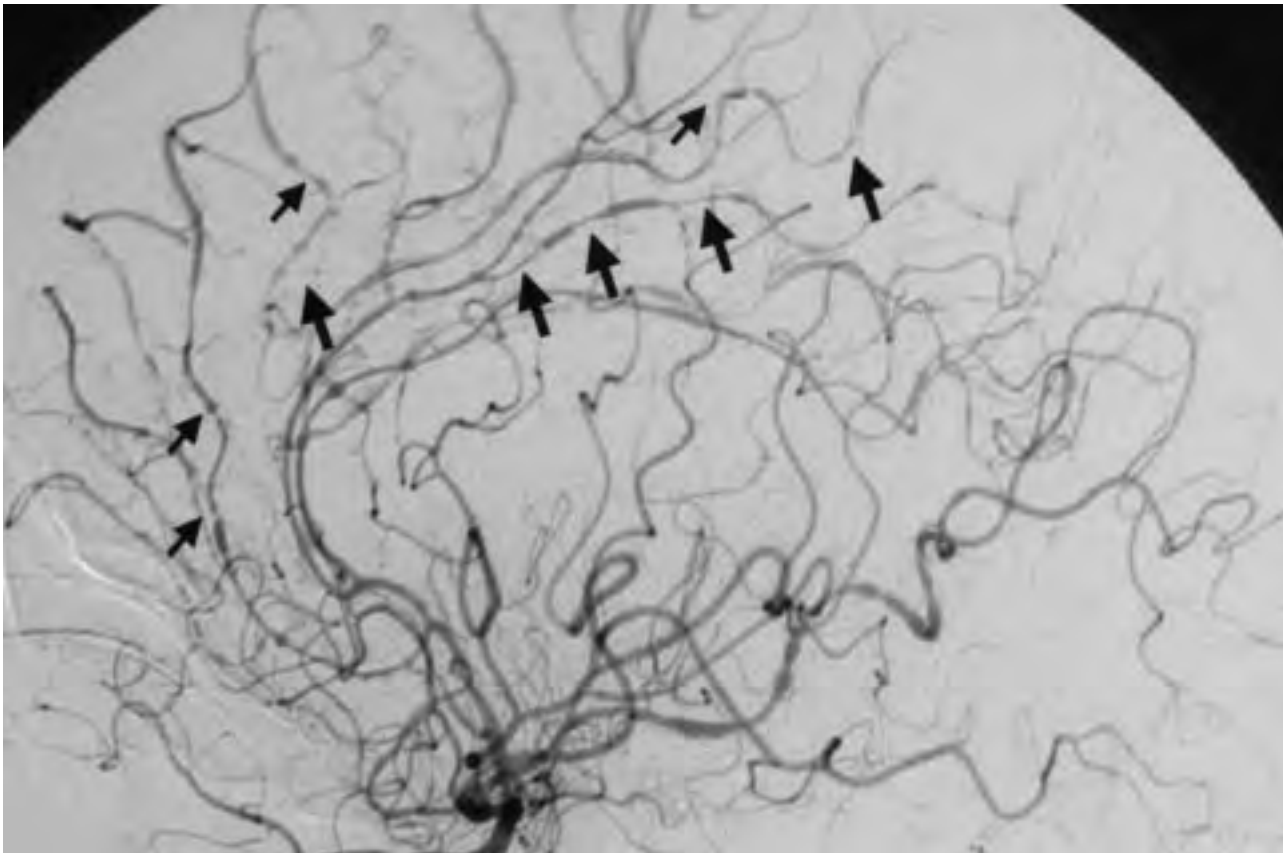

Figure 1. Typical angiographic findings in a patient with biopsy-proven CNS vasculitis. Arrows point to areas of alternating stenosis and ectasia.

ing simple vessel cut off, luminal irregularity in a single vessel or bed (ie, nonbeaded), or an entirely normal angiogram would be low probability. Even when using these conventions, Duna and Calabrese[3] found that the specificity of a high-probability angiogram in a small series of patients with suspected CNS vasculitis was only 26%. With such low specificity, the cerebral angiogram can only secure the diagnosis of CNS vasculitis when the pretest probability is extremely high, implying that all appropriate exclusions[5] have been ruled out and there is a compatible clinical picture.

Lastly, although invasive it has been demonstrated that cerebral angiography is a safe technique[11] and can be performed serially to follow disease activity when necessary.[6,12]

Biopsy

Biopsy of CNS tissues would logically be considered the ultimate gold standard of diagnosis, but clearly the procedure is limited by several factors. First, while data from other conditions have demonstrated that the procedure can be done with minimal morbidity and mortality,[5] it is highly invasive and carries certain risks. Successful biopsy also requires a willing and experienced neurosurgeon, who may not always be available. When the procedure is performed, the technical aspects must be tailored to the individual patient. For example, in patients with suspected granulomatous angiitis of the CNS, the procedure of choice is open wedge biopsy of the tip of the nondominant temporal lobe with sampling of the overlying leptomeninges.[13] Alternatively, directing the biopsy to an area of leptomeningeal enhancement when present may serve to increase the sensitivity. Even when all the technical limitations have been factored in, CNS vasculitis is notoriously a patchy disease, with data from previous reviews suggesting as many as 25% of biopsies may be falsely negative.[10] Finally, even when vasculitis is seen in sampled tissues, it is imperative to perform special stains and cultures for occult infections that may produce secondary vascular inflammation.

In a recent series[14] of 30 consecutive biopsies for suspected CNS vasculitis performed at a single institution, the false-negative rate of biopsy was 16%, yielding a sensitivity of about 84%, which, we believe, is reasonable. When comparing biopsy to other diagnostic modalities such as MRI and angiography, the predictive value of brain biopsy in this study was 90-100%, versus 37-50% for angiography and 43-72% for MRI. These authors concluded that wedge biopsy of cortical and leptomeningeal tissues is central to the multidisciplinary approach to patients with suspected CNS vasculitis.

Pitfalls

Clearly the greatest pitfall in the diagnosis of CNS angiitis is overreliance on neuroradiography, especially

angiography, without performing the necessary and extensive exclusions of mimicking conditions. Failure to biopsy because of concerns of how to handle regarding false negatives is also a pitfall because it does not consider a) the profound morbidity of long-term high-dose immunosuppression given empirically, and b) the ability of biopsy to detect conditions other than CNS vasculitis that have radically different treatments. Finally, it must be appreciated that no single specialist has the necessary expertise to evaluate and treat all of the potential disorders that may present as suspected CNS vasculitis, and thus a team approach is essential. This team should consist of a physician knowledgeable in the diagnosis and treatment of vasculitis including the use of immunosuppressives, a neurologist with special expertise in cerebrovascular disorders other than vasculitis, a capable neuroradiologist who knows the limitations of his procedures, a neurosurgeon willing to tailor a biopsy for a given patient, and a knowledgeable neuropathologist.

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22-082

CIRCULATING ENDOTHELIAL CELLS AND VASCULITIS

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Objective: To demonstrate the presence of circulating endothelial cells in ANCA-associated small-vessel vasculitis, characterize their phenotype and relate the number of circulating endothelial cells to disease activity measured with the Birmingham Vasculitis Activity Score (BVAS).

Methods: 18 patients with active ANCA-associated vasculitis, 20 patients in remission, 20 healthy controls and 12 patients with non-ANCA glomerular disease were studied. Endothelial cells were isolated from blood with anti-CD-146 coated Dynabeads™. von Willebrand factor (vWF), CD 31 and UEA-1 staining were performed concurrently; tissue factor immunocytochemistry and assays for markers of apoptosis and necrosis were also carried out.

Results: Few circulating endothelial cells were seen in healthy controls (0-20, median 6 cells/ml) and patients with non-ANCA glomerulonephritis (0-21, median 4 cells/ml). Large numbers of circulating endothelial cells were detected in patients with active disease (20-5700 cells/ml, median 136 cells/ml, $p < 0.0001$ compared to healthy controls). Cell numbers fell considerably during successful immunosuppressive treatment. Patients in remission had moderately elevated cell numbers (0-52, median 16 cells/ml). There was a significant correlation between cell numbers and BVAS when all patients with quiescent and active vasculitis were included (Spearman rank correlation, $R=0.68$, 95% confidence interval 0.54-0.78, $p < 0.0001$, $n=78$). The vast majority of cells stained annexin/propidium iodide and tissue factor positive, indicating a necrotic and procoagulant phenotype.

Interpretation: The number of endothelial cells in peripheral blood is a novel marker of ANCA-associated small-vessel vasculitis; moreover, it is conceivable that these necrotic cells elicit an inflammatory response in their own right. Hence, our findings not only yield a new diagnostic tool but may also have considerable pathogenetic importance.

23-051

DIFFUSE ENDOTHELIAL DYSFUNCTION IS A COMMON FEATURE OF SNV

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Systemic vasculitis (SNV) centers around active inflammation in the wall of blood vessels, but the role of the lining endothelial cells has received little attention in assessment of the longer-term outcome. Our preliminary finding of endothelial cell dysfunction (ECD) in SNV (Raza K et al, *Circulation* 2000; 102:1470-1472) raised several questions about its significance, which we address here using two well-established tests of EC-dependent vasodilatation in a series of studies. Brachial artery (BA) function was tested by flow-mediated responses whilst skin microcirculation was assessed by iontophoresis of ACh, with appropriate positive controls for smooth muscle responses to nitrate, in a total of 58 patients.

(1) The occurrence of ECD at the BA was confirmed in 54 SNV patients ($p<0.001$ compared to matched controls). (2) This larger series allowed us to ask whether ECD was syndrome specific. In fact, significant ECD was found in ANCA-related small-vessel vasculitis (WG, MPA, and CSS) as well as cPAN affecting medium arteries. This implies that EC dysfunction in SNV is not restricted to vessels involved in the site of active inflammation. (3) To confirm this, we assessed microcirculation responses in clinically uninvolved skin. These were also significantly depressed in 36 cases of SNV ($p<0.0002$ compared to controls) and again involved both WG and cPAN. (4) To examine the mechanism we first focused on ANCA, which are involved in EC activation and injury. However, ECD did not correlate with either ANCA status or the diagnosis of an ANCA-related vasculitis. Renal involvement was not a significant correlate either. (5) Finally, we asked whether this diffuse ECD represented fixed damage by examining the effects of active therapy. TNF blockade with infliximab in four cases produced significant early improvement, maximum at 48 hours but reverting to baseline by 2 weeks. By contrast, steroid/cyclo pulses did not induce significant short-term changes but were associated with major normalization of EC function over 4/12.

These studies established that diffuse endothelial dysfunction occurs commonly in SNV but is potentially reversible, with important implications for future therapy. The relationship to clinical relapse and the responses to other therapy are under study. Finally, since similar ECD is seen in atherosclerosis, these studies predict an increased risk of cardiovascular events as a late sequel to SNV.

Conventional treatment and outcome of Wegener's granulomatosis and microscopic polyangiitis

DAVID R.W. JAYNE, MD

The systemic vasculitides are usually fatal if untreated, and immunosuppressive therapy now saves lives and salvages organ function. Combination immunosuppressive therapy has changed the outcome of vasculitis to that of a chronic disorder with accumulating morbidity and incapacity; however, current treatments are toxic and contribute to morbidity and mortality. Optimization of therapy and the introduction of new treatments has been facilitated by the antineutrophil cytoplasmic autoantibody (ANCA) test, by consensus statements on classification, by improved understanding of pathogenesis, and by the construction of collaborative trial networks.

CLASSIFICATION

Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are primary systemic vasculitides predominantly affecting small blood vessels. Immune deposits are scanty or absent and circulating ANCA are usually present at diagnosis; ANCA may be negative in localized WG or after therapy. The strong similarities, particularly in renal involvement and ANCA status, between WG and MPA have prompted their collective grouping as ANCA-associated vasculitis (AASV). Renal vasculitis, or idiopathic rapidly progressive glomerulonephritis, has been regarded as an organ-limited form of AASV. Up to 50% of patients with Churg-Strauss angiitis are ANCA-positive, and their clinical phenotype often overlaps with that of WG or MPA. Polyarteritis nodosa is distinct from AASV although cases with involvement of both medium-sized muscular arteries and microscopic vessels are now classified as MPA. Despite agreed, consensus definitions of these vasculitic syndromes, diagnostic criteria have not been unified and vary between clinical trials.[1]

The European Vasculitis Study Group (EUVAS) has sub-classified AASV at presentation with the purpose of designing appropriate therapeutic protocols according to disease severity **(Table 1)**.[2] Further sub-classification, such as by age or ANCA specificity, is likely to be of importance to the treatment of vasculitis in the future.

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APPROACHES TO TREATMENT

The natural history of systemic vasculitis includes acute flares, which can be fulminant; treatment-induced remission without clinical evidence of vasculitis; partial remission with grumbling disease activity; and relapse that can be major, involving vital organs, or minor. In response to these patterns, treatment aims to induce rapid remission with high drug doses at the expense of short-term toxicity, then taper the drug doses or switch to safer agents once remission is achieved and maintain low dose therapy for a prolonged period to prevent relapse. Monitoring of vasculitic activity and the prediction of relapse is of importance during this remission phase. Those with only a partial response to induction therapy are at particular risk from ongoing vasculitis and the dangers of a high cumulative treatment exposure.

REMISSION INDUCTION

Localized disease

WG affecting the upper or lower respiratory tract alone without constitutional disturbance has been treated with prednisolone alone or with the antibiotic combination, sulfamethoxazole/trimethoprim.

Early systemic disease

This subgroup comprises localized WG with constitutional disturbance or WG or MPA which is multi-focal but without threatened organ function.[3] Cyclophos-phamide and steroids has been standard therapy, but several uncontrolled studies have reported disease remission in 60-70% with methotrexate and steroids **(Table 2)**.[4-8] Inability to reduce the steroid dose and relapsing disease have been predictive of more widespread vasculitis after methotrexate therapy.[8] Adverse effects related to methotrexate included pneumonitis, which is difficult to differentiate from pulmonary vasculitis, hepatotoxicity, and myelosuppression, but these events were reversible. The control of early renal

TABLE 1
CLINICAL SUBGROUPING ACCORDING TO DISEASE SEVERITY AT PRESENTATION FOR ANCA-ASSOCIATED VASCULITIS[3]

Clinical subgroup	Constitutional symptoms	Typical ANCA status*	Threatened vital organ function	Serum creatinine (μmol/L)	EUVAS trial[3]
Localized	No	Negative	No	<120	
Early systemic	Yes	Positive or negative	No	<120	NORAM
Generalized	Yes	Positive	Yes	<500	CYCAZAREM
Severe	Yes	Positive	Organ failure	>500 if renal; hypoxia if pulmonary	MEPEX
Refractory†	Yes	Positive or negative	Yes	any	SOLUTION

*ANCA is negative in a minority of generalized and severe renal presentations. †Refractory disease implies progressive disease despite at least six weeks treatment with an appropriate regimen; ANCA may become negative with treatment.

TABLE 2
THERAPEUTIC TRIALS OF METHOTREXATE FOR WEGENER'S GRANULOMATOSIS

Study	Number	Follow-up (months)	Design	Remission rate	Relapse rate	Adverse effects
Sneller 1995[6]	41	?	Prospective open	71%	34%	21, 2 deaths (infective)
de Groot 1996[7]	33	18	Prospective open	-	12%	16 in 12 patients
de Groot 1998[4]	17	25	Prospective open	59% partial 35% full	20%	2
Stone 1999[8]	19		Prospective open	89% partial 79% full	50%	2
Langford 2000[5]	42	76	Prospective open	20/21 with renal involvement	10% renal progression	

vasculitis, hematuria with normal or modest creatinine elevation, with methotrexate is more controversial. Two studies have reported stabilization of excretory function; others have found renal vasculitis to predict refractory, progressive disease after methotrexate.[5,7,8] The NORAM trial is comparing oral methotrexate (20-25 mg/week) to oral cyclophosphamide (2 mg/kg/day), both with the same steroid regimen, for this subgroup and includes patients with MPA.[3]

Generalized/renal disease

The empirical introduction of daily oral cyclophosphamide became popular during the 1970s but was only recently subjected to randomized trial.[9,10] Retrospective data from the North Carolina glomerulonephritis study group compared patients with MPA treated with cyclophosphamide to those treated with steroids alone and found improved renal survival and a lower relapse rate in those receiving cyclophosphamide.[11] Recent studies have aimed to reduce cyclophosphamide exposure by using pulse rather than continuous administration or by switching to an alternative drug once remission has been obtained.

Two open, prospective studies of pulse intravenous cyclophosphamide investigated whether this form of administration might be superior to daily oral administration for induction of sustained remission.[12,13] It did not appear more successful in this setting; those with more extensive organ involvement and high ANCA titers had a poor therapeutic response.[13] In contrast, continuing disease activity despite pulsed, intravenous cyclophosphamide has responded to conversion to a daily oral regimen.[14] Four randomized trials have investigated whether pulsed cyclophosphamide is safer and as effective as daily oral administration for the induction of remission.[15-18] None were sufficiently powered to make any conclusions about efficacy in controlling vasculitis, although one study clearly showed a higher relapse rate after intravenous pulse use.[16] All of the studies concluded that adverse effects were more frequent with daily oral cyclophosphamide although this was only the primary end-point in the study by Adu.[15] The studies by Guillevin and Haubitz were both stopped early due to more adverse effects in the daily oral arms.[16,17] The high number of adverse events has been associated with the steroid dose used in these trials and with the protocols for tapering cyclophosphamide.[19] A meta-analysis has summarized results from these trials **(Table 3)**.[20] The CYCLOPS study is currently comparing the efficacy of daily oral to pulsed cyclophosphamide for renal vasculitis in 160 patients.[2]

Severe renal disease

The delayed diagnosis of renal vasculitis increases the risk of the development of renal failure by the time of presentation. Progression to end-stage renal failure is not in-

evitable, and recovery of renal function is possible in many. The addition of pulsed methylprednisolone or plasma exchange or both to cyclophosphamide and oral prednisolone has been advocated to increase the chances of renal recovery. A pooled analysis of existing data suggests that plasma exchange may be superior in this regard but numbers are small and inclusion criteria and immunosuppressive regimens varied **(Table 4)**.

The MEPEX trial is comparing the rates of renal recovery for those with an initial creatinine over 500 μmol/l (6 mg/dl) between the addition of 3 g of intravenous methylprednisolone and seven plasma exchanges, in addition to daily oral cyclophosphamide and prednisolone.[3] Plasma exchange aims to deplete circulating pathogenic autoantibodies; other effects, such as the removal of cytokines, complement, and coagulation factors, and less well-defined immunoregulatory phenomena may also contribute to its therapeutic effects. In a randomized study of 32 patients with Wegener's granulomatosis of varying severity, plasma exchange improved outcome.[21]

Plasma exchange dosing remains empirical; factors likely to be of importance which merit further study include persisting ANCA positivity, ongoing inflammation reflected by C-reactive protein, urinary sediment and repeat renal biopsy. Renal function at presentation remains the strongest predictive factor for renal outcome; however, active lesions on biopsy confer a superior and chronic lesions an inferior prognosis.[22,23] A subgroup of renal vasculitis patients present with dual positivity for ANCA and autoantibodies to the glomerular basement membrane (GBM) with linear IgG deposition in the glomeruli on biopsy. In this setting, renal disease is more severe and renal recovery less common, and concomitant pulmonary hemorrhage is more frequent than presentation with ANCA alone. Prospective therapeutic studies have not been performed although the presence of anti-GBM has argued for the use of plasma exchange. ANCA-associated vasculitis is the most frequent cause of diffuse pulmonary hemorrhage, usually in the context of the pulmonary renal syndrome, and this presentation carries a high mortality.[24,25] No prospective therapeutic studies are available although both plasma exchange and methylprednisolone have been used in retrospective reports.[24,26]

■ REMISSION MAINTENANCE

Over 50% of patients will have a relapse of vasculitis, and this possibility has a major influence on their long-term care.[27] The efficacy of azathioprine for remission maintenance in vasculitis has been previously reported, with relapse rates of 11-30%; smaller studies have used azathioprine in remission induction protocols.[15,28-30] The CYCAZAREM trial compared azathioprine to continued cyclophosphamide for prevention of relapse, following induction of remission with oral cyclophosphamide and steroids for three to six months.[31] There was no difference in relapse rate, 16%, up to the end of the study, 18 months from treatment onset, and there was a trend to fewer serious adverse events in the azathioprine arm.[31] A surprising result of this study was the high remission rate with oral cyclophosphamide and prednisolone in this subgroup. Apart from the withdrawal of ten patients prior to the start of the remission phase, largely due to death or treatment intolerance, all patients entered clinical remission. Thus the 'standard' induction treatment with oral steroids and cyclophosphamide appears effective, but toxicity was high, with over 160 adverse events reported and a serious or life-threatening adverse-event rate of 26%. Reversible leukopenia was the most common adverse effect; azathioprine hypersensitivity was reported in 7% and has features similar to a relapse of vasculitis, which has caused difficulties in diagnosis.[32,33]

The optimal duration of maintenance therapy is undetermined. For those presenting with renal vasculitis, a current trial (REMAIN) is comparing two years to four years of azathioprine and prednisolone.[2] Other factors of potential importance to the duration of remission therapy are persistent ANCA positivity, a history of relapsing disease and the severity of presenting disease. Alternative remission-maintaining drugs include methotrexate (see above), cyclosporin, mycophenolate mofetil and leflunomide.[34, 35]

■ RELAPSE

The diagnosis of relapse requires a similar approach to investigation of the initial presentation, but clinical features may be modified by concurrent immunosuppression, and there is a complex relation between infection and relapse. Vasculitis may occur as a consequence of infection, such as endocarditis, and intercurrent infection can provoke relapse of primary vasculitis.[36-40] Alternatively, infection may be a consequence of immunosuppressive treatment or secondary to structural damage to an epithelial surface caused by previous vasculitis, where it may mimic relapse.[41] Colonization of the upper respiratory tract by *Staphylococcus aureus* in Wegener's granulomatosis increases the risk of disease relapse, and this observation has drawn attention to the possibility of an infectious etiology for this vasculitis, first suggested by Wegener.[39,42] Long-term antibiotic therapy with sulfamethoxazole/trimethoprim in Wegener's granulomatosis reduced the risk of respiratory tract relapse in a placebo-controlled study when added to conventional immunosuppression.[43] The treatment duration was two years, and the difference in relapse frequency was largest in the first six months; a high rate of drug intolerance was observed. Sulfamethoxazole/trimethoprim was less effective in controlling disease activity in Wegener's granulomatosis when used in place of immunosuppression at various stages of disease in a prospective study of 72 patients.[44] An association between ANCA titer and relapse exists but the strength of this association and the role that changes in ANCA should play in dictating treatment remain controversial.[27,45] PR3-ANCA positivity at diagnosis appears to be associated with a higher risk of relapse when compared to MPO-ANCA.[46] An early study in Wegener's granulomatosis randomized patients in remission to treatment intensification or no change in therapy on the basis of a rise in ANCA titer.[47] Subsequent relapse was frequent in the latter group and was not seen in the group treated on the basis of the ANCA titer; the cumulative exposure to immunosuppression was lower in the treatment intensifica-

TABLE 3
CRITICAL ANALYSIS OF TRIALS COMPARING 'PULSE' TO DAILY ORAL CYCLOPHOSPHAMIDE[20]

	Daily oral	Pulse	Comparison
Remission rate	77%	93%	Odds ratio 0.3
Relapse rate	29%	42%	Odds ratio 2.2
Infection	58%	39%	Odds ratio 0.24
Death	22%	20%	No difference
End-stage renal failure	15%	17%	No difference
Cyclophosphamide dose	34 g	17 g	$P < 0.001$

TABLE 4
RENAL SURVIVAL IN PROSPECTIVE TRIALS INCLUDING PATIENTS PRESENTING WITH RENAL FAILURE DUE TO RENAL VASCULITIS. TREATMENT WITH OR WITHOUT PLASMA EXCHANGE

	No. pts	Plasma exchange	No plasma exchange
Glockner[65]	12	5/8	3/4
Pusey[66]	19	10/11	3/8
Cole[67]	11	3/4	2/7
Levy[68]	20	9/11	5/9
Guillevin[16]	8	4/6	1/2
Haubitz[17]	22	6/12	2/10
(Jayne)†	26	9/16	4/10
Total	88	46/68 (67%)*	20/50 (40%)

*$P < 0.05$; †(Jayne) refers to unpublished data

tion group.[47] Other studies have consistently reported a high frequency of ANCA positivity at the time of relapse, and an increased relapse risk in those with persistent ANCA positivity during remission or in those whose ANCA becomes positive during remission.[28,29,48] While further interventional studies based on ANCA specificity and persistence are anticipated, a common response to the increased risk of relapse is to reduce the period between clinic reviews in order to diagnose relapse as early as possible.

■ ADVERSE EFFECTS

The toxicity of treatment contributes to the chronic morbidity and mortality of vasculitis. The National Institutes of Health experience with Wegener's granulomatosis reported a contribution of treatment toxicity to permanent damage in over 50% of their patients.[49] The CYCAZAREM trial revealed an adverse-effect frequency of 1.1 episode per patient with 26% having severe or life-threatening adverse effects within the first 18 months.[31] Infectious adverse effects are the most common cause of death or severe morbidity and their frequency is associated with age and concomitant steroid dosage.[50] *Pneumocystis carinii* pneumonia rates of up to 20% have been found, which have prompted advice for routine prophylaxis with low-dose sulfamethoxazole/trimethoprim in centers where this infection is common.[3,16,51] Urothelial toxicity of cyclophosphamide metabolites is known to cause cystitis and bladder cancer. In the largest cohort to be studied to date, 73/145 developed non-glomerular hematuria and seven (5%) bladder cancer.[52] These patients were collected over a time period when prolonged daily oral cyclophosphamide was standard therapy. The frequency of hematuria was related to the duration or total dose of cyclophosphamide with a 50% rate after 40 months or 120 g.[52] None of the 72 patients without hematuria developed bladder cancer. Of particular concern is the rise in bladder cancer risk with longer follow-up, which was estimated in this study to be 5% at 10 years and 16% at fifteen years.[52] Hemorrhagic cystitis is rare in pulse cyclophosphamide-treated patients, being reported in only one case from the reviewed studies. A Swedish study found an 11-fold increase in bladder cancer rates in patients receiving oral cyclophosphamide for more than one year, and an increase in dermatological malignancy related to azathioprine and steroid exposure.[53] Gonadal failure is associated with the total cyclophosphamide dose and is therefore likely to be more frequent in daily oral regimens. This toxicity has been assessed in Lewis rats when comparable oral regimens led to significantly greater changes in testis histology and reduced conception rates.[54] The human corollary was reflected in male FSH levels that were higher with oral regimens, indicating greater gonadal suppression.[54]

A high incidence of steroid-induced bone disease has been an inevitable consequence of the prolonged exposure to high-dose steroids.[49] Steroid-induced bone disease is common due to the high cumulative exposure and age of the patient population. No protective effect with salmon calcitonin was found in a relatively small randomized trial of steroid-induced bone disease.[55] Patients with chronic inflammatory disease have increased incidence of cardiovascular disease, which is likely to be further exacerbated by steroids, due to effects on blood pressure, glucose and lipid metabolism and possibly other mechanisms.[56] This problem remains to be quantified in vasculitis.

■ OUTCOME

The outcome of vasculitis has been assessed by death, development of end-stage renal failure, disease relapse and the acquisition of irreversible damage.[57] In a retrospective review of 55 cases of Wegener's granulomatosis, Walton reported a mortality of 80%, largely due to renal failure.[58] Leib found a reduction in five-year mortality to 50% with steroids in polyarteritis nodosa and a further reduction with the use of cytotoxics to 12%.[30] Retrospective studies from single centers have reported survival rates with immunosuppressive therapy varying between 75% at 12 months to 87% at eight years, with heterogeneity in disease presentations and therapeutic protocols accounting for much of the difference.[11,49] A large recent review of 155 WG patients highlighted age, renal and pulmonary

involvement as adverse prognostic factors, and the value of a treatment approach adapted to the stage and extent of disease.[59] A retrospective audit of 246 patients with AASV and renal involvement diagnosed in London, UK, between 1995 and 2000 found a standardized mortality rate of 242%. Survival was significantly worse in those presenting with a creatinine over 200 μmol/l, aged over 60, with renal limited vasculitis or those who were dialysis dependent. These results reflect single center experience from the 1990s which reported two-year survivals of 80%, with 20% progressing to end-stage renal failure.[22,53,60-62] Age and creatinine at presentation have also been consistently associated with survival.[22,50,53] The London audit found an end-stage renal failure rate of 27% by two years. Thus, at present, over 40% have a poor outcome by these two simple definitions. Survival in the three initial EUVAS trials has reflected severity of disease (NORAM, 98%; CYCAZAREM, 93%; and MEPEX, 78%).

An inclusive score to record accumulating 'all-cause' damage, the vasculitis damage index (VDI) provides a semi-objective score of incapacity.[63] Vasculitis is the major contributor to VDI in the first six months from onset of disease; subsequently, treatment toxicities, such as diabetes, fractures and infertility, become more important.[57] In the CYCAZAREM study, by 18 months patients had aquired a mean of at least two items of permanent damage. Patient function, as assessed by the generic short-form 36 questionnaire, revealed low perceptions of general health and vitality during remission despite normalization of disease activity scores, with direct consequences on physical and social activity.[31,64] It is unclear whether these continuing symptoms result from subclinical disease activity, ongoing treatment toxicity or the effects of irreversible damage.

CONCLUSIONS

Agreement of diagnostic terminology and the introduction of ANCA testing have stimulated clinical and therapeutic research in WG and MPA. Prospective outcome data from multi-center trials is emerging of direct value in clinical decision making. Current outcomes of vasculitis show a high mortality and renal failure rate, especially in the elderly and in those with renal involvement. Diagnostic delay has a major impact on outcome, and strategies to encourage early suspicion of vasculitis should be encouraged. Survivors suffer relapsing disease and chronic morbidity due to vasculitic damage and the long-term consequences of immunosuppression. Such outcomes provide considerable scope for improvements in therapy in the future.

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RANDOMIZED TRIAL OF CYCLOPHOSPHAMIDE VERSUS METHOTREXATE FOR INDUCTION OF REMISSION IN "NON-RENAL" ANCA-ASSOCIATED VASCULITIS

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Background: This trial (NORAM) aimed to determine whether methotrexate (MTX) was as effective as cyclophosphamide (CYC) for the induction of remission in systemic ANCA-associated vasculitis (AASV: Wegener's granulomatosis and microscopic polyangiitis) without significant impairment of renal function.

Patients and Methods: Patients with newly diagnosed systemic AASV in the absence of life- or organ-threatening disease manifestations and near normal renal function (serum creatinine < 1.5 mg/dl) were included. They were randomized to either oral CYC, 2 mg/kg/day, or oral MTX, 15-25 mg/week. Both limbs received the same concomitant tapering steroid regimen starting at 1 mg/kg/day prednisolone equivalent. All drugs were tapered and withdrawn by 12 months and follow-up continued to 18 months from entry.

Results: Preliminary results are now available: 100 patients from 26 centers in 10 European countries were recruited over 63 months. Fifty-one were randomized to MTX, 49 to CYC. At randomization there was no significant difference in mean age, sex distribution, serum creatinine and BVAS between the limbs. Remission rates at 3 months were 59% and 65%, at 6 months 83% and 84% for MTX and CYC, respectively. Relapse rates and median time to relapse from trial entry were 69% and 13.5 months for MTX and 42% and 15 months for CYC.

Conclusion: MTX and prednisolone achieved a similar remission rate to the standard oral CYC/prednisolone regimen. However, this preliminary data indicates a higher relapse rate in the MTX limb. A final conclusion will require analysis of adverse effect rates and scores for damage and quality of life between the two limbs. This data will shortly become available. Prolonged immunosuppression, beyond the first year of treatment, is probably necessary to reduce relapse.

Treatment of giant-cell arteritis: where we have been and why we must move on

GARY S. HOFFMAN, MD

In 1890, Hutchinson[1] provided an original description of painful inflammation of the temporal vessels. Following this case report, medical insights about temporal arteritis were slow to follow. It was not until 1932 that Horton, Magath, and Brown,[2] at the Mayo Clinic, noted in 2 patients that temporal arteritis was a component of a systemic disease. Biopsy proof of inflammation was presented and distinguished from "periarteritis nodosum."[2,3] The impact of blindness was first realized in 1946,[4] and the first observations regarding efficacy of corticosteroid (CS) therapy were noted in 1950 at the Mayo Clinic by Shick et al.[5] These investigators were the first to demonstrate decrease in blindness in the CS era and even reversal of visual abnormalities in some patients treated shortly after onset of symptoms.[6] How far have we come since those reports?

Since these seminal events, descriptive studies have made physicians keenly aware of classical and even unusual manifestations of giant-cell arteritis (GCA). Descriptions of illness in the elderly emphasize the implications of new-onset atypical or severe headaches, regional jaw and oral pain, or visual symptoms, especially if such symptoms are associated with proximal aching or constitutional abnormalities **(Table 1)**.

When combinations of these features are due to GCA, the Westergren erythrocyte sedimentation rate is elevated in over 90-95% of patients,[7-9] and temporal-artery biopsies reveal a lymphomonocytic or granulomatous infiltrate in at least 50% of temporal-artery biopsies.

Some presentations and disease profiles are very unusual. For example, the histopathologic finding of apparently sterile granulomatous vasculitis with giant cells in a resected aneurysm of the aortic root often leads surgeons to consult medical colleagues. In at least 75% of patients with such a presentation, there is no concurrent evidence of a systemic illness, headaches, or other clues that would support the diagnosis of classical GCA. In fact, such patients may not be elderly. Some have required surgical intervention for severe aortic regurgitation during the fourth decade of life.[10] Do these patients represent "outliers" for classical GCA? We recognize that this type of aortic abnormality may complicate the course of classical GCA in at least 15-20% of patients. However, is it possible that some of these patients have a distinctly different disease from GCA if they are not systemically ill, do not have headaches, visual symptoms, and other classical features of that illness? Should they be treated with CS medications? The answers to these questions are not always clear. However, it is recognized that within this group exist individuals who never received CS or other immunosuppressive therapies, and who have not subsequently developed additional similar vascular events or overt GCA during follow-up periods as long as 12 years.[10] Similar cases of aortitis, with or without giant cells, have been identified in <1-10% of postmortem series. In almost all cases, retrospective review of medical records failed to identify features of GCA or other systemic rheumatologic illnesses.[11,12] Because there is considerable doubt about the value of classifying such patients together with those having typical GCA, they will not be included in subsequent discussion of treatment strategies and outcomes for that disorder.

MEDICAL THERAPY OF GCA OF THE ELDERLY

Authorities agree that once a convincing diagnosis of GCA is assumed, treatment with CS should begin immediately. This sense of urgency is conveyed because of the knowledge that in the pre-CS era, GCA could be complicated by blindness in up to 30 to 60% of cases.[6] In fact, irreversible loss of vision may be a presenting feature in as many as 18% of cases in more current large series.[9,13-17] Risk of blindness increases further among patients with a recent history of amaurosis, unilateral blindness, or stroke.[9]

Prednisone is the most popular form of CS therapy employed. How much prednisone should be used initially? How long should the initial dose be maintained before it is tapered? How long should one expect to treat a patient with GCA? The answers to such questions are as numerous as authorities who have studied GCA. **Table 2** provides a summary of some recommendations. Comparative studies have not been performed that would clearly recommend any one approach above others.

In synthesizing a treatment plan from this literature,

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TABLE 1
GIANT-CELL ARTERITIS: CLINICAL FEATURES (FREQUENCY %)

Author (#cases)	Hunder (94)	Liozan (147)	González-Gay (239)	Chevalet (164)	*Hoffman (98)
Headache	77	NS	83	67	93
Abnormal temporal artery	53	55	72	21	NS
Jaw claudication/pain	51	38	39	16	60
Constitutional symptoms	48	65	70	NS	NS
Polymyalgia rheumatica	34	27	47	49	55
Fever	27	NS	11	46	5
Diplopia	12	NS	7	NS	NS
Amaurosis	5	NS	17	NS	NS
Blindness	13	13	14	NS	18
Stroke	NS	NS	3.5	NS	0
Mean age (yrs)	75	75	73	73	74
Percent female	74	63	56	71	71

* At presentation in this cohort. NS = Not stated.

one may be guided by comorbidities and other risk factors that influence an individual's prognosis or risk of toxicity. For example, in the setting of only headache, polymyalgia rheumatica, and constitutional symptoms, it would appear reasonable to use lower starting doses (eg, 30-40 mg/day) of prednisone, especially if the patient has diabetes, severe atherosclerosis, osteoporosis, or congestive heart failure. However, even if these comorbidities exist, in the setting of threatened or recent (24 to 48 hours) visual loss, higher doses of CS should be employed. Delay in initiation of CS therapy after onset of premonitory visual symptoms has been associated with poor visual outcomes.[15] Treatment may be effective in reversing or halting further visual loss if it is provided in the acute setting.[9,14,15] González-Gay et al[14] noted visual improvement in 7/12 patients who were treated for new-onset ocular symptoms within 24 hours, compared to in 1/17 in whom treatment was delayed more than 24 hours. No patient had improvement if treatment was provided >2 days after visual loss. Patients with visual loss also had an increased risk of stroke.

There is general agreement that once CS therapy has been started, the likelihood of subsequent visual loss is dramatically reduced.[6,13,14] However, one recent study that utilized an aggressive CS tapering protocol noted a prevalence of visual loss in 18% of patients at study enrollment and in an additional 13.8% at 1-year follow-up.[17]

How long to treat?

Whereas some early reports of GCA suggested that treatment may only be necessary for 6 to 12 months, in 1973 Beevers et al[18] recognized the chronic nature of this illness and noted that in many cases CS therapy may be required for several years. Indeed, this is now a widely accepted perception **(Table 2)**. Relapse rates in the course of CS tapering have been reportedly ~30->80% over 1 to 4 years of follow-up.[16-23] No doubt this broad range reflects differences between treatment protocols, definitions of relapse, and possibly even ethnic differences in study cohorts. Regardless of such differences, it is apparent that GCA is not readily controlled in many patients once CS are reduced to low or moderate doses (ie, prednisone 5-15 mg/day). Even after 2 to 3 years of therapy, about 50% of patients remain CS-dependent, a situation that has led to substantial morbidity in an already fragile elderly population. The risk of fractures and cataracts are 5 and 3 times greater, respectively, in patients with GCA compared to age-matched controls not treated with CS.[24] Nesher et al[25] found that among 43 patients followed for a mean period of 3 years, 35% had fractures and 21% had severe infections, which in two-thirds led to death. An important role for CS could be implicated in 37% of all deaths.

Whether mortality rates among patients with GCA are different than that of age- and sex-matched controls remains controversial. Definitive conclusions may not be possible because of the limited ability of published studies to detect differences among subsets of the very elderly, who have high mortality. Nonetheless, it is difficult to argue that when a patient with GCA is found to have died because of granulomatous inflammation, contributing to aortic dissection or rupture, that the illness did not play a role in premature death. These events are not rare. Among 100 cases of GCA followed at the Mayo Clinic, 16 patients had acute aortic dissection, which was fatal in 50%.[26,27] Others have also noted that GCA may contribute to death by stroke, myocardial infarction, or aortic aneurysm rupture. Most recognized disease-related deaths have been early in the course of illness. It has been sug-

TABLE 2
RECOMMENDED USE OF PREDNISONE* IN GIANT-CELL ARTERITIS

Author	Initial Dose(s)	Start Chronic Dose Reduction	Rate of Reduction**	Comments
Graham[19]	80 mg/day × 2 days	Day 10	5 mg/week to 10 mg/day 10 mg/day × 3 months and then slow taper	
Lundberg and Hedfors[20]	19-37 mg/day; if visual or neurologic symptoms present, 37-75 mg/day	NS	NS	• Visual loss proximate to presentation: recommend 1,000 mg methylprednisolone IV • Most patients able to stop CS within 2 years
Nesher[29]	40 mg/day adequate for most	NS	Taper to 10 mg/day by 6 months and 5-7.5 mg/day by 1 year	50% of patients remain on therapy at 3-year follow-up
Chevalet[30]	35-50 mg/day	4 weeks	50% after 4 weeks, then more gradual	Initial doses of methylprednisolone (240 mg IV) do not provide therapeutic advantages
Hunder and Valente[26]	40-60 mg/day	2 weeks	30 or 50 mg/day at week 2, then decrease by 10% every 1-2 weeks until dose = 20 mg/day, then decrease every 2-4 weeks until 10 mg/day. Then decrease by 1 mg/month	50% of patients able to discontinue CS at 2-year follow-up

*Where prednisolone was used, conversion to an equivalent dose of prednisone is provided. **All authors continued taper only in absence of active disease. NS = Not stated.

gested that early deaths were due to inadequate treatment with CS or because CS were administered too late to affect fixed vascular abnormalities.[19,27,28] Although this is likely to be true, GCA or its treatment may in fact contribute to death at any time in the course of illness.

The need for prolonged CS therapy to control GCA, and the goal of reducing disease- and treatment-related morbidity and mortality, has led investigators to explore the use of adjunctive agents to improve outcomes.

A possibly important, but not yet addressed, issue is whether anti-platelet therapies or anticoagulation would improve or worsen outcomes.

■ ADJUNCTIVE THERAPY TO CORTICOSTEROIDS IN GCA

Numerous studies have explored the utility of either methotrexate (MTX) or azathioprine as a means of achieving improved disease control and less dependence on CS therapy. Because many of these studies have either been preliminary, combined GCA and "pure" polymyalgia rheumatica, or used very low doses of second agents, they will not be discussed. However, two recent randomized, double-blind, placebo-controlled studies of weekly MTX have been completed. In both, the rate of CS taper was rapid, so that in the absence of relapse, CS withdrawal could be accomplished in 4 months[23] or 6 months.[17] In both studies, relapses were frequent and the first relapse occurred with equal frequency in the CS-only and CS + MTX groups. However, the frequency of more than one relapse differed between groups in one study and not the other. Jover et al[23] found that MTX diminished second relapses and cumulative CS use, while Hoffman et al[17] did not find MTX to be beneficial. The reason for these different conclusions is uncertain. Consequently, what role MTX or other adjunctive therapies may play in GCA remains unsettled.

Surgical considerations

At least 15 to 20% of patients with GCA will develop clinically significant thoracic aortic (less often abdominal) aneurysms and/or stenoses of arch vessels. Sudden aortic rupture or dissection rarely provides opportunities for effective therapeutic intervention. It is therefore important that physicians who provide care for patients with GCA realize that newly recognized bruits or aortic murmurs may not merely represent atherosclerosis or calcification of valve leaflets. All such findings should be investigated. If an aneurysm or aortic regurgitation is found, it should be evaluated by cardiology and cardiovascular surgery colleagues. The cost-effectiveness of different imaging techniques for sequential large-vessel evaluation and the utility of angioplasty (+/- vascular stents) for aortic arch branch vessel stenoses have not been studied.

THE FUTURE

Our inability to control GCA without producing CS-related morbidity may not represent an impasse. Numerous investigators have demonstrated that the lesions of GCA are, in large measure, driven by macrophages and Th-1–type lymphocytes. Vascular lesions are rich in pro-inflammatory cytokines such as IL-1, TNF, and IFN-γ. It is possible that excessive up-regulation of these mediators is critical to vessel injury. Should that be the case, anti-cytokine therapies that target IL-1 and the Th-1 pathways may prove to be beneficial for GCA.

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Henoch-Schönlein purpura (treatment and outcome)

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Henoch-Schönlein purpura (HSP) is the most common systemic vasculitis of childhood. It is a form of leukocytoclastic vasculitis that is characterized by inflammation and necrosis involving small vessels, predominantly post-capillary venules, capillaries, and arterioles. There is a neutrophil infiltration of the necrotic vessel walls with scattered nuclear debris (leukocytoclasia), hemorrhage, and fibrin deposits. Immunofluorescence microscopy reveals deposits of immunoglobin A (IgA) in capillaries and post-capillary venules as well as complement and fibrin. It is characterized by nonthrombocytopenic purpura, arthritis and arthralgia, abdominal pain, gastrointestinal hemorrhage, and glomerulonephritis. However, clinical manifestations vary, and the extent to which individual patients manifest features of the condition govern, at least in part, the approach to management. Furthermore, even though the characteristic features allow for confident diagnosis, there is overlap with other forms of leukocytoclastic vasculitis that can lead to diagnostic confusion and to therapeutic dilemmas.[1]

CLINICAL FEATURES WITH THERAPEUTIC IMPLICATIONS

Cutaneous manifestations

The palpable purpuric lesions, often on dependent or pressure-bearing areas, usually do not require special therapeutic steps.[2,3] At times, however, they might ulcerate or may present as extensive hemorrhagic bullae.[4] In children younger than 3 years, the clinical picture may be dominated by subcutaneous edema involving the scalp, periorbital area, dorsi of the hands and the feet, and the genitalia.[5] This might manifest itself as acute hemorrhagic edema that has in the past been considered to be a separate entity but is now viewed as a variant of HSP.[6-8] Although spontaneous resolution will eventually occur, the pain, discomfort, and appearances frequently justify therapeutic intervention.

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Gastrointestinal disease

Two-thirds of children with HSP develop gut symptomatology.[9-11] Abdominal pain due to submucosal and subserosal hemorrhage and edema is the commonest feature, but gastrointestinal hemorrhage is common and can be massive and life threatening.[11] Intussusception develops in 4 to 5% of patients, and bowel ischemia and infarction, intestinal perforation, fistula formation, and late ileal stricture are recorded.[11,12] The severity of the abdominal pain and the seriousness of the complications of gut involvement have influenced many clinicians to treat the gut manifestations even though eventual improvement without therapy is well recognized. Hemorrhagic pancreatitis, hydrops of the gall bladder, and pseudomembranous colitis also occur, but infrequently.[13]

Articular features

Joints are involved in 50 to 80% of patients, with the knees and ankles most affected and with wrists, elbows, and the finger joints less often.[2,9,10,14] Nonsteroidal anti-inflammatory drugs have a role, but in severe situations steroids have been used with seeming benefit even though, as emphasized subsequently, resolution occurs without therapy.

Renal manifestations

Renal involvement occurs in 20 to 34% of children with HSP with a wide spectrum of manifestations ranging from microscopic hematuria and mild proteinuria to nephritic/nephrotic syndrome or even rapidly progressive crescentic glomerulonephritis and renal failure.[15-18] It is in the context of the renal disease that the greatest therapeutic endeavors have occurred, yet sadly without definitive randomized controlled trials.

Other features

Orchitis,[19,20] central nervous system involvement with seizures and coma,[21,22] Guillain-Barré syndrome,[23] parotitis, carditis, and pulmonary disease with hemorrhage are reported[24,25] and may require treatment in their own right.

TREATMENT

Treatment of HSP is, in general, supportive. There is a need to maintain hydration, nutrition, and electrolyte balance. Pain relief with simple analgesics is indicated. If hy-

pertension is present, this might require antihypertensive therapy. The role of glucocorticoids is controversial. They can dramatically benefit joint and skin findings (and the pain of orchitis) although they are not usually needed in the management of these features. Allen et al (1960)[26] concluded that in their study painful edema and arthritis resolved with or without steroids within 24 to 48 hours after onset. However, systemic steroids are indicated in patients with severe gastrointestinal hemorrhage,[9,26] for example, as prednisolone orally in a dose of 1 to 2 mg per kg per day for a week, with then gradual reduction over the next two to three weeks. Pulsed intravenous methylprednisolone in doses of 300 to 600 mg per m^2 per dose for 3 consecutive days may also be effective in these circumstances, possibly followed by a gradually reducing oral regimen. However, a number of retrospective studies have shown that abdominal pain resolves eventually with or without steroids but that steroids expedite this process. The role of glucocorticoids in preventing nephritis remains unclear. Some studies have been undertaken with variable results. Buchanec et al (1988)[27] and Mollica et al (1992)[28] concluded from a retrospective and a prospective nonrandomized study respectively that immediate treatment with steroids prevented renal disease. Saulsbury (1993),[29] on the other hand, in a retrospective review concluded that pretreatment with steroids did not prevent nephritis. Hence, at present it is not possible to give precise recommendations but there is a mandate for a well-randomized controlled trial to be undertaken.

Children with HSP who have clinical and histopathological features of moderately severe or severe renal disease have been treated with glucocorticoids with or without cytotoxic drugs. At the present time, no prospective controlled studies have been undertaken that clearly define the approach to treatment in these patients. Counahan et al (1977)[30] reported no benefit in terms of moderately severe nephritis utilizing prednisolone and/or immunosuppressive drugs. Foster et al (2000),[31] studying similar patients, showed that prednisolone and azathioprine therapy was associated with a better outcome compared to untreated patients. Administration of intravenous "pulsed" methylprednisolone in rapidly progressive glomerulonephritis has been shown to have a role if started early in the course of the disease.[32,33] There is also some evidence, in severe cases, that a combination of prednisolone, cyclophosphamide, heparin or warfarin and dipyridamole can result in clinical and histological benefit in severe cases.[34] This approach is similar to that advocated for the management of rapidly progressive glomerulonephritis with crescents histopathologically from any cause, as opposed to being selectively beneficial for the situation as a result of HSP.[35] The use of high- or low-dose intravenous immunoglobulin has been shown in limited studies to stabilize poor renal function or slow progression of renal disease in HSP nephritis.[36,37] Plasma exchange has been advocated by some and has resulted in an encouraging outcome in one study[38] and transient benefit in another,[39] but has still to be confirmed in large trials. In the patient left with persistent proteinuria after the acute disease process has settled, angiotensin converting enzyme inhibitors may have a role.

COURSE AND OUTCOME

In two-thirds of children, HSP runs its entire course within 4 weeks of onset.[30,40] Younger children usually experience a shorter course and will have fewer recurrences than older patients. Approximately 16 to 40% have at least one recurrence, which usually consists of a rash and abdominal pain. The majority of these recurrent episodes take place early in relation to the disease onset but can occur up to two years afterwards.The episodes may recur, seemingly spontaneously, or be associated with intercurrent infections. The prevention of recurrent attacks is sometimes attempted, and anecdotally a period of alternate-day low-dose prednisolone may help; some have advocated the use of dapsone in this situation.[41]

The overall prognosis is good. Significant morbidity is associated with disease of the gastrointestinal tract in the short term and in the long term with nephritis. The clinical and pathologic features are, to an extent, predictive of the long-term outcome. In patients who present with a nephritic, nephrotic, or nephritic/nephrotic syndrome, 44% have hypertension or impaired renal function on long-term follow-up, whereas 82% who present with hematuria (with or without mild proteinuria) are normal.[42] Children with renal manifestations in the acute phase require prolonged follow-up. This is especially important if there was extensive crescentic involvement on initial renal biopsy.

Long-term studies confirm that renal failure and hypertension can develop up to ten years after the disease onset. Overall 1 to 5% of children with HSP progress to end-stage renal failure. These patients account for approximately 10% of children entering into end-stage renal failure programs.[16,17] Renal transplantation has been successfully undertaken. Histologic features recur in one-third to one-half of all patients, but clinical recurrence and graft loss are uncommon.[43,44]

CONCLUSION

As can be seen, Henoch-Schönlein purpura is a disorder that in the majority of circumstances spontaneously remits in time with no more than supportive measures required therapeutically. However, there is a significant morbidity associated with the disease of the gastrointestinal tract in the short term and with nephritis in the long term. No clear guidelines exist in terms of treatment, but steroid therapy probably has a role in the initial phases of the disease for gut and other complications and may have a protective contribution in preventing the development of nephritis or modifying its course. For established glomerulonephritic disease, steroid and immunosuppressive therapy may be indicated, but controlled trials are needed to establish this. In the context of crescentic nephritis with a rapidly progressive glomerulonephritic picture clinically, the aggressive approach utilized for this pattern of illness would seem to be indicated in view of the serious renal prognosis with which it is associated.

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Targeted therapies in systemic vasculitis

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In the near future, the ability to target specific components of the immune system that have gone awry has the potential to revolutionize the treatment of systemic vasculitis. By targeting aberrant or dysregulated parts of the immune system, "biologic" interventions offer the prospect of fewer adverse effects and greater efficacy than conventional treatments. However, many challenges must be overcome before the potential benefits of this new class of therapies can be realized **(Table 1)**. I will address several issues related to biologic therapies in systemic vasculitis:

- Hurdles to the development of targeted therapies
- Challenges in the evaluation of efficacy
- Candidate targets
- Results of early studies
- The path to progress.

TABLE 1
HURDLES TO THE DEVELOPMENT OF TARGET THERAPIES IN SYSTEMIC VASCULITIS

- Knowledge of immunoregulatory defects incomplete
- Understanding of genetic/epidemiologic/environmental risk factors poor
- Uncertain relationships between disease and potential microbial pathogens
- Few animal models
- Reluctance of patients to enroll in randomized trials
- Complexity of disease assessment
- Difficulty in determining incremental effectiveness of new therapies compared with conventional treatments
- Length of time required for rigorous trials
- Market forces

HURDLES TO THE DEVELOPMENT OF TARGETED THERAPIES

The development of biologic therapies in systemic vasculitis confronts major intellectual challenges. The most daunting of these is that, without exception, definitions of the underlying immunoregulatory defects in the systemic vasculitides are still incomplete. The contributions of genetic predispositions (inborn or acquired), epidemiologic risk factors (age, gender, ethnicity), and environmental exposures to the development of vasculitis remain poorly understood. Although there are clear precedents for microbial pathogens causing systemic vasculitides (eg, hepatitis B and polyarteritis nodosa; hepatitis C and mixed cryoglobulinemia), the relationships between most forms of vasculitis and potential microbial pathogens are still only speculative. Finally, the absence of adequate animal models for most types of vasculitis is a major impediment to the development and assessment of new treatment approaches.[1]

Economic hurdles exist, as well. Based on estimates of the prevalence of giant-cell arteritis (GCA) and polymyalgia rheumatica (PMR) alone, the prevalence of vasculitis in the United States easily exceeds half a million cases.[2] However, the common perception is that all forms of vasculitis are rare. The pharmaceutical industry is far more likely to devote resources to the development of therapies for diseases that have larger perceived markets.

CHALLENGES IN THE EVALUATION OF EFFICACY

For any new therapy, the determination of efficacy requires randomized trials. The first challenge in the evaluation of novel therapies for vasculitis, therefore, is to enroll sufficient numbers of patients into clinical trials. With regard to GCA/PMR (the most common form of systemic vasculitis in the developed world), the perception among many practicing physicians is that this disorder does not require referrals to specialists at academic medical centers. This is because for decades, glucocorticoids have been a remarkably effective therapy for the treatment of GCA (albeit a toxic one), and there have been few new therapies introduced. Since academic centers have had nothing new to offer patients in terms of treatment, the understandable position of most practitioners has been that "my prednisone works as well as yours." In order to optimize enrollment in trials of new vasculitis therapies, therefore, the intervention must be both truly novel and not widely available.

A second challenge to the evaluation of efficacy is reluctance on the part of patients to be randomized. (This is *not* a drawback that is exclusive to trials of biologic agents in systemic vasculitis). The tendency of patients with dread diseases—and often of the physicians who treat

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them—is to embrace all new therapies as superior, even before these therapies have been tested adequately. This may be particularly true for systemic vasculitis, because of the enormous potential toxicities associated with most conventional therapies. As the experience with tumor necrosis factor (TNF) inhibition in multiple sclerosis illustrates, however, *newer is not always better.* (Quite contrary to expectations, multiple sclerosis patients experienced dramatic *worsenings* after treatment with anti-TNF agents. These drugs are now contraindicated in this disease.) The experience with anti-CD40 ligand therapy in systemic lupus erythematosus illustrates this lesson again (investigations of this agent were terminated abruptly because of life-threatening thrombotic events). Because of the many redundancies, counter-regulatory mechanisms, and unknowns that characterize our current state of knowledge about the immune system, the belief that "newer is better" may be particularly dangerous with regard to biologic therapies.

Another major challenge to evaluating the efficacy of new treatments in vasculitis is the complexity of disease assessments (eg, activity, remission, and damage). Vasculitides are the prototypes of multi-organ system diseases. Consequently, distilling the concept of "active disease" into a number as quantifiable as the counts of swollen or tender joints is difficult. The 1990s observed substantial improvements in the methods of vasculitis assessment, with the creation and validation of several disease assessment indices.[3–6] *All* of these indices are imperfect yardsticks of disease activity, but they are the best clinical measurements currently available. Teaching investigators to use these instruments in a uniform fashion in clinical trials is no small task.

Although conventional therapies for systemic vasculitis are toxic, they *are* effective, at least in the short-term. (The principal shortcomings of conventional treatments are their side effects, not their lack of efficacy.) Daily cyclophosphamide and high doses of prednisone, for example, lead to significant improvement in >90% of patients with WG.[7] Thus, determining the *incremental effectiveness* of new medications—if any—is not easy. Furthermore, new therapies must be employed *in addition to* the old ones: failure to use therapies known to be effective in these potentially lethal diseases would not be ethical.

The effectiveness of conventional therapies in the control of disease and the requirement for using these "old" medications along with new agents to be tested have another implication for the determination of efficacy: proving that a new medication works requires a lengthy study, with prolonged patient follow-up. Given the relatively crude state of outcome measures in vasculitis, hard endpoints—sustained remission, number of disease relapses over time, and death—require time to accumulate. The length of time required to test a new agent adds substantially to the expense of performing such trials and heightens the bar when it comes to performing trials of high quality.

Finally, market forces also complicate the evaluation of new treatments' efficacy. *The dose of a biologic agent that is effective for one disease may be subtherapeutic for another.* Thus, when testing a new therapy for a specific disease, one would like to be certain of employing the optimal dose. However, pharmaceutical sponsors, eager to get their products into general use—whether approved for a given indication or not—are usually unenthusiastic about dose-finding studies in relatively uncommon conditions.

SEVERAL POSSIBLE BIOLOGIC APPROACHES TO TREATMENT

There are many possible candidate molecules for biologic approaches to treatment. A partial list is shown in **Table 2**. Many of these potential targets are drawn from our current understanding of GCA. Although there remains much to learn about GCA, research over the past decade has highlighted many potential targets for biologic intervention in this disease.

Tumor necrosis factor

Tumor necrosis factor (TNF) is a critical mediator of inflammation in a variety of conditions. TNF release, principally by macrophages, leads to activation of the vascular endothelium, including the expression of adhesion molecules and the upregulation of class II major histocompatibility (MHC) molecules. These events orchestrate the recruitment of inflammatory cells and increase production of immunoglobulins and complement proteins. As a major cytokine in the Th1 inflammatory pathway, TNF stimulates the release of other pro-inflammatory cytokines, including interleukins (IL)-1, -6, and -8. At least two different approaches to the inhibition of TNF are now commercially available. Others will be shortly. The early results of treating WG with TNF inhibition are discussed below.[8]

Interferon-gamma

IFN-γ, a cytokine produced by Th1 lymphocytes and natural killer cells, induces class II MHC expression and morphologic changes in both endothelial cells and macrophages. IFN-γ also increases the expression of adhesion molecules on endothelial cells, and has effects that are synergistic with those of TNF.[9,10]

In giant-cell arteritis, IFN-γ produced by CD4+ T-cells within the adventitia appears to drive the inflammatory response. Strong evidence supports the concept of GCA as an antigen-driven, T-cell mediated disease, and the adventitia appears to be the site of immunologic recognition events.[11] IFN-γ+ T-cells appear to be recruited to the adventitia by a specific antigen or antigens (which, of course, remain unidentified). From this location, the T-cells—via the production of IFN-γ—orchestrate a cascade of inflammation that permeates the entire vessel wall, culminating in some patients in ischemic events that result from luminal occlusion (eg, anterior ischemic optic neuropathy).

IFN-γ is an appealing target for a biologic intervention because of the central role it appears to play in both GCA (and WG; see below). The implications of blocking IFN-γ, however, are presently unclear. Data emerging from a mouse model of large vessel arteritis indicate that strategies for blunting the inflammatory response (eg, by the in-

TABLE 2
POTENTIAL TARGETS FOR BIOLOGIC INTERVENTIONS IN SYSTEMIC VASCULITIS

- Tumor necrosis factor
- Interferon gamma
- Matrix metalloproteinases and reactive oxygen species
- Platelet-derived growth factors and vascular endothelial growth factor
- Interleukin-6
- The interleukin-10/interleukin-12 balance
- Interferon-alpha
- Interleukin-1/interleukin-1 receptor antagonist
- CTLA-4 and other co-stimulatory molecules

hibition of IFN-γ) are likely to lead to the persistence of the inciting agent/antigen,[12] with consequences that may be ultimately deleterious to the host.

Interleukin-1 and interleukin-6

In addition to TNF, several other macrophage products constitute potential targets for biologic interventions. In GCA, for example, both circulating macrophages and those homing to the site of antigen recognition in the adventitia produce IL-1β and IL-6.[13] These cytokines probably account in large measure for the profound constitutional complaints, polymyalgia rheumatica symptoms, and elevated erythrocyte sedimentation rates so characteristic of many GCA patients.

In addition to its secretion by monocytes/macrophages, IL-6 is secreted by vascular smooth muscle cells and endothelium in response to TNF and IL-1. IL-6 is a potent activator of acute phase response proteins, stimulates the hypothalamic-pituitary-adrenal axis, helps propagate Th1 cytokine responses, and has recently been implicated in the pathogenesis of atherosclerosis.[14] In vitro, animal, and human studies of this molecule over the past decade have implicated it in the pathogenesis of a variety of vasculitides, including GCA, Takayasu's arteritis (TA), rheumatoid vasculitis (RV), vasculitis associated with SLE, Wegener's granulomatosis (WG), and microscopic polyarteritis (MPA).

Plasma concentrations of IL-6 are increased during flares of GCA, TA, WG, and RV.[15-18] In general, IL-6 concentrations parallel disease activity in these disorders. Temporal artery biopsy specimens in GCA reveal an increase in IL-6 producing cells within the arterial media (macrophages) and intima (fibroblasts). Following the treatment of GCA, IL-6 levels normalize.[15] Recent evidence, however, suggests that IL-6 suppression by conventional GCS doses in GCA is incomplete, and that IL-6 elevations correlate with disease flares.[19] IL-6 may be a more sensitive indicator of persistent vascular inflammation than the ESR, and persistently elevated IL-6 levels may indicate patients who will require additional treatment. Trials of anti-IL-6 therapies are under way in RA. Whether the inhibition of IL-6 production and monocyte activation will result in clinical and immunologic improvement in patients with vasculitis is an intriguing question.

Matrix metalloproteinases and reactive oxygen species

In GCA, macrophages lining the media and media-intima border synthesize other products under the direction of INF-γ: matrix metalloproteinases (MMP) and reactive oxygen species (ROS).[11] MMP, which play an important role in joint destruction in inflammatory arthritis, probably contribute substantially to the fragmentation of the internal elastic lamina in GCA. MMP are also required for the mobilization of smooth muscle cells (ultimately contributing to luminal occlusion). ROS production leads to lipid peroxidation and destruction of cellular membranes. Whether or not these targets can be inhibited by specific approaches—and whether such approaches would have meaningful clinical effects—both remain to be seen.

Platelet-derived growth factors and vascular endothelial growth factor

Macrophage (and multi-nucleated giant cell [MNGC]) products also lead directly to arterial failure and clinical events in GCA. Products elaborated by these cells lead to smooth muscle migration, intimal hyperplasia, and luminal occlusion. Intimal proliferation is mediated by the in situ production of platelet-derived growth factors A and B (PDGF-A & -B) and vascular endothelial growth factor (VEGF), all of which are produced by MNGC.[20] The presence of MNGC correlates strongly with the concentration of IFN-γ within the arterial wall.[21] Therapies targeting intimal proliferation could serve as treatments adjunctive to those designed to abolish "inflammatory" elements of the immune response.

The interleukin-10/interleukin-12 balance

IL-10 downregulates lymphocyte activity in vivo by suppressing macrophage activation. This cytokine is secreted by helper T-lymphocytes, macrophages, and keratinocytes. The inhibition of macrophages by IL-10 leads to a decrease in plasma levels of IL-1, TNF, and IL-12, and ultimately to the suppression of Th1 activity. Conversely, IL-12, produced by activated macrophages, is a potent activator of CD4+ T-cells and natural killer cells and is downregulated by IL-10. Because of the major roles of IL-10 and IL-12 in the regulation of the Th1 inflammatory pathway, manipulation of these cytokines offers the opportunity to alter the inflammatory milieu in ways beneficial to patients.

Monocyte activation and skewing of the Th1:Th2 ratio have been demonstrated in a variety of human vasculitides. As noted, PBMCs isolated from patients with active WG secrete increased amounts of the Th1 cytokines IL-12, IFN-γ, and TNF. Moreover, in vitro levels of IFN-γ are decreased by the exogenous administration of IL-10,[22] suggesting a possible therapeutic role for IL-10. To date, however, IL-10 has not been employed in significant numbers of patients with vasculitis.

CTLA-4 and other co-stimulatory molecules

Current experimental approaches to the induction of immunological self-tolerance in autoimmune disorders such as SLE involve the use of biologic agents to block

molecules that promote T-cell activation. In general terms, these strategies are intended to disrupt "co-stimulatory" pathways.[23] Such strategies may also be applicable to certain forms of vasculitis. Potential targets within co-stimulatory pathways include the B7 stimulators of CD28, B7-1, and B7-2.

Among the many molecules involved in co-stimulation, molecules of the B7:CD28/CTLA4 pathway are described most completely. CTLA4-Ig is a soluble chimeric protein consisting of the extracellular domain of human CD152 and a fragment of the Fc portion of human IgG1.[24] CTLA4-Ig binds to both B7-1 and B7-2 molecules on antigen-presenting cells, thereby blocking the CD28-mediated co-stimulatory signal for T-cell activation. There is early evidence, based on studies of candidate genes, that WG may be an appropriate disease in which to test this approach.[25]

■ RESULTS OF EARLY STUDIES

Anti-TNF investigations in Wegener's granulomatosis

Preliminary results of etanercept use in vasculitis include data from a six-month open-label study of 20 WG patients.[8] This trial was conducted to evaluate the safety of etanercept combined with the potentially hazardous conventional therapies used to treat WG. (Prior to this trial, etanercept had never been employed in combination with cyclophosphamide.) Etanercept (25 milligrams subcutaneously twice a week) was added to standard therapies for WG that were prescribed according to disease severity. All patients enrolled had histories of refractory WG: the mean time since original diagnosis was 63.6 months (range: 14-189 months), and 14 patients (70%) had never achieved remissions permitting the successful discontinuation of GCS. Sixteen patients (80%) had limited WG at entry, and 4 (20%) had severe disease. Eighteen of the patients (90%) were receiving GCS and 18 (90%) were receiving another immunosuppressive agent (8 methotrexate, 6 cyclophosphamide, 4 azathioprine). However, 14 of the 20 patients (70%) had etanercept added as the only new therapeutic variable.

The most common etanercept-related adverse event was the occurrence of injection site reactions. Eight injection site reactions occurred in 5 patients (25% of all patients enrolled, but <1% of all injections). All injection site reactions were mild. Two patients had a combined total of 5 hospital admissions (1 patient had 4 admissions), but none were attributable solely to etanercept-related adverse events. One patient with severe subglottic stenosis developed pneumococcal tracheobronchitis and subsequently had a localized *H zoster* infection. Nineteen patients (95%) remained on treatment at 6 months, the single exception being a patient who developed progression of orbital (retro-bulbar) disease at 4 months. There were no deaths.

Although the principal purpose of this open-label trial was to investigate the safety of etanercept in WG, preliminary indications of treatment efficacy were sought in comparisons of disease activity scores at entry and 6 months. The Birmingham Vasculitis Activity Score for WG (BVAS/WG)[5] was used to measure disease activity. The mean BVAS/WG at entry was 3.6 (range: 1-8). At 6 months, the mean BVAS/WG score decreased 3.0 points, to 0.6 ($P < 0.001$; 95% confidence interval: –4.0, –2.1). Among the 14 patients in whom etanercept was the only new treatment variable, the mean BVAS/WG score declined 2.7 points, from 3.1 at entry to 0.4 at 6 months ($P < 0.001$; 95% confidence interval: –4.5, –1.8). The mean daily prednisone dose in this subset decreased from 12.9 mg at entry to 6.4 mg at 6 months, but this comparison did not achieve statistical significance (difference: –6.5; $P = 0.19$; 95% confidence interval: –16.6, +3.6). Sixteen of the patients (80%) achieved BVAS/WG scores of 0 at some point during the trial. *However, intermittently active disease was observed in 15 patients (75%)*. There were 3 severe flares during the course of the trial (two flares of pre-existing orbital disease and one de novo flare of glomerulonephritis).

Randomized trials to assess the efficacy of etanercept in WG have begun. The Wegener's Granulomatosis Etanercept Trial (WGET), a randomized, double-blinded, placebo-controlled study, is under way at 8 medical centers in the United States.[26] In this trial, patients are randomized to either etanercept and placebo in addition to conventional WG treatments (which all patients receive at entry). The conventional treatments are tapered after the achievement of remission. The principal outcome measure is the ability of etanercept to maintain disease remissions. Enrollment in WGET is now 75% complete, but no outcomes related to efficacy are available at this time.

A single-center trial (randomized but unblinded) is being conducted at the National Institutes of Health. This trial involves the combined use of etanercept and methotrexate (versus methotrexate alone) for patients with non-life-threatening WG. All patients receive etanercept and methotrexate initially. At 6 months, those patients in remission are randomized to either continue etanercept, or to stop receiving etanercept but to continue methotrexate.[27] Follow-up in this trial continues at the present time. No efficacy data are currently available.

■ THE PATH TO PROGRESS

Despite the challenges noted above, investigations in systemic vasculitis have made tremendous advances in recent years. Both the International Network for the Study of the Systemic Vasculitides (INSSYS) and the European Union Vasculitis Study Group (EUVAS), organizations with overlapping memberships but separate funding sources, have completed large randomized trials of non-biologic therapies in systemic vasculitis.[28,29] Under the auspices of INSSYS, WGET—the first multi-center, randomized, double-blinded trial of a biologic agent in vasculitis—is presently under way.

To advance the therapy of vasculitis, randomized, double-blind, placebo-controlled trials will be required. Continued improvements in outcome measures will facilitate the rigorous conduct of clinical trials. Evaluations of new therapies should include small studies aimed at determining the optimal dose for larger trials.

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25-070

CAMPATH 1-H (ANTI-CD52) FOR REFRACTORY VASCULITIS: RETROSPECTIVE CAMBRIDGE EXPERIENCE 1989-1999

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CAMPATH 1-H is a humanized anti-CD52 monoclonal antibody designed to deplete lymphocytes. Its compassionate use in the treatment of refractory or relapsing multisystem autoimmune disease in Cambridge between 1989 and 1999 is reviewed. This aimed to induce sustained treatment-free remission and minimize cumulative immunosuppressive and steroid exposure. One hundred twenty-one patients received CAMPATH 1-H. Diagnoses were Wegener's granulomatosis 63, Behcet's disease 18, polyarteritis nodosa 8, Sjogren's syndrome 7, microscopic polyangiitis 5, Churg-Strauss angiitis 2, other 29. Mean age was 47 years, disease duration 5 years, 60% were female and mean follow-up was 36 months. Prior to treatment, immunosuppressives were withdrawn and prednisolone reduced to 10 mg/day. Patients received CAMPATH 1-H 135 mg over five days and prophylactic antiviral, antibacterial and antifungal therapy. Treatment was repeated for persisting or relapsing disease.

At one year, 22 patients had died (18%) and data was incomplete on 9. Of the remaining 90 patients, 75 (83%) were in remission with prednisolone less than 10 mg/day and no immunosuppressive, and 15 (17%) had persisting disease activity. The number of courses of CAMPATH 1-H required to achieve remission was one in 41 patients (55%), two in 26 (35%) and three in four (5%) (not known in four). Adverse events were: infusion reactions 50 (41%), infection 39 (32%), and new automimmune disease 20 (17%). At follow-up 37 had died (31%); causes of death were sepsis 18, uncontrolled disease 11, malignancy 5, cardiovascular event 8, and other 2. The association of death with CAMPATH treatment was considered probable in 9, possible in 14, unrelated in 13 and not known in 1. Age and creatinine > 150 μmol/l were independently associated with death (both $p < 0.001$). Relapse occurred in 32 (43%), with a mean time to relapse of 23 months from initial treatment. CD4 counts were 145, 167 and 285 × 106 at 3, 12 and 48 months' follow-up, respectively.

Lymphocyte depletion with CAMPATH 1-H can achieve remission in refractory vasculitis. Its use is associated with infusion reactions, infections and autoimmune events and may contribute to mortality in high-risk subgroups.

Inflammation in acute coronary syndromes

MARK ROBBINS, MD, AND ERIC J. TOPOL, MD

The late Russell Ross's assertion that atherosclerosis is an inflammatory disease is now strongly supported by clinical, basic, and pathological research calling for an evolution in thought concerning the evaluation and treatment of acute coronary syndromes (ACS).[1-5] The initial insult is endothelial injury and subsequent dysfunction via the deleterious effects of the known cardiac risk factors such as oxidized LDL, infection, hyperglycemia, hypertension, hyperhomocysteinemia, or smoking. Irrespective of the cause of endothelial damage, the resultant activation and proliferation of inflammatory cells, smooth muscle cells, generation of cytokines, growth factors, and many other substances lead to the progression of atherosclerosis. The presence and degree of inflammation and procoagulant state, defined by elevated CRP, fibrinogen, interleukin (IL)-1, IL-6, TNF-α, adhesion molecules, plasminogen activator inhibitor (PAI-1), tissue factor, and composition of the atherosclerotic plaque have been strongly associated with an increased risk of future cardiac events.[6-9] Thus, the perpetuation of the inflammatory response likely plays a pivotal role in the pathobiology and vulnerability of the atherosclerotic plaque.

PATHOBIOLOGY OF INFLAMMATION, ATHEROSCLEROSIS, AND ACS

Endothelial function

The endothelium lies in a critical location between the remaining vascular wall and the circulating blood thereby functioning as the pivotal barrier that protects the arterial wall from injury. This critical monolayer of cells is pluripotential, carrying out the following functions: 1) provision of a nonthrombotic surface; 2) maintenance of vascular tone through the production and release of nitric oxide (NO), prostacyclin, and endothelin; 3) regulation of growth factors and cytokines; 4) provision of a nonadherent surface for leukocytes and platelets; and 5) the modification of lipoproteins as they transverse its permeable barrier.[5] Injury to this monolayer plays a key role in the initiation and progression of the atherosclerotic lesion by increasing adhesive cell surface glycoproteins such as vascular cell adhesion molecule-l (VCAM-1) and intracellular adhesion molecule (ICAM), adherence, migration, and activation of leukocytes, and smooth muscle cells, production of cytokines, chemokines, and growth factors, as well as the reversal from an antithrombotic to a prothrombotic state.[4,10-12]

Adhesion molecules

Cell-cell interactions are a vital component in the pathogenesis of inflammation. Collectively known as cell adhesion molecules, three distinct families exist—the selectins, the integrins, and the immunoglobulin superfamily each with its own specific role in the inflammatory process. The process entails tethering and rolling of leukocytes on the activated endothelium, leukocyte activation, and ultimately firm adhesion and transendothelial migration along a chemotactic gradient generated by mediators of inflammation.[13,14]

Selectins are expressed on the cell surface of leukocytes (L-selectin), platelets (P-selectin), and endothelial cells (E-selectin). Upon activation from inflammatory cytokines, mainly TNF-α and IL-1, cell surface expression of each selectin is enhanced.[15-17] This process is vital in the early phase of inflammation mediating leukocyte recruitment and transient endothelial cell to leukocyte interactions (tethering and rolling phase). The subsequent steps of firm adhesion and migration of leukocytes is predominantly mediated through the interaction of integrins [leukocyte function associated antigen-1 (LAF-1), macrophage antigen-1 (MAC-1), very late activation antigen-4 (VLA-4) and GPIIb/IIIa receptor], the immunoglobulin superfamily [vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and intercellular adhesion molecule-2 (ICAM-2)] and potent stimulation by inflammatory cytokines including IL-1, IL-4, IL-8, TNF-α, INF-γ, and chemokines such as chemotactic protein-1.[13,14] In addition to the cell adhesion molecules on endothelial cells, leukocytes, and platelets, ICAM-1 and VCAM-1 are expressed on smooth muscle cells.[18] The interaction between leukocytes and smooth muscle cells contributes to smooth muscle cell migration and proliferation, cellular composition of the atherosclerotic plaque, and an increased expression of monocyte tissue factor mRNA, all of which are likely to be vital in influencing plaque stability.[18,19] An additional component that ties inflammation

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and the prothrombotic state involves the adhesion of activated platelets to the endothelium through the P-selectin-GPIIb/IIIa receptor interactions with subsequent platelet aggregation and thrombus formation.[20]

Growing evidence supports that the presence of increased cell adhesion molecules in serum or vascular tissue may reflect ongoing active vascular remodeling due to persistent inflammation. Elevated serum levels of the soluble form of the VCAM-1 receptor (sVCAM-1) has been associated with the extent of atherosclerosis in patients with peripheral vascular disease.[21] In patients with coronary artery disease, elevated levels of the soluble ICAM-1 (sICAM-1) has been found to be inversely proportional to HDL levels and associated with the presence of other coronary risk factors, unstable angina, myocardial infarction, and importantly to increased risk of future myocardial infarction in apparently healthy men.[22,23] Interestingly, immunohistochemical evaluation of coronary athrectomy tissue has shown P-selectin but not E-selectin, or ICAM-1 was expressed significantly greater in the setting of unstable angina versus stable angina.[24] This reflects an augmented response between an endothelial cell adhesion molecule and the activated platelet linking thrombus formation and unstable coronary syndromes.

Treatment strategies available based on the inhibition of cell to cell interactions have shown promise in the treatment of chronic inflammatory diseases, and recently coronary artery disease.[13,25] This should not be surprising given the marked similarities that exist between the pathophysiology of inflammatory diseases, such as rheumatoid arthritis, and atherosclerosis **(Table 1)**. ASA and other NSAIDS affect the expression and function of cell adhesion molecules, and have been shown to inhibit many phases of the adhesion cascade.[18] Direct antagonism via monoclonal antibodies and selectin-blocking agents against ICAM-1 and L-selectin has been shown to reduce neutrophil accumulation and myocardial injury in experimental animal studies.[26,27] New approaches using antisense oligonucleotides to inhibit mRNA translation for cell adhesion molecule expression, and inhibition of gene expression by synthetic DNA molecules and triplex-forming oligonucleotides have shown conceptual promise in animal studies.[13]

TABLE 1
SIMILARITIES BETWEEN ATHEROSCLEROSIS AND RHEUMATOID ARTHRITIS

	Atherosclerosis	Rheumatoid arthritis
Macrophage activation		
TNF-α	↑	↑
Metalloproteinases	↑	↑
Interleukin-6	UA ↑	↑
Mast cell activation	↑	↑
T-cell activation		
CD+DR+	UA ↑	↑
CD4+CD28–/INF+	UA ↑	↑
TH1/TH2 balance	TH1 ↑	TH1↑
B-cell activation	0 or ↑	0 or ↑
CRP	↑	↑↑
Adhesion molecules	↑	↑
Endothelin	↑	↑
Neoangiogenesis	↑	↑

Source: Modified, with permission, from Pasceri and Yeh, "A tale of two diseases: atherosclerosis and rheumatoid arthritis," Circulation 1999; 100(21):2124-2126.

■ CELLULAR AND HUMORAL MEDIATED RESPONSE

Monocytes and macrophages

Monocytes, the circulating precursors of tissue macrophages, are essential in the progression of atherosclerosis and are found in all stages of atherosclerotic lesions.[4,28] Their recruitment and infiltration through the endothelium into the intima are tightly coupled to the humoral activity of the T-lymphocyte. The colocalization of CD4+ T-cells and macrophages and the abundant expression of HLA II molecules in atherosclerotic lesions is strong evidence for the role of cell-mediated immunity in the development and progression of atherosclerosis. Population size of CD14dimCD16a+ peripheral blood monocytes has been shown to correlate with degree of hypercholesterolemia and is dramatically reduced with lipid lowering therapy.[29] This phenotypic expression, in contrast to other phenotypes of monocytes, is shown to express high levels of inflammatory cytokines such as TNF-α whereas the anti-inflammatory IL-10 is low or absent. In addition, these cells are further characterized by an upregulation of cell surface adhesion molecules, suggesting an increased capacity for cell to cell interactions.[30]

The degree of macrophage infiltration has been shown to distinguish between unstable and stable coronary lesions. The preferential localization of macrophages in high-flow shoulder regions of the atherosclerotic plaque correlates with areas at highest risk for plaque instability. In contrast to controls, infiltrates of CD68-positive macrophages and CD3- and CD8-positive T-cells were statistically associated with the severity and frequency of superficial plaque inflammation and rupture.[31-33] This plaque instability, in part, stems from metalloproteinase (MMP-1 and MMP-2) production and release by activated macrophages within the inflamed atherosclerotic plaque.[34]

T-Lymphocyte

Antigen-presenting macrophages induced T-cell activation and results in inflammatory amplification through T-cell release of TNF-α, and INF-γ, further activating macrophages, platelets, and smooth muscle cells.[35] Levels of the main specific immune markers CD4+ and CD3+/DR+ T-cells, IL-2, and IgM have all been reported to be higher in unstable than in stable angina patients.[36] In addition, a higher percentage of IL-2 receptor positive T-lymphocytes in culprit lesions of patients with acute coronary syndromes indicate recent activation and amplification of the immune response within plaques. These

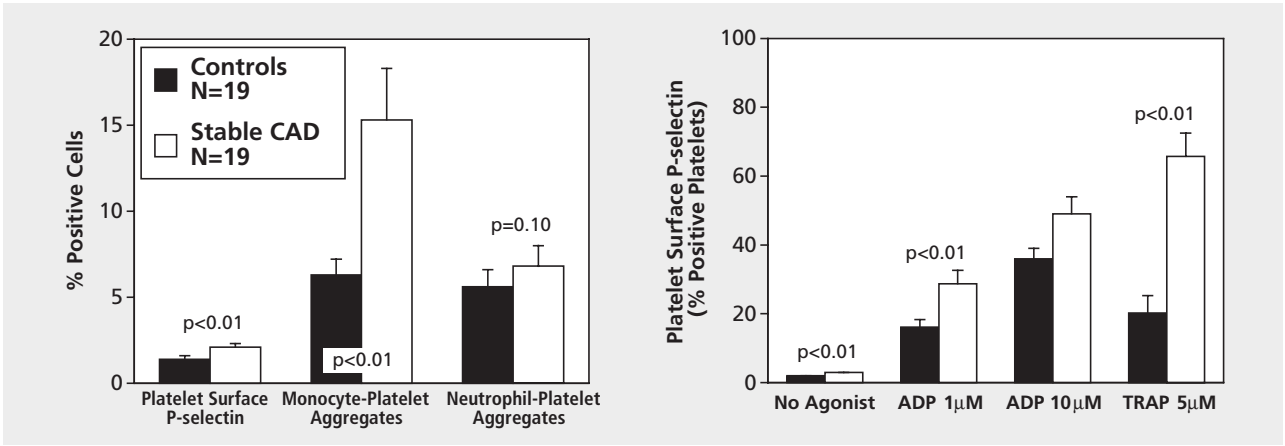

Figure 1. Augmented platelet activity and response to agonist in patients with stable coronary artery disease versus normal controls. (Reprinted with permission from the American College of Cardiology Foundation *Journal of the American College of Cardiology*, 1998, vol 31, pp 352-358.)

findings support the concept that a burst of inflammatory products could initiate or accelerate the onset of an acute coronary event.[37]

Mast cell

Mast cells have been recently identified to inhabit the vulnerable shoulder regions of the atherosclerotic plaque and to be associated with plaque erosion and rupture.[38,39] The population size of mast cell in athrectomy tissue correlates with the clinical severity of coronary syndromes. Their presence in the adventitia of ruptured plaques has led to the postulate that histamine release may provoke coronary spasm and contributes to the onset of myocardial infarction.[40,41] Mast cells have a primary role in the perpetuation of the inflammatory response in atherosclerosis, characterized by the production of TNF-α and neutral proteases (tryptase and chymase).[42,43] TNF-α stimulates macrophages and smooth muscle cells to produce two prometalloproteinases—prostomelysin and procollagenase. Subsequent activation of prometalloproteinases by mast cell produced tryptase and chymase leads to fibrous cap degradation and plaque destabilization.[44]

Neutrophil

As previously discussed, macrophages and T lymphocytes are the predominant cellular components of local inflammation within the atherosclerotic plaque. Neutrophils, although found sparsely in atherosclerotic plaques, play an integral part in the acute inflammatory response to tissue injury and have been implicated as a major factor in tissue damage in response to ischemia and reperfusion.[45] TNF-α, IL-8, IL-6, platelet-activating factor, and leukotrienes enhance neutrophil recruitment to ischemic and reperfused myocardium by augmenting cell adhesion molecule expression. The extent of accumulation has also been correlated to the degree of tissue injury.[46,47] A systemic activation of neutrophils has been reported in patients with angiographically documented coronary artery disease as compared with normal controls and a subset of trauma patients providing further proof for a chronic systemic inflammatory state in patients with atherosclerosis.[48]

Platelet

Traditionally, platelets have not been classified as inflammatory cells, but recent discoveries have led investigators to believe that platelets are critical constituents that tie in both inflammation and thrombosis. The presence of serologic markers of platelet activation is well established in the setting of an ACS.[49-51] Inflammatory cytokines induce the translocation of the cell adhesion molecule P-selectin to the surface of the platelet membrane, facilitating interactions among platelets, endothelial cells, and monocytes. Monocyte expression of tissue factor is induced by P-selectin and may be an initiator of thrombosis in areas of vascular injury.[52]

An initial step to answer the question of whether platelet activation is a result of or results in the development of an ACS was recently reported by Furman et al[53] In a flow cytometric analysis patients with stable coronary artery disease were shown to not only have increased levels of circulating activated platelets with enhanced P-selectin expression, but also to have an increased propensity to form monocyte-platelet aggregates **(Figure 1)**.[53] Additional evidence to implicate platelets as inflammatory mediators is the recent finding of their expression of CD40L. This transmembrane protein found on constituents of both cellular and humoral components of the inflammatory system is structurally related to TNF-α. CD40L is rapidly expressed by activated platelets and induces the expression of chemokines and cell adhesion molecules by endothelial cells thus provoking cell attraction, activation, and migration into the arterial wall.[54]

■ MARKERS AND MEDIATORS OF INFLAMMATION

C-Reactive protein (CRP)

Although many markers of inflammation have been associated with adverse cardiovascular outcome, CRP has been evaluated in every clinical phase of coronary disease. It therefore provides a superlative avenue to thoroughly discuss the prognostic significance of inflammatory mark-

ers in cardiovascular disease. CRP is an acute-phase reactant whose concentration in blood rises dramatically in response to nonspecific inflammatory stimuli. It has been convincingly linked to cardiovascular disease, initially in sera of patients after acute myocardial infarction and recently in the wall of human coronary arteries possibly linking its presence directly with the development of atherosclerosis.[55-57] Whether the association reflects a casual or direct interaction, elevated levels of CRP are associated with a worse prognosis in the full spectra of atherosclerotic disease.

In the setting of a Q-wave myocardial infarction, Anzai et al[58] reported that elevated levels of CRP were associated with cardiac rupture, left ventricular aneurysm formation, and 1-year cardiac death. Even though CRP was found to be an independent predictor of these events, there remained a confounding correlation to extent of cardiac enzyme elevation in those patients without revascularization procedures.[58] Therefore, CRP levels in this study may have reflected infarct size and subsequent risk for adverse outcome.

Tommasi et al[8] reported on the prognostic value of CRP levels in patients with a first acute myocardial infarction, uncomplicated in-hospital course, absence of residual ischemia, and normal left ventricular function. Only increased CRP levels were independently associated to the incidence of patients who developed cardiac events (cardiac death, new-onset angina, and recurrent myocardial infarction) **(Figure 2)**.[8] Importantly, there was no correlation between CRP levels and extent of rise of cardiac enzymes.

Although numerous studies have shown that an elevated CRP in the setting of unstable angina and non–Q-wave myocardial infarction is associated with worse prognosis,[6,7,59-61] Biassicci et al[62] reported on the prognostic significance of CRP elevation in patients with unstable angina without myocardial injury. They excluded those patients with elevated levels of cardiac enzymes at entry to avoid the interplay of myocardial necrosis on CRP and future events. They reported that an elevated discharge CRP was strongly associated with recurrent coronary instability and myocardial infarction **(Figure 3)** and, interestingly, 42% of patients had persistent elevation of CRP 3 months after hospital discharge. Adjunctive evidence that elevated CRP levels possess predictive power exceeding their association with myonecrosis is their independent and additive prognostic value to markers of myocardial injury, such as troponin T and I.[63,64]

In the Thrombolysis in Myocardial Infarction (TIMI) IIA trial, a dose-ranging trial for enoxaparin in UA and NQMI, elevated CRP correlated with increased 14-day mortality **(Figure 4)**. Most importantly, these findings existed even in patients with a negative rapid troponin T assay, thereby dissociating myonecrosis from CRP's prognostic power.[64] Milazzo et al[65] reported that in patients undergoing CABG a preoperative elevation of CRP has prognostic significance **(Figure 5)**. CRP levels <3 mg/L and ≥3 mg/L were associated with new ischemic events in 4% vs. 25% of patients, respectively.

In the setting of percutaneous coronary revascularization, a hyperresponsive reaction of the inflammatory system, defined by elevation of CRP, IL-6, and serum amyloid A after angioplasty, was recently presumed to portend a worse prognosis.[66] Gaspardone et al[67] confirmed this by showing a persistent elevation in CRP 72 hours after coronary artery stenting (excluding patients with periprocedural myocardial infarction) pinpointed all patients who later suffered an adverse outcome. In contrast, no cardiac events occurred in those with normal levels at 1 year follow-up **(Figure 6)**.

Ex vivo studies have recently introduced the concept that detecting heat release by inflammatory cells within an atherosclerotic plaque may predict future instability and rupture.[68] Stefanadis et al,[69] using a thermography catheter, demonstrated heterogeneity in heat production of 20%, 40%, and 67% in atherosclerotic plaques of patients with stable angina, unstable angina, and acute myocardial infarction, respectively. Most importantly there was a significant correlation between thermal heterogeneity and baseline CRP **(Figure 7)**.[69]

More conclusive evidence that chronic, indolent inflammation plays a principal role in the development and progression of atherosclerosis has come from the long-term follow-up of patients with no known atherosclerotic disease but increased levels of CRP. Among 14,916 apparently healthy men participating in the Physician's Health Study an elevated level of high-sensitivity CRP (HsCRP), which detects CRP levels as low as 0.175 mg/L, added to the predictive value of elevated lipids in predicting first myocardial infarction **(Figure 8)**.[70] Similarly, in the Women's Health Study, those who developed cardiovascular events had higher baseline CRP levels than control subjects, with the highest levels at baseline being associated with a five- and seven-fold increase in any vascular event and combined stroke or myocardial infarction, respectively.[71]

Additional evidence that CRP levels are strong predictors of future cardiac events in apparently healthy men was recently published from the Monitoring Trends and Determinants in Cardiovascular Disease Study (MONICA). Patients in the highest quintile of CRP level had a 2.6-fold increased risk of suffering a fatal or nonfatal myocardial infarction or sudden cardiac death.[72] These findings strongly support the pivotal role that inflammation plays in the destabilization of atherosclerosis.

The question remains what if any direct role CRP plays in the development of atherosclerosis. A possible explanation supporting CRP as an indirect cardiovascular risk factor is that it reflects inflammation related to coronary vessel pathogens, extent of atherosclerosis, myocardial necrosis, myocardial ischemia, and activity of circulating proinflammatory cytokines.[73] Direct evidence for CRP's role in the pathogenesis of atherosclerosis is that its presence in the arterial wall predicts severity of atherosclerosis and that it is able to bind to damaged membranes and lipids, activate complement, and stimulate production of tissue factor from activated macrophages.[74-77] Irrespective of its pathologic role, there is overwhelming evidence that CRP, a sensitive marker of inflammation, is a powerful predictor of future cardiac events in patients with Q-wave

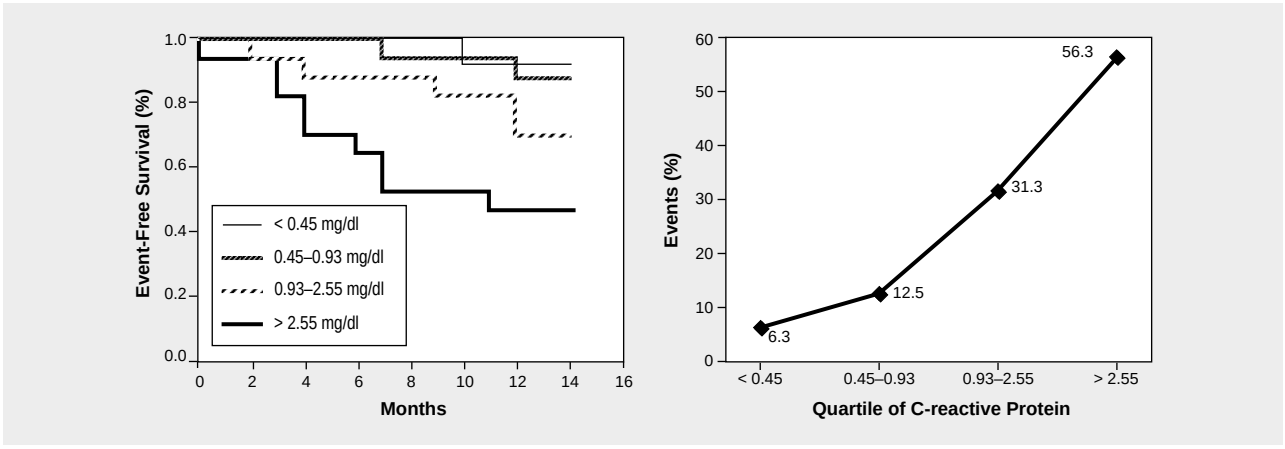

Figure 2. (Left) The event-free survival with respect to level of CRP in patients after an uncomplicated myocardial infarction. (Right) The distribution of events per quartile of CRP elevation. (Reprinted from the *American Journal of Cardiology*, vol 83, Tommasi et al, "C-reactive protein as marker for cardiac ischemic events in the year after a first, uncomplicated myocardial infarction," pp 1595-1599, Copyright 1999, with permission from Excerpta Medica.)

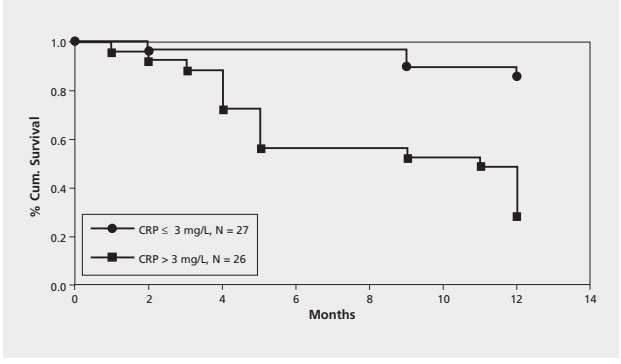

Figure 3. Cumulative event-free survival in patients with unstable angina, negative cardiac enzymes, and elevated discharge CRP. (Reprinted, with permission, from Biasucci et al, "Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability," Circulation 1999; 99(7):855-860.)

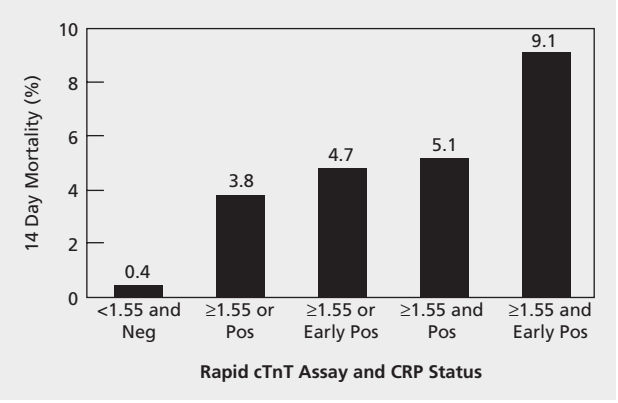

Figure 4. Independent and additive predictive value of CRP and cTNT (early positive defined by being positive in <10 minutes) on 14-day mortality. (Reprinted with permission from the American College of Cardiology Foundation *Journal of the American College of Cardiology*, 1998, vol 31, pp 1460-1465.)

MI, non-Q MI, unstable angina, stable angina, in patients who have undergone CABG and percutaneous coronary stenting, and recently in apparently healthy men and women. Recently the FDA has approved the use of a high-sensitivity CRP assay (Behring) as a prognostic test in the evaluation of patients with or expected atherosclerosis. This test has been most studied in those patients without clinically apparent atherosclerosis as a predictor of future cardiovascular events based on tertile of elevation.

Tumor necrosis factor-α (TNF-α) and other mediators

TNF-α is a pleiotropic proinflammatory cytokine with a wide range of effects that extend across a spectrum of pathologic conditions. Present in atherosclerotic lesions,[78] TNF-α appears to be one of the most important influences on the progression of atherosclerosis. Its upregulation is known to mediate and amplify a multitude of interactions resulting in progressive inflammation, plaque destabilization, and prothrombotic tendencies[79-87] **(Table 2)**. Treatment with a chimeric mAb to TNF-α has been shown to suppress inflammation and improve patient well-being in rheumatoid arthritis. Administration of anti-TNF-α Ab was recently shown to rapidly downregulate a spectrum of cytokines (IL-6), cytokine inhibitors (TNF receptors p75 and p55), and acute-phase proteins (amyloid A, haptoglobin, and fibrinogen).[88] This potent suppression of markers and mediators of inflammation may have tremendous potential in preventing progression of atherosclerosis.

IL-6 and IL-1 Ra (IL-1 receptor antagonist) not only have been shown to be elevated in the setting of ACS, but also are associated with increased risk of in-hospital events.[89] IL-6, produced by a variety of inflammatory cell types, has been shown to remain elevated up to 4 weeks after a myocardial infarction. Its properties increase fib-

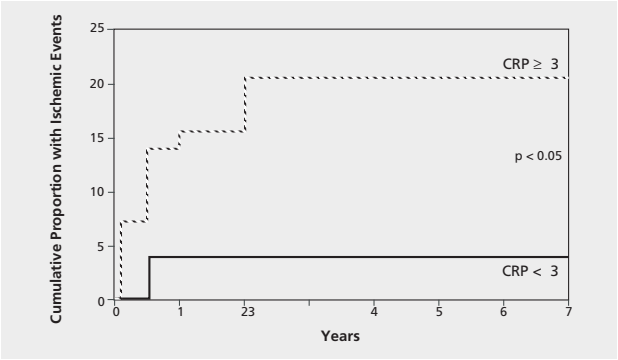

Figure 5. Cumulative proportion of ischemic events in patients with elevated CRP prior to coronary artery bypass grafting. (Reprinted from the *American Journal of Cardiology*, vol 84, Milazzo et al, "Elevated levels of C-reactive protein before coronary artery bypass grafting predict recurrence of ischemic events," pp 459-461, Copyright 1999, with permission from Excerpta Medica.)

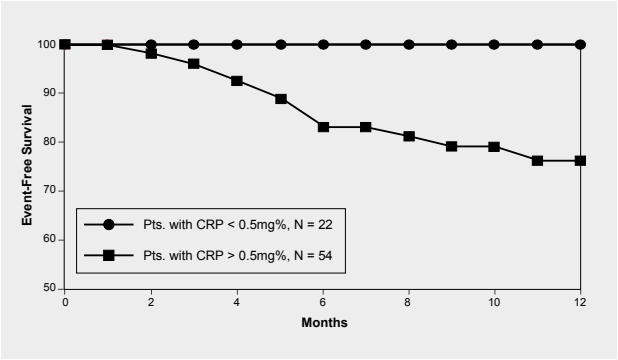

Figure 6. Event-free survival with respect to persistent elevation of CRP >72 hours after coronary stenting. (Reprinted from the *American Journal of Cardiology*, vol 82, Gaspardone et al, "Predictive value of C-reactive protein after successful coronary-artery stenting in patients with stable angina," pp 515-518, Copyright 1998, with permission from Excerpta Medica.)

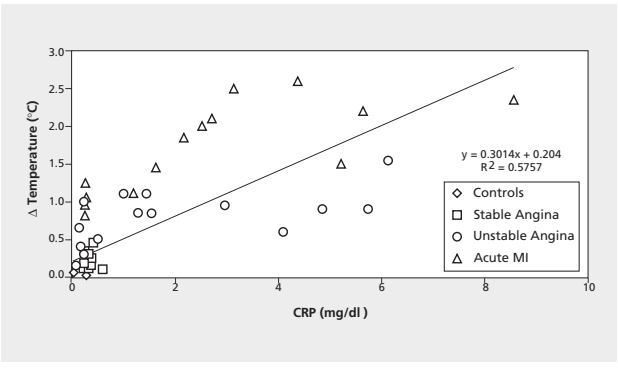

Figure 7. The correlation between thermal heterogeneity and level of CRP in control, stable angina, unstable angina, and acute myocardial infarction patients. (Reprinted, with permission, from Stefanadis et al, "Thermal heterogeneity within human atherosclerotic coronary arteries detected in vivo," Circulation 1999; 99(15):1965-1971.)

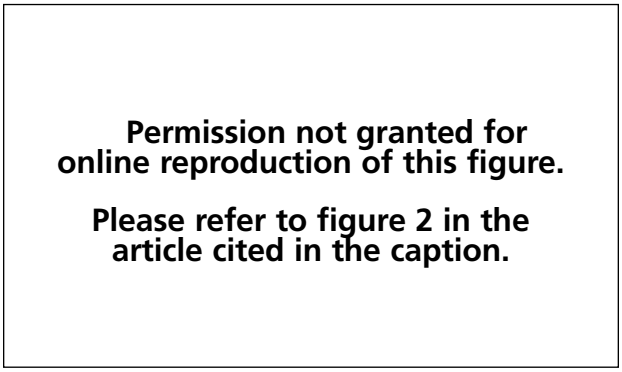

Figure 8. Predictive value for lipoprotein(a), total homocysteine, total cholesterol, fibrinogen, t-PA antigen, ratio of total cholesterol to HDL, CRP, and CRP plus total cholesterol HDL ratio. (From Ridker PM et al, Ann Intern Med 1999; 130:933-937, with permission.)

rinogen and PAI-1, promote adhesion of neutrophils and myocytes during myocardial reperfusion, and produce a negative inotropic effect on the myocardium.[90-94] Pannitteri et al[95] reported that levels of IL-8 not only are elevated in the setting of acute myocardial infarction but that they precede the levels of IL-6 and parallel the kinetics of CPK. IL-8 is a powerful trigger for firm adhesion of monocytes to vascular endothelium, may play a potential atherogenic role by inhibiting local inhibitors of metalloproteinases in atherosclerotic plaques, and stimulates smooth muscle cell migration.[96,97] IL-4 and IL-13 have been shown to enhance the ability of activated human monocytes to oxidize LDL, thus potentiating its toxic effects.[98] OxLDL induces IFN-γ production by T-helper-1–like cells, which are known to inhibit local collagen synthesis by SMC, stimulate expression of tissue factor and CD40, and selectively induce MCP-1.[98-100] Many other cytokines have been implicated in immunity, inflammation, thrombosis, and angiogenesis.[101]

The above discussion underscores the vast trafficking, redundancy, and interplay of the cytokine system. Each mediator, though, must work through specific receptors and ultimately regulate gene expression of proteins vital to the potentiation and regulation of the inflammatory cascade. Nuclear factor κ-B (NF-κB), peroxisome proliferator-activated receptor activators (PPARs), CD40 receptor and its-ligand, and inducible cyclooxygenase enzyme (Cox-2) are avidly being investigated as we attempt to discover the final common pathway of inflammation and its role in atherosclerosis.

NF-κB

NF-κB is a transcription factor located in the cytoplasm of many cells as an inactive complex associated with a specific class of inhibitory proteins, called IκB. This complex binds and prevents nuclear translocation and DNA binding of NF-κB.[102] In response to inflammatory stimuli IκB is eventually degraded and NF-κB is released and transported to the nucleus. In the nuclei, NF-κB can initiate or regulate early response gene transcription by binding to promotor or enhancer regions.[103] NF-

TABLE 2
PROINFLAMMATORY AND THROMBOTIC PROPERTIES OF TNF-α

Inflammatory properties
Regulation of macrophage colony-stimulating factor
Regulation of cell adhesion molecules
Modulation of smooth muscle cell phenotype
Induction of IL-1 mRNA
Inhibition of endothelial cell apoptosis
Plaque destabilization
Induces smooth muscle cell interstitial collagenases
Neutral effect on tissue inhibitors of metalloproteinases
Thrombotic properties
Augments transcription and expression of tissue factor
Decreases in activity of thrombomodulin-C and tissue-type plasminogen activator
Increases production of plasminogen activator inhibitor
Increases release of Von Willebrand factor

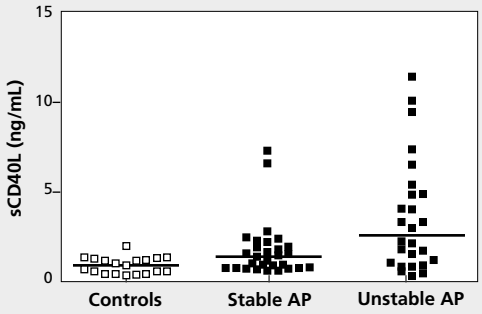

Figure 9. Levels of soluble CD40L in normal controls, stable angina, and unstable angina patients. (Reprinted, with permission, from Aukrust et al, "Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina," Circulation 1999; 100(6):614-620.)

κB is known to regulate or be regulated by genes involved in every aspect of the proinflammatory cascade.[104,105] TNF-α and IL-1 are two important inducers, contributing to a positive feedback loop for NF-κB activation. As a consequence, there is a continuous upregulation of cytokines and perpetuation of inflammation.[103] NF-κB has been implicated in a variety of inflammatory diseases, such as allograft rejection, rheumatoid arthritis (RA), asthma, and inflammatory bowel disease.[104] In RA, NF-κB is overly expressed in synovial tissue, associated with surface expression of cell adhesion molecules, production of cytokines, and upregulation of the inducible isoform of cyclo-oxygenase (Cox-2). These processes are parallel to those found in atherosclerotic lesions.[104]

NF-κB activity is enhanced by known cardiac risk factors such as very low-density lipoprotein, OxLDL, hyperglycemia, and elevated levels of angiotensin II. On the contrary, its activity is inhibited by HMG-CoA reductase inhibitors, antioxidants, and gallates (phenolic compounds found abundantly in red wine).[106-111] Recently, Ritchie[112] reported data showing that NF-κB is activated in patients with unstable angina without evidence of myonecrosis and is therefore potentially linked in plaque disruption. Immunosuppression with glucocorticoids, gold, cyclosporin, FK506, and, importantly, aspirin and salicylates is known to inhibit NF-κB. Kopp et al[113] demonstrated that aspirin inhibits NF-κB activity by preventing the degradation of IκB, while Weber et al[114] established aspirin's ability to inhibit TNF-α–stimulated NF-κB activity.

CD40 and CD40L

CD40 is a phosphorylated 49-kDa glycoprotein expressed on B-lymphocytes, fibroblasts, monocytes, platelets, epithelial cells, and endothelial cells.[115] CD40L, also named CD154 or gp39, belongs to the TNF family of cytokines. The presence of CD40 and CD40L has been found in human atheroma, and their association is implicated with expression of cell adhesion molecules, cytokines, matrix metalloproteinases, and tissue factor.[54,115] Anti-CD40L has been shown to regulate autoimmune diseases such as lupus nephritis, skin and cardiac allograft rejection, and multiple sclerosis in experimental models.[116-118] Mach et al[119] reported a reduction in aortic atherosclerotic lesion size, fewer T-lymphocytes and macrophages, and a decreased presence of cell adhesion molecules in atheroma in cholesterol-fed mice lacking the LDL receptor when treated with anti-CD40L antibody.[119] Aukrust et al[120] recently reported elevated levels of CD40-CD40L in patients with angina pectoris. Patients with unstable angina had significantly higher levels than those with stable angina, allowing the authors to conclude that presence of CD40-CD40L may have a pathologic role in plaque destabilization and the development of ACS[120] **(Figure 9)**.

PPAR

Peroxisomal proliferator-activated receptors (PPARs), including PPAR-α, PPAR-γ, and PPAR-δ, are a group of nuclear transcription factors playing a key role in adipogenesis and lipid metabolism.[121] Recently, modulation of the development and progression of atherosclerosis has been substantiated by research that appears to link PPAR activity with the regulation of inflammation and plaque stability by their interactions with macrophages, endothelial cells, smooth muscle cells, and metalloproteinases. Ricote et al[122] found PPAR-γ to be upregulated in activated macrophages and to inhibit gelatinase B, nitric oxide synthase, and scavenger receptors. OxLDL has been shown to induce PPAR-γ expression in macrophages, resulting in monocyte differentiation and enhanced uptake in OxLDL.[123,124] Max et al[125] recently reported elevated levels of PPAR-γ expression on monocytes in human atherosclerotic lesions as compared to normal controls. Furthermore, PPAR-γ stimulation leads to a concentration-dependent decrement in monocyte-derived metalloproteinase activity. Finally, PPAR-α and -γ have been implicated in the induction of macrophage apoptosis through

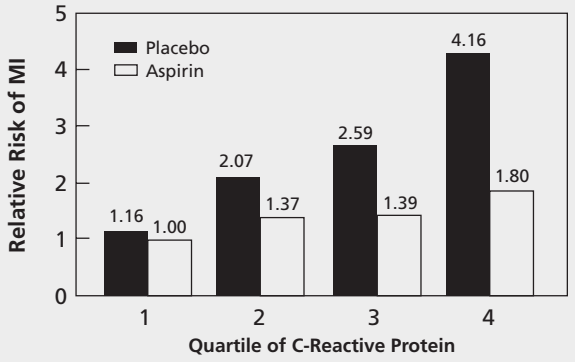

Figure 10. Relative benefit of ASA with respect to quartile of CRP. Data are shown allocated to ASA (open bars) and placebo (solid bars). (From Ridker et al, "Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men," N Engl J Med 1997; 336:973-979, with permission. Copyright © 1997 Massachusetts Medical Society. All rights reserved.)

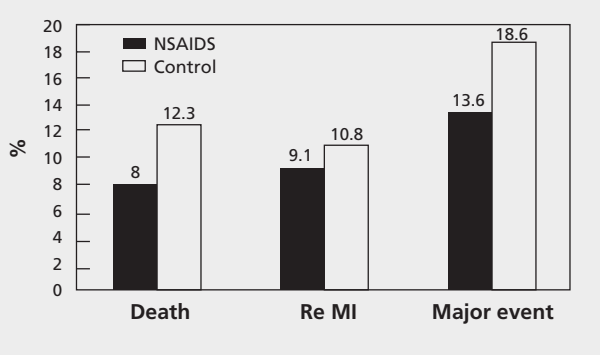

Figure 11. Cardiovascular events allocated to background of NSAID (solid bars) and control (open bars).

inhibition of NF-κB antiapoptotic pathways.[126] Endothelial cells also appear to be under the influence of PPARs by the regulation of leukocyte/endothelial cell interactions. Jackson et al[127] demonstrated an inhibitory effect of stimulated PPAR on endothelial cell expression of VCAM-1. In addition, stimulated PPAR-α has been shown to inhibit TNF-α-mediated endothelial cell VCAM-1 expression, COX-2 expression, IL-1 induced production of IL-6, and thrombin-induced endothelial-1 production.[128-130]

Key stimulatory PPAR ligands are naturally occurring prostaglandins, as well as synthetic antidiabetic and antilipidemic drugs. Gemfibrozil, a finofibrate and stimulator of PPAR-α, has recently been shown to dramatically reduce IL-1-induced production of IL-6, expression of COX-2 in human smooth muscle cells, and cardiovascular events in patients with low HDL levels. Importantly, this reduction in cardiovascular events was independent of LDL levels.[130,131] Troglitazone, an insulin sensitizer and PPAR-γ ligand, demonstrates a range of anti-inflammatory and potential plaque-stabilizing activities such as PPAR-γ–induced inhibition of macrophage metalloproteinases.[125]

Currently, the complex activities of PPARs and their ligands are not completely understood, although ligands with positive effects on lipid lowering (finofibrates) and glycemic control (troglitazone) would suggest that these transcriptional factors are clinically beneficial and mainly antiatherogenic.

Cyclo-oxygenase-2 (COX-2)

There are two distinct isoforms of cyclo-oxygenase (COX-1 and COX-2). These enzymes are necessary in the conversion of arachidonic acid to prostaglandin G2 and H2, which are potent agonists to the inflammatory cascade.[132,133] The ability of ASA and other NSAIDs to inhibit inflammation through their regulation of COX-1 was first described by Vane in 1971.[132] It was not until 1991 that an inducible form of the COX enzyme was discovered, COX-2.[134] Although a weak COX-2 inhibitor, aspirin and most available NSAIDs by virtue of their preferential COX-1 inhibitory effects, provide minimal anti-inflammatory action at doses not associated with significant side effects. COX-2 receptors are scantly expressed in the gastrointestinal tract or platelets and therefore likely provide augmented inflammatory control with few adverse effects.[135] COX-2 is felt to be the principal isoform that participates in inflammation and has been recently found to be widely expressed in atherosclerotic tissue.[136,137] Macrophage COX-2 mRNA expression has been shown to be induced by inflammatory cytokines such as INF-γ, TNF-α, and lipopolysaccharide, while other cytokines with anti-inflammatory properties, such as IL-10, have been shown to inhibit its induction.[138,139]

Speir et al[140] recently demonstrated a reduction in reactive oxygen species generation in CMV infected smooth muscle cells when pretreated with NSAIDs. This reduction was thought mainly to be due to inhibition of the COX-2 enzyme.[140] Although most investigations have described COX-2 as a proinflammatory mediator, recent reports by Cockerill et al[141] and Bishop-Bailey et al[142] have provided evidence for its anti-inflammatory potential. They demonstrated that HDL enhanced the expression of COX-2–dependent prostaglandin-I2, which is known to inhibit platelet and leukocyte activity. In addition, inhibition of IL-1-β resulted in upregulation of COX-2 and downregulation of the cell adhesion molecule ICAM-1. Although questions still remain, anti-inflammatory treatment such as aspirin, with its unquestionable beneficial effects, and a recent retrospective analysis of NSAIDs in patients after myocardial infarction that demonstrated a reduction in cardiac mortality and adverse events **(Figure 10)**,[143] suggest the potential for augmented clinical benefit with more potent and selective cyclooxygenase inhibition.

■ THE FUTURE OF INFLAMMATION CONTROL IN ACS

Aspirin, initially thought of mainly as an antiplatelet drug in the battle with atherosclerotic heart disease, is becoming more recognized for its anti-inflammatory properties. In addition to aspirin's COX-1 and weak COX-2 activity,

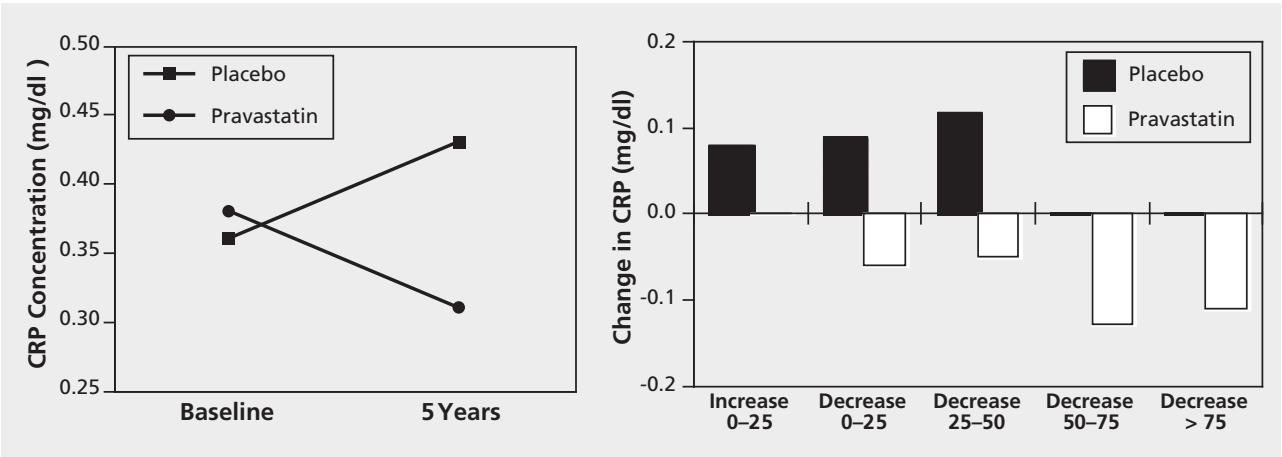

Figure 12. (Left) CRP at baseline and 5 years for patients treated with pravastatin or placebo. (Right) The change in CRP according to LDL level. Data are shown allocated to pravastatin (open bars) and placebo (solid bars). (Reprinted, with permission, from Ridker et al, "Long-term effects of pravastatin on plasma concentration of C-reactive protein," Circulation 1999; 100(3):230-235.)

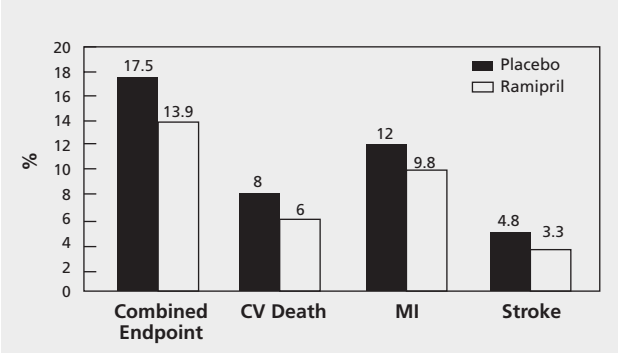

Figure 13. Cardiovascular endpoints allocated by patients receiving ramipril (open bars) or placebo (solid bars).

the inhibition of NF-κB activity is achieved by inhibiting both the degradation of IκB and effects of TNF-α. Clinical evidence to support aspirin's anti-inflammatory role has been reported by Ridker et al.[144] Aspirin reduced first MI in the Physicians Health Study, and this effect was directly related to the baseline CRP level[144] **(Figure 11)**. In addition, the recent negative results of the oral IIb/IIIa receptor inhibitor may be explained in part by the lack of aspirin's anti-inflammatory properties in the group receiving sole oral IIb/IIIa treatment.

A paradigm shift in thought may be evolving in favor of the anti-inflammatory properties of aspirin being more salient than its relatively weak antiplatelet effects in the reduction of ischemic cardiac events. HMG-CoA reductase inhibitors have been shown to dramatically reduce cardiovascular mortality and morbidity although the reduction in events is not linear with the reduction of LDL cholesterol below 125 mg/dL.[145] In an analysis of the Cholesterol and Recurrent Events (CARE) trial, Ridker et al[146] reported a significant 22% drop in CRP over a 5-year period in those treated with pravastatin versus placebo. Interestingly, CRP rose even in the placebo-treated arm which realized a reduction in LDL cholesterol **(Figure 12)**. Evidence continues to mount suggesting an anti-inflammatory role for HMG-CoA reductase inhibitors as these agents have been shown to alter regulation of DNA transcription, regulate natural-killer-cell cytotoxicity, inhibit platelet-derived growth factor-induced DNA synthesis, and decrease macrophage production of metalloproteinases.[146-149] ACE inhibitors have recently been demonstrated to possess potent anti-inflammatory properties that may explain their regulating effects on atherosclerotic driven endpoints. ACE inhibitors have been shown to exhibit antiproliferative and antimigratory effects on SMC and leukocytes, restore endothelial function, modulate platelet effects, and promote endogenous fibrinolysis.[150] The Heart Outcomes Prevention Evaluation (HOPE) study, a study of patients with vascular disease and no known heart failure, reported a dramatic and significant decrease in cardiovascular death, MI, and stroke in patients treated with ramipril versus placebo **(Figure 13)**.[151] Finofibrates and insulin sensitizers such as troglitazone are stimulators of PPAR receptors and are currently receiving attention for their anti-inflammatory and antiatherogenic potential. Unfortunately, not all methods of inflammatory control have realized a positive clinical outcome. Prevention of reperfusion injury in patients presenting with ACS by inhibiting leukocyte adhesion was recently reported from the HALT MI study. There was no significant reduction in infarct size and unfortunately a significant increase in infection rates in those randomized to high dose of the CD11/CD18 inhibitor.[152] This trial underscores the careful balance needed between adequate anti-inflammatory control and clinically significant immunosuppression.

Even though CRP and HsCRP have been shown to predict risk of future adverse cardiovascular events in virtually all patient subgroups, treatment options are limited to drugs not specifically heralded for their anti-inflammatory properties. Novel downstream approaches with the use of TNF-α, CD40L, NF-κB, and COX-2 inhibitors are under in vitro and animal investigations to

determine their potential role in the battle against atherosclerosis. Treatment of atherosclerosis as an inflammatory disease should first focus on those pathogens known to initiate and propagate this disease, such as hypercholesterolemia, hypertension, diabetes, hyperhomocysteinemia, smoking, and possible infection. The second approach should be to uncover the etiology of the nearly 50% of patients who present with an ACS without known cardiac risk factors. Finally, further investigation is needed to determine the clinical efficacy of adjunctive anti-inflammatory therapy on the background of pathogen directed treatment.

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Kidney transplantation and ANCA-associated vasculitis

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Although the outcome of treatment has improved considerably, about 20% of patients with ANCA-associated vasculitis (AASV) will develop renal insufficiency and will have to be treated with renal replacement therapy.[1] One of the options for these patients is renal transplantation. Renal transplantation has become a standard renal replacement therapy worldwide, and allograft survival rates have steadily improved over the last decades. The average cadaveric graft is now projected to function more than 13 years and the average live-donor graft for more than 21 years.[2] This means that we will not only be confronted more often with the question of whether kidney transplantation is the right option to choose for the patient with AASV and renal insufficiency, but that we also can expect to see a greater number of patients with recurrent glomerulonephritis after kidney transplantation. The relevance of the problem was recently emphasized by data from Australia.[2] In this country, from 1979 to 1988, the incidence of all-cause graft loss fell by 45.5 per 1,000 transplants (95% CI, 40.9 to 50.2), largely because of a fall in the incidence of graft loss caused by acute rejection. In contrast, the incidence of recurrent disease rose by 1.3 per 1,000 (95% CI, 0.6 to 2.1). In line with these findings, it has also been shown that recipients of human leukocyte antigen (HLA)-identical living related donor grafts rarely experience rejection, however at the expense of a high rate of recurrent glomerulonephritis.[3] In a study from the Netherlands, recurrence of glomerulonephritis was present in 36 to 42% of those biopsied, resulting in 24% of graft losses.[3]

It is clear that information on the long-term course of AASV patients after transplantation is crucial for practicing physicians, to guide their patients in making the choice for or against renal transplantation. In addition, the ongoing development by pharmaceutical companies of new immunosuppressive drugs for application after kidney transplantation may inspire us to try new immunosuppressive protocols for induction or maintenance treatment of AASV before the disease has caused renal insufficiency. For example, studies with mycophenolate mofetil have been reported[4] and are ongoing.

In this short review I will discuss indications and contraindications for renal transplantation in AASV patients, recurrence of vasculitis after transplantation, and the impact of post-transplant immunosuppression on immunoregulation in AASV.

INDICATIONS AND CONTRAINDICATIONS FOR RENAL TRANSPLANTATION

It is well established that quality of life improves significantly after kidney transplantation as compared to hemodialysis or peritoneal dialysis treatment. Only rather recently could it be shown that transplantation also results in a significant prolongation of patient survival.[5] It is very difficult to demonstrate that this is also true for AASV patients. In a recently published single-center retrospective study,[1] transplanted AASV patients were younger than those remaining on hemodialysis. This makes the better patient survival observed in the transplanted patients difficult to interpret. It would require a multicenter case-control study with transplanted AASV patients as cases and AASV patients on the waiting list as controls to document a beneficial effect of transplantation on patient survival or relapse rate. To the best of my knowledge, such a study has not been reported. It has to be doubted that such a study is feasible.

Are AASV patients especially at risk for opportunistic infections or other transplantation-related complications? Although this subject has never formally been studied, patients with severe atrophy of the nasal mucosa and local infections or with a limited bone marrow reserve after repeated courses of cyclophosphamide therapy are prone to develop infections after transplantation that are difficult to treat. One should therefore be reluctant to put such patients on the waiting list for kidney transplantation. In addition, it has been reported that, in such patients, reactivation of CMV infection after transplantation may lead to thromboembolic complications such as venous thrombosis of the extremities and pulmonary embolism.[6]

How relevant are ANCA-titers in the pretransplant work-up? The utility of ANCA as a predictor of relapse has generated some controversy. We found that ANCA titers, even of IgG subclasses, or detected with the newest catching ELISAs, failed to predict relapses.[7] Reports of successful renal transplantation in the face of a positive myeloperoxi-

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dase (MPO)-specific ANCA were presented by Noel et al in 1993,[8] by Grotz et al in 1995,[9] and Frasca et al in 1996.[10] In a pooled analysis of recurrence after transplantation in 39 AASV patients,[11] there was no statistically significant difference in the relapse rate between those with and without circulating ANCA at the time of transplantation. So, unlike the situation in anti-GBM disease, where persisting anti-GBM antibodies are associated with a much higher recurrence rate, positive ANCA titers in patients who are clinically well are not a contraindication for transplantation.

ANCA-associated disease is known to occur in families,[12] and the occurrence of AASV after nephrectomy in a kidney donor has been described.[13] Are AASV patients good candidates for living, related transplantation? Since there is no registry of living donors related to AASV patients, this question is difficult to answer. One could specifically screen for AASV-related signs and symptoms and measure ANCAs and other autoantibodies in potential donors related to AASV patients. In case of positivity, another donor should be sought.

■ RECURRENCE

A comprehensive, pooled analysis of all reported series of AASV using the terms *transplantation, vasculitis, Wegener's granulomatosis,* and *microscopic polyangiitis,* was published in 1999[11]; nine reported series and patients from Lund and the author's own series from the Chapel Hill region were included, covering a total of 127 patients. In order to avoid reporting bias, only case series (including more than one patient) were included. Doing a PubMed search on January 12, 2002, I was unable to find additional later case-series.

The major findings of this important study[11] are as follows:

Recurrent AASV occurred in 22 of 127 patients, corresponding to a relapse rate of 17.3%. The average time to relapse was 30.9 months, ranging from 4 to 89 months. (There are single case reports of relapses later after transplantation.[14]) Of the 21 patients with recurrent disease for whom clinical information was available, renal involvement occurred in 12 patients, whereas 10 patients had relapsing disease affecting extrarenal organs only. Recurrent vasculitis affecting the upper respiratory tract occurred in eight patients, the lungs in six patients, the gut in two, the skin in four, the joints in four, and the eyes in two.

The length of renal replacement therapy prior to transplantation was available on 7 patients who suffered a relapse and 41 patients who did not relapse. There was no statistically significant difference in the distribution of time on dialysis prior to transplantation between relapsers and nonrelapsers. Of the 16 patients with relapse for whom treatment information was available, 12 received cyclophosphamide, 3 received azathioprine (in addition to cyclosporin A and prednisone), and 1 received high-dose methylprednisolone alone. In 11 patients remission could be induced. There was no statistically significant difference in the relapse rate between patients treated with cyclosporin A and those not receiving cyclosporin A. Relapses occurred in 20.4% of patients with Wegener's granulomatosis compared with 15.7% of patients with microscopic polyangiitis or necrotising crescentic glomerulonephritis (NS). Similarly, patients with C-ANCA were no more likely to suffer a relapse than patients with P-ANCA (20 vs 17.2%).

The relapse rate in this analysis (17%) is somewhat lower than the expected rate reported in nontransplant patients, which ranges from 30 to 45%.[15,16] In the series from the Hammersmith Hospital, London, UK, the vasculitis relapse rate after transplantation was only 0.02 per patient per year as compared to approximately 20% on dialysis.[1] These data suggest that maintenance immunosuppression after kidney transplantation lowers relapse rates of AASV. How could standard post-transplant immunosuppressive therapy have such a favorable influence?

■ IMPACT OF POST-TRANSPLANT IMMUNOSUPPRESSION ON IMMUNOREGULATION IN AASV

It is well known that current immunosuppressive drugs, applied after kidney transplantation, mainly affect cellular immunity compared with humoral immunity. Relevant preexisting alloreactive antibodies, such as anti ABO-blood-group antibodies, must therefore be removed by invasive procedures such as plasmapheresis, combined with splenectomy, to make successful transplantation possible. Interestingly, azathioprine maintenance immunosuppression gives much better long-term results in these patients compared with cyclosporin A.[17] It is therefore not very likely that the favorable effect of immunosuppressive drugs, such as corticosteroids or calcineurin-inhibitors, on the vasculitis relapse rate is mediated by an effect on the humoral immune response or inhibition of the production of preexisting ANCA. Theoretically, this could be different for azathioprine or mycophenolate mofetil.

Is there evidence that dysregulated cellular immunity is present in AASV?

Although cellular immunity against ANCA-antigens as a pathogenetic mechanism was postulated early after the discovery of ANCA,[18] it has been difficult to demonstrate that specifically in patients with AASV there is increased reactivity of lymphocytes with proteinase 3- of myeloperoxidase–antigen or antibody-derived peptides.[19,22]

The first convincing evidence of a dysregulation of cellular immunity in AASV was described in 1992 by Schmitt et al.[20] They measured soluble interleukin-2 receptor (sIL-2R) levels in 102 patients with Wegener's granulomatosis. Levels of sIL-2R were elevated in all patients, even in the absence of disease activity. However, levels of sIL-2R were significantly higher in patients who had relapses than in those who did not. These findings were later confirmed and extended.[21-23] All patients with AASV show signs of disturbed cellular immunity, such as reduced CD28 expression on CD3-positive cells and increased expression of the early T-cell activation marker CD 69 on CD3-positive cells, as well as of CD 38 on CD8-positive cells. These abnormalities persist during immunosuppressive therapy.

In a recent survey of our patients with AASV, we found a significantly increased expression of CD25 on CD4-positive cells also in patients without signs of disease activity; the pattern of the phenotypic lymphocyte subpopulation distribution appeared to be highly specific for AASV (Neumann I et al, Abstract, this meeting). It is unlikely that this T-cell abnormality is secondary to increased antigen-presenting activity by myeloid cells, since the latter

phenomenon can only be demonstrated during active disease.[24] The persistence of dysregulated cellular immunity during remission in AASV might explain the relapsing course observed in most patients. Furthermore, the successful treatment of AASV with agents that directly affect T-cells such as monoclonal antibodies to CD4 and CD52[25] or rabbit anti-thymocyte globulin (ATG)[26] supports the hypothesis that dysregulated cell-mediated immunity is a proximal event in the pathogenesis of AASV. It will therefore be interesting to see what effects newer immunosuppressive agents applied after kidney transplantation with effects on cellular immunity—such as anti-ILR-2 monoclonal antibodies, deoxy-spergualin, or rapamycin—will have on lymphocyte phenotype and recurrence rates of AASV in transplanted patients.

CONCLUSIONS

Kidney transplantation is a realistic therapeutic option for patients with renal insufficiency and AASV. Ongoing disease activity, persisting infections, or irreversible damage caused by previous immunosuppressive therapy are contraindications for transplantation. There is no reason to believe that the duration of dialysis therapy or the nature of the AASV and/or ANCA will have a profound impact on the relapse rate after transplantation. Since AASV may occur in relatives, care must be taken to rule out AASV in potential living related organ donors. Prognosis for patient and graft survival after transplantation in AASV is good, and relapse rates are lower compared to hemodialysis.

The favorable effect of kidney transplantation on relapse rate could possibly be mediated through post-transplant maintenance immunosuppression. Since dysregulated T-cell immunity is likely to be key to the pathogenesis of AASV, kidney transplantation could therefore be considered a model for learning which new immunosuppressive drugs (often used in large industry-sponsored trials) have the strongest favorable effect on the course of the disease. Immunosuppressive drugs used to treat or prevent transplant rejection could be tried as induction or maintenance treatment in new AASV patients. A prospective randomized trial for maintenance therapy in patients in remission (IMPROVE-PROTOCOL) with mycophenolate mofetil has recently been started in Europe.

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Surgical treatment of Takayasu's disease

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ABSTRACT

Takayasu's disease affects young females in the second and third decade of life. During the chronic phase, the aorta and its major arteries become stenotic, causing significant sequelae. Surgical treatment is possible with expectation of good results. The author advises treatment of stenotic arteries that are potentially dangerous or that adversely affect lifestyle with either percutaneous transluminal angioplasty or surgery. The author emphasizes the difference between surgical procedures for atherosclerosis versus a procedure for Takayasu's disease.

I. INTRODUCTION

Takayasu's disease (TD) is an unusual illness that affects young females in the second and third decade of life by causing inflammation of the major large arteries.[1] The disease has two phases. The acute phase presents with systemic signs and symptoms of inflammation including fatigue, weight loss, fever, loss of appetite, and pain over involved arteries. This acute phase is infrequently diagnosed and sometimes confused with a viral illness. The disease then progresses to a more chronic phase. During this time stenotic lesions develop in the thoracic and abdominal aorta and its major branches. The patient then presents with signs and symptoms depending on the location of the involved arteries. This chronic stage could cause differences in blood pressure in the upper extremities; bruits over the carotid and subclavian arteries and abdominal aorta; weak distal pulses; angina; myocardial infarction; hypertension; and transient ischemic attacks or strokes.

Although there is a growing experience with magnetic resonance angiography and CT angiography, standard arteriography is still the preferred method of diagnosis.[2-5] Since this disease can affect arteries above and below the diaphragm, it is recommended that all arteriographic studies on patients with the diagnosis of TD include the major arterial system from the arch of the aorta to both femoral arteries. Characteristic findings on arteriography include long, tapered stenoses that show a smooth intimal lining that involves the entire artery and not just the bifurcations. The subclavian, axillary, carotid, and to a lesser extent the abdominal aorta and renal arteries are the arteries most commonly involved.

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The decision to recommend surgery can be difficult. These patients are young and frequently less tolerant of lifestyle changes forced upon them by TD. Involvement of a major arterial system makes them susceptible to significant medical sequelae including stroke, hypertension, congestive heart failure, and myocardial infarction. However, the surgery itself is complicated and has its own risks and complications. Obviously the final decision will require careful consultation between the attending physicians and the patient.

Basic principles of surgical treatment

Takayasu's disease is not atherosclerosis. Therefore surgical treatment is different for patients with TD than for patients with arterial problems associated with atherosclerosis. The following are general principles to be considered in patients with TD who are candidates for surgery:

1. Patients with TD are frequently young. One should not assume that because the patient is young, he or she may not have significant medical problems. The multi-arterial involvement by TD could mean that the individual has significant renal disease, cardiac disease, and other problems that could affect the overall surgical outcome. Therefore, a complete preoperative evaluation is essential.
2. It is important that surgeons be conservative with the management of patients with TD. Long-term prognosis for patients with TD properly treated is actually quite good.[6] Surgery should be used only if there is a very significant problem that would affect a patient's prognosis or significantly interfere with the patient's lifestyle.
3. Percutaneous transluminal angioplasty (PTA) has become an effective alternative to surgery.[6] Initially, there was considerable concern about the long-term overall results of PTA. Dilating a chronic lesion might initially be successful, but long-term follow-up might show a restenosis. Restenosis does occur, but with the development of stents, the long-term incidence of restenosis may be considerably less. Therefore, in the initial management of patients with TD who are candidates for operation, consultation with appropriate radiologic interventionists to determine the feasibility of PTA is appropriate.
4. The basic surgical procedure is a bypass operation. The bypass should always be between arteries uninvolved with TD. Arteries that appear normal on arteriography should be used for the proximal and distal anastomoses. It is believed that the incidence of anastomotic problems in arteries that are without dis-

ease will be considerably low. However, note that even if the artery looks normal on arteriography, there is still a 44% incidence of microscopic involvement with TD, so these patients could still have anastomotic problems in the future.[7]

5. Endarterectomy is almost never performed on patients with TD. The pathology involves all three layers of the artery, and removing the intima and part of the media is difficult and at times impossible.
6. Emergency or urgent surgery is usually not necessary and should be avoided. In general, patients with TD develop chronic lesions over a long period of time and, therefore, do have the benefit of collateral formation.
7. Surgery should be avoided during the acute phases of the illness. Most reports suggest but do not prove that anastomatic problems are less likely if the patient's illness is quiescent.

II. CEREBROVASCULAR DISEASE

The major branches of the aortic arch are the arteries most commonly affected with TD. Patients with atherosclerosis commonly have a stroke due to emboli originating from the carotid bifurcation. However, patients with TD do not have focal narrowings of atherosclerosis but instead have long tapered narrowings that may suddenly occlude, causing a stroke.

To understand this, consider the normal anatomy of the aortic arch. The three main arteries off the arch are the innominate, the left carotid, and the left subclavian. All three vessels provide important blood flow to the brain. The innominate gives off the right carotid and the right vertebral, so essentially all circulation for the right side of the brain comes from the innominate. The left carotid and the left subclavian, which gives off the left vertebral, provides circulation to the left side of the brain. Involvement of these major arteries and their branches is quite common. It is not unusual to have patients in their initial arteriography show occlusion of one or more of these major arteries. Therefore, if one or more arteries are occluded and other arteries are involved, sudden occlusion of the involved arteries could cause a catastrophic loss of blood flow to the brain. In the author's original presentation, four patients presented with cerebrovascular accidents and all four of these patients had occlusion of one or more thoracic aortic arch arteries.[8]

Patients who present with these multiple occlusions of the major thoracic aortic arch arteries present a significant dilemma. They may not be symptomatic. Their arteries may be involved but not occluded. Nevertheless, the author feels that if there is significant narrowing or occlusion of major arteries such as the innominate, left carotid, subclavian, or their branches, a bypass should be done originating from the aortic arch to the arteries distal to the disease to improve blood flow and prevent a stroke if one of the narrowed arteries suddenly occludes.

In the past, the author was reluctant to recommend PTA for treating patients with involvement of the major arteries of the aortic arch. However, more recently, PTA and stenting appear promising. Therefore, it would be advisable to consider PTA particularly for lesions involving either the right or left common carotid artery. These lesions tend to be long, tapered narrowings that can be treated with PTA and stent placement.

III. LOWER EXTREMITIES

The abdominal aorta below the diaphragm is the artery most commonly involved in patients with TD. The distribution of the disease is quite peculiar.[9] It usually begins below the renal arteries and then ends just above the aortic bifurcation. There is a unique sparing of the distal aorta. These patients can develop severe claudication, which is poorly tolerated in these young patients. Since the development of disease in the abdominal aorta is slow over a period of time, collaterals frequently develop. Therefore, it is not common to have severe ischemic symptoms such as rest pain, ulcerations, or gangrene.

The patient with abdominal aortic involvement presents interesting challenges. Most abdominal bypass operations for patients with atherosclerosis begin in the abdominal aorta below the renal arteries and go to either both femorals or iliac arteries. However, since the abdominal aorta below the renal arteries is frequently involved with TD, it is inappropriate to originate a bypass from that part of the aorta. The aorta between the diaphragm and the renal arteries is possible as an inflow source but is technically difficult. The author recommends that the proximal anastomosis begin at the thoracic descending aorta and the distal anastomosis to the left iliac artery. Since the distal part of the aorta is spared, a bypass to one iliac artery can perfuse both legs. The procedure is all done retroperitoneally, which is tolerated well with excellent relief of symptoms and good long-term results.

IV. UPPER EXTREMITIES

The axillary and subclavian arteries are commonly involved with TD, reducing blood flow to both upper extremities. Since muscle mass in the upper extremities is relatively small, symptoms related to exercise of the upper extremities are unusual, as is gangrene and ulcerations in the hands. A significant problem, however, in patients with involvement of the subclavian or axillary arteries is the inability to obtain reliable blood pressure measurements. Hypertension is one of the frequent medical complications of TD, usually due to renal artery or mid-aortic involvement. But with both arms involved with TD, it is impossible to obtain reliable blood pressures, and therefore the diagnosis and treatment of patients with hypertension is a major problem. An attempt to revascularize the upper extremities in order to obtain reliable measurements of blood pressure is a major indication for intervention.

The subclavian steal syndrome can occur in patients with occlusion of the subclavian arteries proximal to the origin of the vertebral artery. Blood is "stolen" from the brain because it flows retrograde down the vertebral artery to supply the arm. Although the incidence of subclavian involvement is high, the "steal" syndrome is unusual because involvement of the arteries is proximal and distal to the origin of the vertebral arteries. But more recently, a few isolated cases of subclavian steal have been reported.[10]

Fortunately, PTA of the subclavian or axillary arteries is becoming more common, particularly with the availability of stents. This procedure is relatively easy to per-

form and seems to yield overall good results.[6] If percutaneous transluminal angioplasty is not possible, then revascularization of the upper extremities should be performed.

V. RENAL ARTERY DISEASE

Hypertension secondary to renal artery disease is common. The morbidity and mortality from TD frequently is due to unrecognized and untreated hypertension. Congestive heart failure, left ventricular hypertrophy, and myocardial infarction can occur in these relatively young patients. Therefore, any patient with TD and hypertension must have the renal arteries evaluated. Magnetic resonance angiography and Duplex scan of the abdominal aorta and its branches can be an appropriate screening device.

Percutaneous transluminal angioplasty is now the preferred method for treating patients with renal artery stenosis.[11-14] Unlike other arteries involved with TD, the renal artery usually has a short segmental lesion. Therefore, the angiographer would have relatively little difficulty in placing a balloon in that area, dilating it, and then placing a stent to guarantee success of the dilatation. In the past, the results of renal artery dilatation were questionable because of the frequency of restenosis and recurrence. However, the use of the stents may be helpful in reducing the overall incidence of restenosis, and this method can revascularize both renal arteries with improvement in blood pressure.

If percutaneous transluminal angioplasty cannot be performed or is unsuccessful, then surgical bypass is indicated. Usually, bypasses of renal arteries originate from the abdominal aorta below the renal arteries. However, if this is involved with TD, this approach is not possible. The hepatic and/or the splenic arteries have been used as inflow to either renal artery as a method to provide improved blood flow and reduce the incidence of hypertension.

VI. ANEURYSMS

Aneurysms are relatively uncommon in patients who are born and raised in the United States. This is different than experiences in both Japan and India in which abdominal and thoracic aortic aneurysms are relatively common.[15,16] It is not quite clear why there is such a difference. The incidence of rupture of either the abdominal or thoracic aortic aneurysms is not known. It would appear to be quite low on the basis of published reports. However, one must understand that these young patients may have 40 to 50 more years of life expectancy, and therefore the risks of aneurysm rupture over that period of time might be quite significant.

Repair of abdominal and thoracic aortic aneurysms is indicated if they reach sizes greater than 5 cm. The new endovascular approach may provide an interesting alternative to patients with abdominal aortic aneurysms from TD. Currently, the author is not aware of any successful placement of endovascular grafts in patients with either abdominal or thoracic aortic aneurysm from TD.

Aneurysms also occur in the innominate and carotid arteries. This presents technical problems, since resection and replacement of these arteries can be difficult with a relatively high incidence of stroke. These cases must be handled individually depending upon the extent of involvement and the symptoms.

VII. SURGICAL RESULTS

Surgical results for operations on patients with TD are good. There is a very low morbidity and mortality. There is always a concern of the development of anastomotic aneurysms in arteries that have been used for either inflow or outflow bypasses. The author's experience has been that anastomotic aneurysms are relatively uncommon.[8] Constant surveillance of patients who have undergone these procedures is necessary. Anastomotic stenoses, however, do develop. It is not quite clear whether they are due to the recurrence of TD at the level of the anastomosis or other reasons.[8] Nevertheless, they can be treated with either another surgical procedure or possibly even dilatation.

VIII. CONCLUSION

Takayasu's disease is a difficult problem confronting the vascular surgeon. Most vascular surgeons have very little experience with this entity. It would not be unusual for a surgeon to see no more than one or two patients with this disease throughout his or her entire career. Nevertheless, the vascular surgeon does possess the skills and the experience necessary to provide the appropriate procedure with expectation of good results in those patients who have serious problems due to TD. However, he or she must be aware of the differences between patients with TD and those patients with atherosclerosis.

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New approaches to the management of subglottic stenosis in Wegener's granulomatosis

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OBJECTIVES

1. Describe new modifications in the management of laryngeal manifestations of Wegener's granulomatosis (WG).
2. Present a conservative yet aggressive method for long-term management of subglottic stenosis.
3. Present and demonstrate how tube-free speech-ready tracheostomy can be applied to the more challenging cases of WG.

The specific etiology and pathophysiology of subglottic stenosis in Wegener's granulomatosis is not clearly understood. It is postulated that this segment of the airway is particularly vulnerable to pathological processes affecting the microcirculation, as it is a junction between two embryological growth centers. Support for this argument is found in the anatomy of the stenosis being localized to the subglottic region at the junction of two microcirculations. The true vocal cords and the trachea above and below the stenotic ring are almost completely spared, which further reinforces the microcirculation hypothesis. This ring-like inflammatory process develops approximately 1.5 to 2 cm below the true vocal cords and above the trachea resulting in progressive airway obstruction. The normal mucociliary transfer system is disrupted and tracheobronchial secretions are trapped at the level of the stenosis and below it, resulting in secondary infections. The forced airflow through this narrow segment creates turbulence, which is aggravated by coughing and attempts to clear secretions. The overall effect is to further irritate the involved tissue, precipitating a vicious cycle that may progress to life-threatening airway obstruction.

The subglottic stenotic lesion is membranous in most cases and is limited to a short or narrow segment of the lower larynx. Similar stenotic lesions may appear at the junction between the trachea and the right or main bronchi and sometimes even further down the tracheobronchial tree, at other areas where different microcirculations meet. In some instances, the stenotic process partially or completely resolves with systemic treatment such as steroids and immunosuppressant medications. In more stubborn cases, a local surgical intervention can reestablish a stable and secure airway and promote local healing that may control and possibly prevent persistent or recurrent stenosing processes. Contrary to treatment of laryngotracheal stenosis caused by different etiologies such as trauma, local treatment of subglottic stenosis in WG must also focus upon subduing or reversing the manifestations of the systemic disease. The surgical method of treatment must also take into account the unique nature of the pathological process, which is predominantly caused by vasculitis. Certain surgical modalities such as laser surgery, stents, and reconstructive procedures have higher tendencies to aggravate circulation disorders and may be contraindicated. Given their rheumatologic diagnosis and the systemic medications taken for it, many of these patients are not candidates for complex reconstruction or resection in any case.

Every possible measure should be taken to restrict and avoid potential damage to the vocal cords, the trachea, the bronchial tree, and the lungs in the course of the surgery and anesthesia. We present the current methods of treatment conjointly adopted by the Departments of Rheumatology and Otolaryngology at The Cleveland Clinic Foundation (CCF). The principles follow the original guidelines laid by Drs. Lebovics and Hoffman during their tenure at the NIH in Washington, DC. Certain technical modifications have been adopted over time.

Patient population

The patients differ according to their degree of subglottic involvement by the systemic disease, the stage of the disease, and, when applicable, previous treatments. Fiberoptic laryngotracheal bronchoscopy determines the degree and extent of the involvement at the level of the subglottic segment of the airway. Computed tomography

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performed in the axial and tangential planes is performed, especially in those chronic and previously treated cases that are more complicated. Consultation and coordination with the treating rheumatologist determines the scheduling and planning of surgical interventions. On the rare occasions when patients present with acute life-threatening airway obstruction even before the final diagnostic process is completed, emergent intervention is performed. Otherwise, we usually coordinate a thorough and prompt effort to establish the diagnosis and detailed clinical findings while the patient is prepared for surgery. These efforts include physical examinations, radiologic exam as described above, and appropriate laboratory studies. Prior planning with the anesthesiologists is imperative to ensure uncomplicated success in these sometimes very challenging cases.

■ ENDOSCOPIC TREATMENT

Using a specially designed articulating laryngoscope with ports for jet ventilation, the larynx is exposed and the patient is maintained under general anesthesia utilizing jet ventilation. Photographic documentation of the larynx and trachea is routinely achieved. In initial or virgin cases, a biopsy is taken to verify the diagnosis. Telescopic or flexible fiberoptic endoscopy is performed to assess the full extent of the tracheobronchial tree and rule out additional lesions. The stenotic lesion is injected submucosally with a long-acting corticosteroid suspension such as methylprednisolone. The solution is infiltrated along the submucosal-perichondrial plane. A total of 80 mg is injected. Under microscopic magnification, four to six lysing longitudinal incisions are made in a star-like fashion, employing sharp metal microlaryngeal blades. These incisions release the constricting stenotic ring and break it up, widening the diameter of the airway and simultaneously preserving islands of intact mucous membrane between the incisions. This epithelium is intended to regenerate and re-surface the expanded lumen. Progressive serial dilatations are performed using semi-rigid, flexible, smooth Malloney dilators lubricated by topical steroid cream. If bleeding occurs, it is controlled by topical application of 1:1,000 adrenaline, and the lesion and tracheobronchial tree are irrigated. The next stage of the procedure involves repeated topical applications of mitomycin-C over a 6-minute period with the intent to further inhibit fibrosis and re-stenosis. The airway is suctioned out and cleared of reactive secretions and blood. Finally, 4% Lidocaine is squirted down the tracheobronchial tree, to act topically to prevent postsurgical reactive laryngospasm as the patient is awakened. A bolus of systemic steroids is administered intravenously during the procedure. The procedure is performed in full coordination with the anesthesiologists throughout each stage. Postoperatively, the patients receive respiratory treatments in aerosol form for the twelve hours after the procedure and are discharged the following morning.

Throughout this group's experience, we have never used laser surgery on subglottic stenosis caused by WG. Incidentally, the remote cases in which patients were treated with laser surgery in other institutions prior to their referral to the CCF developed complicating secondary stenosis that required more extensive surgical intervention to overcome the severe secondary superimposed damage, most probably induced by management with laser and other surgical methods. These patients required laryngotracheal reconstructive procedures, or had to undergo establishment of permanent tracheostomies. In our experience, then, laser therapy is contraindicated is this patient population.

Results

To date, over fifty patients have been managed with this endoscopic dilatation procedure. Twenty-six have rheumatologic diagnoses, twenty-five with WG and one with pemphigoid. Thirty patients have needed repeated similar procedures. Only six patients suffering from WG with severe and complicated subglottic stenosis, all complicated or aggravated by unsuccessful surgical treatments including laser surgery, have had to undergo the surgical establishment of a long-term tube-free tracheostomy or laryngotracheal reconstruction. The only complication encountered during or following the endoscopic dilatation was secondary to the jet ventilation mode of providing anesthesia. Pneumothorax was encountered in one patient with known pulmonary involvement of her WG with history of past episodes of pneumothorax. This particular patient underwent three additional dilatation procedures since the pneumothorax without repetition or recurrence of this complication.

■ PERMANENT TUBE-FREE SPEECH-READY TRACHEOSTOMY

This procedure is a radically new approach to the management of chronic airway obstruction. When indicated, it provides safe, secure airflow through a bypass of the glottic or subglottic narrowing with preservation of the patient's voice, cough mechanism, and the swallowing process. Once the tracheostoma has healed, usually three weeks after the surgical procedure, the patient is free of the pain, discomfort, and complications of the indwelling tracheotomy tube which is otherwise a prerequisite for the maintenance of conventional tracheotomy. Within two months postoperatively, 70% of the patients with long-term tube-free tracheostomy develop the ability to constrict their tracheostomal opening for unaided production of normal voice and effective cough. A supplementary sling procedure is available to the minority group that fails to achieve effective constriction and sealing of the stoma for the purpose of producing speech and normal cough.

■ SUMMARY

Further development of conservative endoscopic procedures at the CCF has provided patients with dependable surgical adjuncts to the systemic medications of WG. In most instances, a single surgical dilatation procedure stabilizes the patients at an airway diameter that exceeds 50% of the norm, thereby rendering the patient almost asymptomatic at rest and minimally restricted during exercise. The more chronic patients presenting with history of previous surgical procedures performed on their larynx

and trachea, usually in other institutions, have been observed to require consecutive treatments after the original treatment, enjoying shorter symptom-free intervals. We have not encountered any local complications such as damage to the vocal cords, altered voice, or compromised structural integrity of the larynx and the trachea. The procedure has been found to be effective and well-tolerated as a means for treating, maintaining, and rehabilitating patients with chronic airway obstruction, particularly in those that have been initially treated by our service, and those that were managed from the very beginning of their disease. However, even the more difficult and complicated cases clearly demonstrate an improvement in their condition through the above treatment protocol, and the interval between treatments gradually increases in this group as well.

The more aggressive long-term tube-free tracheostomy procedure, usually performed only on difficult and select patients with severe complications, has proven itself to be a highly gratifying procedure, achieving a permanent mode of management for these patients which safely allows for almost complete freedom from symptoms combined with good tolerance and functional rehabilitation.

Video documentation will serve to further demonstrate the beneficial effects of both these modes of treatment.

Sinonasal complications of vasculitic diseases

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Systemic vasculitides may affect multiple organs in the body. Certain diseases, such as Wegener's granulomatosis or Churg-Strauss syndrome, are particularly prone to destructive effects in the nose and paranasal sinuses secondary to the underlying autoimmune process. These disease manifestations may occur externally, with alteration in the facies and appearance of patients with major destruction of the architecture of the bony paranasal sinuses. Results of these processes include infections that are multiple and vary from symptoms including pain, headache, and discharge to fevers and contiguous infections or potentially major organ system disasters. The orbit as well as the central nervous system are prime targets, and this paper will deal with some of those issues on a global scale using mostly patients with Wegener's granulomatosis as a model.

ANATOMY

Prior to understanding the disease processes of the sinonasal tract, one must understand both the gross and microscopic anatomy of these organs, with its complex architecture and tunneling, as well as the normal physiology of sinus and nasal homeostasis. This certainly helps to facilitate one's understanding of the infectious and inflammatory complications one may see in clinical practice.

The nose generally consists of lateral bony pyramids, also known as the nasal bones, with a midline structure coming perpendicular from the base of the skull known as the perpendicular plate of the ethmoid. Anteriorly, this bony plate attaches to two cartilaginous structures known as the vomer as well as the quadrangular cartilage. The floor of the nose is part of the roof of the hard palate, and this bony floor also separates these two anatomic compartments. The mucous membranes of the nose consist of a glandular epithelium, with a mixed respiratory glandular mixture that secretes large amounts of mucins, immunoglobulins, lactoferrins, and other immunological and active chemicals to help facilitate the cleansing of the upper airways. Laterally on the nasal wall are three turbinates, with the inferior being the largest and the superior the smallest. Under the inferior turbinate anteriorly exits the nasal lacrimal duct, which acts as a conduit for ocular secretions to leave the face of the globe and drain through the nose. Under the middle turbinate is the middle meatus, which generally drains the frontal sinuses as well as the anterior ethmoid complex of the maxillary sinus, and superiorly is the sinus ostium draining the posterior ethmoid cells. The sphenoid sinus drains directly into the back of the nose through its rostrum or anterior face. The natural ostium is classically at approximately a 23° angle from the floor of the nose.

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EXTERNAL FEATURES

Patients with granulomatous vasculitis, be it Wegener's or Churg-Strauss syndrome, may present with a cosmetic and functional deformity known as a "saddle-nose deformity." This is obvious from an external examination of the nose, and it consists of a depression and retraction of the midportion of the nose, often with tenting of the skin in that depressed region. Saddle deformities cause rotation of the nasal tip with an increase in the nasolabial angle and in very severe cases may have it approach 180°. In these patients, the lower lateral cartilages of the nose usually survive, thus producing a tip and opening that is more normal in appearance and often functionally viable. The etiology of a saddle-nose deformity from an anatomic perspective is loss of the dorsal septal cartilage.

The most common cause of saddle-nose deformity in the United States is iatrogenic, be it a complication of rhinoplasty or certain types of nasal septal surgery. Mechanical trauma, as well as blunt trauma in addition to chemical trauma, such as cocaine abuse, are other etiologies associated with this type of anatomic defect. The intranasal examination in these cases may often reveal a nasal septum with a perforation. In many patients with vasculitis, no perforation may be noted; however, there is clearly loss of the quadrangular cartilage. Other granulomatous diseases of a nonvasculitic origin can cause a similar picture, and these include acid-fast infection of the nose as well as diseases of unknown etiology, such as sarcoidosis.

Survival of the lower lateral cartilage is crucial from both a cosmetic and functional perspective, and any type of surgical restorative approach will generally rest on replacing that which has been lost anatomically. Since it is septal cartilage and a dorsal support that is lost, most surgi-

cal approaches for reconstruction will focus on that avenue. The upper lateral cartilages often survive in these patients, although they may be disfigured and partly eroded through the inflammatory destruction that occurs.

In patients with Wegener's granulomatosis who have a saddle-nose deformity, no clear-cut etiology for the cartilaginous destruction has been proven, although one may speculate as to its origin, and part of this paper will suggest a possible cause. It is well known that cartilage has low metabolic needs and hence uses a small blood supply, but nevertheless it does need blood, oxygen, and nutrients in order to survive. This blood flow generally comes to the cartilage through the perichondrium, which also can facilitate chondrogenesis with new chondrocytes, in an active process similar to that seen in bone from its periosteum and the osteoclastic osteoblastic cycles. With all that said, cartilage has a very tenuous blood supply, and small infringements on its vascular support will cause necrosis and destruction. This is well established in other cartilaginous structures, such as the ear, and diseases such as relapsing polychondritis and in infectious etiologies of the ear, such as *Pseudomonas*-based infections. The laryngeal cartilages are also susceptible to inflammation, stenosis, and destruction from both infectious and inflammatory reactions, and these diseases include etiologies such as glanders (*Pseudomonas mallei*), as well as acid-fast disease, chemical trauma (acid and lye ingestion), blunt and penetrating trauma, and complications of external beam irradiation which cause a local microvasculopathy.

In the case of patients with granulomatous vasculitis, the upper lateral cartilage to some degree, and the lower lateral cartilages to a greater degree, obtain their blood supply to the perichondrium through the overlying soft tissues and skin as well as the underlying vestibular skin. These skins are more of a squamous type of epithelium and generally less likely to be subject to a respiratory disease such as Wegener's or Churg-Strauss. My inference, therefore, is with less soft tissue inflammation in this region, the blood supply to the perichondrium for the lower lateral and usually to the upper lateral cartilages is preserved. The nasal septum, however, has more of a respiratory component to its mucoperichondrium, which is more likely to be susceptible to an attack of active Wegener's. The inference here is that since this tissue is more likely to be inflamed, the blood supply to the quadrangular cartilage and the anterior portion of the nasal septum is more likely to be compromised, particularly superiorly, with a resultant slow and insidious cartilaginous destruction. Externally, this manifests itself as a saddle-nose deformity. The nasal bones are covered by soft tissue and skin and as such are probably less susceptible to interruptions of blood flow due to inflammation of respiratory tissues.

I have seen cases through the years of patients with Wegener's granulomatosis, particularly those newly diagnosed, who recently underwent some sort of a septal procedure, either for biopsy or for functional reasons, and claim that they immediately lost their nose, ie, developed a saddle deformity after the surgery. While this is anecdotal at this time, I suspect there is already present a level of inflammation within the mucoperichondrium and by disturbing the tenuous blood supply during lifting these flaps off of the cartilage, one is essentially committing the nose to a cartilaginous breakdown. The upshot is that except in extreme and compelling cases, one should avoid any type of elective nasal septal surgery in patients with systemic vasculitis unless the patient is clearly in remission and there is no evidence of disease activity within the mucous membranes in the nose.

Reconstruction of the saddle deformity in Wegener's granulomatosis patients is a medical issue subject to debate; this author has experience with nearly ten such cases. I have also had the benefit of seeing the work of other physicians where more extensive types of rhinoplasty were performed and with unfortunately devastating consequences. The basic principle that should be considered is one of replacement of that tissue which is lost and to minimize any manipulations of other tissues. This is including but not limited to the upper and particularly the lower lateral cartilages. I do not believe there is value in dissecting them off of the soft tissue base that supports their vasculature, and in a similar vein, any type of bony work in the nose should be avoided.

In order to do a proper bony osteotomy, one needs to strip the periosteum off of the nasal bones in order to break them and reset them into their new configuration. In an analogous fashion to what was discussed earlier regarding cartilages, one may iatrogenically cause a loss of blood supply to the underlying bones with potentially catastrophic results from a cosmetic perspective. I remember one patient quite well who underwent a total of six procedures and ended with three major holes in the midface and region of the nose and was left with a residuum of lower lateral cartilage and nothing else. She dealt with the problem by keeping the areas covered with bandages as she walked in public.

■ DISEASE OF THE PARANASAL SINUSES

When Wegener described his first four cases of necrotizing granulomatous vasculitis in four autopsy specimens,[1] the classic description was one of inflammation within the kidneys, lungs, and paranasal sinuses. In the NIH review of 158 patients by Dr. Gary Hoffman,[2] essentially two-thirds of the patients with Wegener's granulomatosis will develop some issue related to the paranasal sinuses at some point during their illness. As opposed to the nasal cavity and nasopharynx, the paranasal sinuses have essentially a pure line of ciliated respiratory epithelium. This is one of the primary recipients of the so-called Wegener's attack in my opinion. The paranasal sinuses anatomically are invaginations within the bony skeleton of the face and head that are lined with a ciliated columnar respiratory epithelium. They secrete glandular material including lysozyme and lactoferrin as well as immunoglobulin-G and secretory immunoglobulin-A. They act as a reservoir for humidification of air going through the nasal cavity as well as a heating and cooling system for that same air. This finely tuned homeostatic system persists, and the pathophysiology of Wegener's granulomatosis revolves around inflammation of this thin lining. As the lining becomes inflamed, the small passages that allow for the outflow of fluid become

blocked, and one experiences a backup and stasis of fluid. At this point, it is not a tremendous leap of faith to assume that static fluid will eventually become infected, causing pain and the myriad of symptoms associated with acute sinusitis. As there are four paranasal sinuses on each side of the face, any permutation one can conceive of is possible for disease activity as well as signs and symptoms of sinus disease.

Additionally, anatomic considerations for each sinus dictate the clinical presentation as well as the possibility of any extra-sinus complications. In addition to stasis and backup of fluid with infection, scarring and hyperreactivity of the respiratory epithelium or mucosa becomes common with resultant mucoperiosteal thickening. This becomes infected, which stimulates the inflammatory response from both the humeral and cellular components, with subsequent activation of cytokines causing the cycle to grow and cascade in a continuing downward spiral. Treatment of disease in the paranasal sinuses at this point is directed towards restoring normal mucociliary flow and transport if possible, and if not possible then to establish new conduits in the areas of drainage to allow for surgical and mechanical cleaning postoperatively. It goes without saying that concurrent treatment of the systemic illness with the appropriate immunosuppressive therapies under the direction of the rheumatologist, immunologist, or pulmonologist is critical to breaking the cycle of inflammation and infection at two separate points.

The unique anatomy of the frontal sinus explains the clinical presentation of infection in these patients as well as potential complications. The sinuses are innervated by branches of the supraorbital nerve, and patients will present with pain over the frontal bone as well as possible headache and even fever. The anterior table of the frontal sinus has marrow within the bone, and chronic festering infection that does not drain through the nasofrontal duct can infect the bone marrow anteriorly, causing an osteomyelitis known as Pott's puffy tumor. Looking posteriorly from the frontal sinus, one must realize that the anterior cranial fossa is bordered by the posterior table of the frontal sinus, connected by valveless diploic veins. Purulent infection of the frontal sinus can therefore travel retrograde through these valveless veins and allow for the transmission of septic emboli to the meninges. Intracranial complications are therefore possible, including but not limited to meningitis, epidural empyema, subdural empyema, as well as brain abscess. Treatment of the frontal sinus is geared towards reestablishing draining, either endoscopically or through an incision from the undersurface of the frontal bone in the region not containing bone marrow, thus limiting the chance of an osteomyelitis. Intracranial complications often require combined therapy, with both a sinus surgeon and neurosurgeon in attendance.

The sphenoid sinus is similar to the frontal sinus in that its anatomic location predisposes the patient to potential intracranial disease. It sits under the Turkish saddle with the pituitary gland above with its anterior and posterior divisions. Laterally out of the sinus, you have the third and fourth cranial nerves in addition to the first branch of the trigeminal nerve and the abducens nerve. The optic nerves traveling posteriorly towards the chiasm impinge on the lateral wall of the sphenoid sinus and can often be seen endoscopically through the sphenoid sinus. Additionally, the internal carotid artery passes posterior to the sphenoid sinus, and in up to one-quarter of patients may even be dehiscent. Significant disease of the sphenoid sinus, therefore, must be addressed promptly and aggressively from both a microbiologic and inflammatory perspective. Surgical drainage anteriorly through the nose is a minimum, often required in order to deal with pain, fever, and potential orbital problems and intracranial complications.

In most patients with Wegener's granulomatosis, the most common sinuses involved are the maxillary and ethmoid sinuses. The anterior ethmoid system usually drains with the frontal sinus and infundibulum draining the maxillary sinus through the middle meatus out from under the middle turbinates. Disease can occur internally in the nose along its lateral wall in the region of the middle turbinate, blocking off the entire system or causing additional inflammation within an already narrowed ostiomeatal complex. The lateral border of the ethmoid sinus is the medial border of the orbit. Again, one does not require a major leap of faith in order to sense that potential complications of infection or inflammation of the ethmoid sinus can travel into the orbit.

Orbital complications of sinusitis include cellulitis, both the pre- and postseptal types, and they may include, but are not limited to, blepharitis, conjunctival injection, displacement of the globe, medial rectus palsy, diplopia, visual changes, and in a worst-case scenario, blindness. In a similar vein, treatment is always geared towards reestablishing drainage, either through an external or intranasal approach, in addition to antimicrobial therapy and systemic treatments. The maxillary sinus may also contribute to pain and tooth discomfort; however, it rarely causes any life-threatening complications and is often addressed through either a Caldwell-Luc approach under the lip through the canine fossa or via a medial meatal antrostomy approach to drain the sinuses.

Functional endoscopic sinus surgery, touted over the last 10 to 15 years as the future of sinus surgery, is probably not the best approach to dealing with patients who have active vasculitic disease. In these patients, the respiratory epithelium is inherently inflamed and the primary focus of inflammatory disease; it is often difficult to surgically restore normal mucociliary flow. Classical surgical techniques such as a Caldwell-Luc, external fronto-ethmoidectomy (Lynch procedure), as well as a frontal sinus obliteration may have increased utility in the surgical management for patients with active vasculitis.

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Pathogenesis—Immune Predisposition and Infectious Etiology of Systemic Vasculitis

26-019

SHORTENING OF TELOMERES: EVIDENCE FOR REPLICATIVE SENESCENCE OF T-CELLS DERIVED FROM PATIENTS WITH WEGENER'S GRANULOMATOSIS

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Background: Replicative senescence describes the fact that somatic cells undergo a finite and predictable number of cell divisions before entering an irreversible state of growth arrest. Thus, replicative senescence is a reliable indicator of preceding and persistent activation of the cellular immune response because it is invariably associated with clonal expansion of B- or T-lymphocytes. Since repeated cell division results in a progressive shortening of telomeres, determination of telomere length by Southern blotting is a useful tool to analyze replicative senescence.

Results: Based on these considerations, we analyzed DNA derived from T-cells of patients suffering from Wegener's granulomatosis. Shortened telomeres, in addition to telomeres of normal length, were detected in patients with disease for five years or more (n=9), but not in patients with newly diagnosed disease. Because T-cells in culture undergoing replicative senescence become negative for CD28, the major T-cell co-stimulatory receptor, its expression on T-cells of patients with Wegener's granulomatosis was tested. Reduced expression of CD28 was noted, particularly in patients with disease for more than five years and shortened telomeres. In conclusion, our data provide evidence that a portion of T-cells had undergone replicative senescence, which in turn indicates clonal expansion of T-cells as consequence of activation.

27-047

PRESENCE OF AUTOANTIBODIES IN RELATIVES OF PATIENTS WITH PRIMARY SYSTEMIC VASCULITIDES (PSV)

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The presence of autoantibodies in relatives of patients with PSV has been occasionally reported.

Objective: To transversally evaluate the presence of different autoantibodies in relatives of a PSV cohort.

Methodology: Three groups were studied: Group I: 188 first-degree relatives (mother-25, father-18, sister-66, brother-45, son-9, daughter-25) of 77 PSV patients. Group II: 77 PSV patients (Wegener's granulomatosis-32, Behçet's disease-17, Takayasu arteritis-8, microscopic polyangiitis-7, polyarteritis nodosa-7, Henoch-Schönlein purpura-4, giant cell arteritis-1, cutaneous PAN-1). Group III: 65 healthy sex- and age- to case-matched controls. The presence of the following autoantibodies was evaluated: antinuclear and anticytoplasmic, rheumatoid factor, PR3-ANCA, MPO-ANCA, anti-dsDNA, anticardiolipin (IgG, IgM, IgA), anti-β2-GPI, anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase. Statistical analysis: χ^2 with Yates correction or two-tailed exact Fisher test. The probability of having PSV according to the presence of each autoantibody was calculated as odd ratio (OR).

Results: No differences regarding sex and age distribution was seen between groups. Differences in antibody prevalence between patients and their first-degree relatives were as follows (patients vs. their relatives, respectively): **RF:** 16/75 (21.3%) vs 12/188 (6.4%), OR 3.97 (CI 95% 1.78-8.9), $p < 0.002$. **PR3-ANCA:** 13/74 (17.6%) vs 4/187 (2.1%), OR 9.8 (CI 95% 3.06-31), $p < 0.0009$. **MPO-ANCA:** 5/75 (6.7%) vs 2/187 (1.1%), OR 6.6 (CI 95% 1.25-34.8), $p < 0.023$. **Anti-β2-GPI:** 6/74 (8.1%) vs 42/187 (22.5%), OR 0.3 (CI 95% 0.12-0.75), $p < 0.008$. For the rest of the antibodies tested there were no differences between PSV patients and their first-degree relatives. When compared to healthy subjects, anti-β2-GPI and anti-Tg were the only antibodies increased in relatives (22.5% vs 0%; $p < 0.009$ and 17% vs 6.3%; $p < 0.039$, respectively).

Conclusions: The prevalence of anti-β2-GPI autoantibodies was higher in relatives of patients with PSV than that seen in patients and healthy non-related individuals. The low OR suggests a lesser probability to develop PSV. In contrast, for RF, PR3-ANCA and MPO-ANCA a high OR may indicate an increased probability for the development of PSV in relatives in whom these autoantibodies are present. The significance of the presence of these autoantibodies in relatives or patients with PSV is unknown. Follow-up of these subjects is being performed.

28-064

POLYMORPHISMS OF CANDIDATE GENES IN ANCA POSITIVE VASCULITIS

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Genetic factors have often been suspected in ANCA-associated small vessel vasculitis, but only few have proven to be of importance. Deficiency of alpha-1-antitrypsin, the main inhibitor of proteinase 3 (PR3), and an increased frequency of the C3F allele have been found to correlate with PR3-ANCA positive vasculitis.

We have searched for associations between ANCA-associated small vessel vasculitis and polymorphisms in the genes for three candidate molecules: IL-1Ra, Fcγ-RIIa and CTLA-4. IL1RN*2, an allele of a polymorphism in intron 2 of IL-1Ra, has been detected in increased frequency in various inflammatory and renal diseases. In CTLA-4, a microsatellite in exon 3 has been associated to autoimmune disease, for instance Wegener's granulomatosis. The R/H131 polymorphism in Fcγ-RIIa has been connected to autoimmune and infectious disease. Patients at our departments with positive ANCA tests during the period March 1991 and December 1998 were iden-

tified, and blood samples were collected after informed consent. Patients were categorized according to ANCA serology using ELISA and to disease phenotype using the "Chapel Hill" nomenclature. Of the 109 patients, 51 had Wegener's granulomatosis (WG) and 58 had microscopic polyangiitis (MPA), 61 had PR3-ANCA and 46 had MPO-ANCA. Genotypes were determined using PCR technique. Fisher's exact test was used for statistic calculations.

	MPA	WG	MPO	PR3	Total	Contr.
IL1RN*2	0.198	0.304	0.206	0.287	0.248	0.255
Fcγ-RIIa H	0.474	0.480	0.435	0.525	0.477	0.482
CTLA-4 long	0.647	0.657	0.641	0.656	0.651†	0.558

† = p<0.05.

This study confirms and extends earlier observations concerning CTLA-4. An increase was found for the long alleles, which are considered to be linked to T-lymphocyte activation. The increase was moderate and reached statistical significance only in the whole study population, but frequencies were similar in both serology and phenotype subgroups. No significant increases were found for the IL1-Ra and Fcγ-RIIa polymorphisms.

29-072

IMMUNOFLUORESCENCE FINDINGS IN KIDNEYS OF SCG/KJ MICE: A MODEL OF PAUCI-IMMUNE CRESCENTIC NEPHRITIS?

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SCG/Kj mice are a recombinant inbred strain of mice spontaneously developing crescentic GN, systemic vasculitis and MPO-ANCA, and have therefore been suggested as an animal model for ANCA associated pauci-immune crescentic GN. We evaluated the development of renal lesions in 24 SCG/Kj mice at 8, 10, 12, 14, 16, 24 and 40 weeks by light and immunofluorescence (IgG, IgM, IgA, C3) microscopy. MRL/lpr mice served as controls. In all animals the typical picture of a diffuse immune complex GN was found, demonstrating initially significant mesangial deposition of IgG, IgM and C3 as early as 8 weeks. Intensity increased with age and became strongly positive for all three Ig and C3 in the mesangium and along peripheral capillary loops. Interestingly, IgA was more dominant in the SCG/Kj strain compared to observations in MRL/lpr mice. Crescent formation also began early at week 10 and was affecting about 90% of the specimens from week 12 to 40 correlating with the amount of proteinuria. Significant Ig deposition was already present early in the course and only a weak correlation was found between glomerular Ig deposition and proteinuria. In conclusion, the SCG/Kj strain of mice provides an animal model for spontaneous ANCA positive crescentic GN. The massive presence of mesangial or glomerular immunoglobulin deposits, however, differs from the usually pauci-immune pattern in man. Thus, SCG/Kj mice seem not to be a representative model for human ANCA positive crescentic nephritis.

30-079

ANTI-MYELOPEROXIDASE ASSOCIATED PAUCI-IMMUNE FOCAL SEGMENTAL GLOMERULONEPHRITIS IN RATS

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The presence of MPO-ANCA in humans is associated with a pauci-immune focal segmental glomerulonephritis. We have developed a novel model of this disease in rats.

Wistar Kyoto rats were immunized with purified human MPO (50 micrograms im). Over 2 to 4 weeks all rats developed anti-myeloperoxidase antibodies as confirmed by ANCA IIF (on rat and human neutrophils) and ELISA (titers 60 - 100%). Hematuria (dipstick 1+ to 4+) was detected in 95% by week 5, accompanied by mild proteinuria, mean 6.22 mg/day (0.08-34.3) vs controls 0.17 mg/day (0.001-0.67). Kidney sections taken from rats killed at 8 weeks showed glomeruli with segmental inflammation (83% of rats) and occasional fibrinoid deposits (20%), tubular red cell casts (100%) and tubulo-interstitial inflammation (100%). Lung sections showed evidence of fresh hemorrhage and hemosiderin deposition (83%). Immunofluorescence microscopy revealed no deposits of IgG and only scanty tubular deposits of C3.

To investigate the effect of a local renal immune stimulus one group of rats was immunized with MPO as previously described, followed by a sub-nephritogenic dose of rabbit anti-rat glomerular basement membrane antibody at 5 weeks. After 1 week these rats developed macroscopic hematuria and marked proteinuria, mean 40.0 mg/day (0.81-106.1) vs control rats given anti-rat GBM alone, mean 3.1 mg/day (0.45-6.28). Kidney histology revealed segmental inflammation in 100%, fibrinoid necrosis in 80%, and crescents in 80% of rats.

This novel rat model of ANCA associated focal segmental glomerulonephritis should facilitate the further study of human small vessel vasculitides.

31-112

SEROLOGIC AND MOLECULAR PARVOVIRUS B19 (B19) ANALYSES IN ANCA-ASSOCIATED VASCULITIDES: A CASE-CONTROL STUDY

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Objectives: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS) are ANCA-associated vasculitides of unknown etiology. Because B19 has been associated with various vasculitides and with ANCA, we examined its potential role in ANCA-associated vasculitides.

Methods: We tested the sera from 13 selected patients with

newly diagnosed ANCA-positive WG, MPA or CSS. Every case was matched to 3 healthy controls according to age (±3 yr) and gender. All sera were tested for specific IgG and IgM antibodies (Ab) to B19 (3rd-generation ELISA, Biotrin, France) and DNA was analyzed by polymerase chain reaction (PCR). Cases and controls were compared with respect to the presence of B19-specific AB (IgG and/or IgM) and DNA detection by PCR.

Results: The 13 patients (mean age: 50.1 ± 11.1 yr, M/F sex ratio: 1.6) comprised 6 WG, 6 MPA and 1 CSS. ANCA were distributed as follow: cytoplasmic labeling and/or PR3-ANCA, n = 7, and perinuclear labeling and/or MPO-ANCA, n = 6. IgG Ab to B19 were equally detected in the sera of cases (77%) and controls (79%) (OR = 0.84, p = 0.84). All 13 cases and 39 controls were negative for IgM Ab and B19 DNA.

Conclusion: These results suggest that neither acute nor persistent B19 infection is an etiological factor of ANCA-associated vasculitides. However, a potential pathogenic role of B19 in individual cases cannot be excluded.

32-121

FREQUENCY OF FUNCTIONAL IL-10 AND TGF-β GENE-POLYMORPHISMS IN ANCA-ASSOCIATED VASCULITIS (AAV)

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Rationale: Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are anti-neutrophil cytoplasmic (ANCA) associated primary systemic vasculitides (AAV) of unknown origin. Since the immunopathogenesis of MPA suggests a strong Th2 response giving rise to the hypothesis that genotypes suppressing Th1 responses or augmenting Th2 responses are more frequent in MPA than in WG, TGF-β_1 and IL-10 genes are suspected to modify the course of AAV. The purpose of this study was to identify any association between genotype frequencies of functional polymorphisms (PMs) of the genes of these two cytokines and AAV.

Methods: 161 patients with AAV and 153 healthy blood donors were genotyped for a biallelic PM in codon 25 of the TGF-β_1 gene using PCR, and for the biallelic PM at position -1082 of the IL-10 gene using the amplification refractory mutation system - PCR methodology.

Results: For TGF-β_1 codon 25 PM no significant difference was found between control and any of the patient groups. For IL-10 (-1082) PM we found a significant shift towards the homozygous AA genotype in WG and in MPA. This significance was significantly more impressive in MPA. Moreover, we found a gender-associated significant difference in MPA for IL-10 (-1082) PM. In this group the AA homozygous genotype was more frequent in females compared to males.

Conclusion: On the basis of the analyzed cohorts a significant contribution of the named TGF-β_1 codon 25 PM to the susceptibility defining genetic backgrounds of AAV appears unlikely. However, the significant differences suggest a role of the enhanced IL-10 (-1082) PM in WG and MPA with a newly described gender difference in MPA.

Pathogenesis—Patterns of Injury: Implications for Pathogenesis

33-038

T CELL RECEPTOR V-BETA REPERTOIRES IN SYSTEMIC VASCULITIDES OF CHILDHOOD

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Introduction: Despite conflicting evidence and much debate, superantigenic stimulation of the immune system in Kawasaki disease (KD) remains an attractive hypothesis since there is considerable overlap between the clinical and immunological phenotypes of KD and classical superantigen-mediated diseases such as the toxic shock syndrome. Moreover, although there are limited data in adults suggesting that SAgs may be involved in the initiation of other primary systemic vasculitides, no such data exist for children.

Methods: To investigate the possible etiological role of SAgs, this study examined peripheral blood TCR Vβ repertoires in children with KD (n=6), polyarteritis nodosa (PAN, n=23), Wegener's granulomatosis (WG, n=1), and microscopic polyangiitis (MPA, n=1). 20 normal children and 30 children with non-vasculitic inflammatory disease or recipients of renal allografts served as controls and disease controls, respectively. 3 color FACS analysis of peripheral blood mononuclear cells stained with conjugated monoclonal antibodies to CD3, CD4, CD8, and 17 different Vβ families was performed.

Results: The mean % of CD4+ T cells bearing Vβ2 was significantly increased in the KD group versus controls and disease controls (p=0.03 and p=0.01, respectively). Individual KD patients were also noted to have CD4+ T cell Vβ expansions other than Vβ2 (Vβ5.1 n=2; Vβ12 n=1). 60% of the primary systemic vasculitis patients had one or more TCR Vβ expansions in the CD4+ lymphocyte population, compared with 30% of the controls (p=0.02-0.05), and 36% of the disease controls (p=0.05-0.1). Unlike KD, however, the pattern of Vβ families expanded in individual patients was more diverse, perhaps indicative of the involvement of several different SAgs. Follow-up studies of 7 primary systemic vasculitis patients demonstrated a normalization of the CD4+ T cell Vβ repertoire following induction of remission of vasculitis.

Conclusion: Our preliminary data provide indirect evidence for an etio-pathogenetic role for SAgs in KD and primary systemic vasculitides affecting children.

34-053

PERIPHERAL BLOOD- AND GRANULOMA CD4+CD28– T-CELLS DISPLAY CYTOKINE PRODUCTION RESTRICTED TO IFN-γ AND TNF-α IN WEGENER'S GRANULOMATOSIS

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Objective: Expansion of T-cells lacking CD28 expression has been reported in Wegener's granulomatosis (WG). We addressed the question, whether the fraction of peripheral blood and granuloma CD28– T-cells within the CD4+ T-cell population is a source of Th1 like cytokine production in WG.

Methods: 12 patients with active, generalized, biopsy-proven WG were analyzed. We assessed surface antigens and intracytoplasmic cytokine expression of peripheral blood fractions of CD28– and CD28+ T-cells within the CD4+ T-cell population by flow-cytometry (FACS). Cytokine secretion was additionally confirmed by an enzyme-linked immunosorbent assay (ELISA). Immunohistologic studies were performed on biopsies from the respiratory tract.

Results: The fraction of CD28– T-cells within the CD4+ T-cell population was significantly expanded compared with healthy controls (mean 14.4 vs. 2.1%, P<0.01). CD57 (differentiation marker) and CD18 (β_2 integrin) were upregulated on CD4+CD28– T-cells and generally missing on CD4+CD28+ T-cells. CD25 (IL-2Rα) was missing on the CD4+CD28– subset but found on their CD4+CD28+ counterparts. CD4+CD28– T-cells displayed a cytokine expression restricted to IFN-γ- and TNF-α, whereas CD4+CD28+ T-cells displayed a broader cytokine expression including IL-2. Immunohistologic analysis using serial sections demonstrated that the majority of CD4+ T-cells lacked CD28 and expressed IFN-γ- and TNF-α within granulomatous lesions.

Conclusion: CD4+CD28– T-cells appeared highly differentiated, displayed a Th1-like cytokine production restricted to IFN-γ- and TNF-α. β2 integrins, i.e. CD18, may promote recruitment of CD4+CD28– T-cells into granulomatous lesions, where they support granuloma formation by their restricted cytokine production. Moreover, IFN-γ- and TNF-α producing T-cells within the expanded peripheral blood and within the granuloma CD4+CD28– population may represent an important target of anti-TNF-α directed therapies.

35-055

A HUMAN IN VITRO GRANULOMA MODEL FOR WEGENER'S GRANULOMATOSIS (WG)

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Objective: Chronic granuloma formation occurring in one or several organs and mostly accompanied by systemic vasculitis are hallmarks of Wegener's granulomatosis. Although animal models mimicking the vasculitic pathogenesis exist, currently no model is available to investigate granuloma formation in WG. The aim of our study was to develop an in vitro granuloma model that is suitable for examining mechanisms of granuloma formation in WG.

Methods: PBMC and PMN were isolated from healthy controls and WG patients (n=5). 6 x 10^5 cells PBMC + PMN in a ratio of 10/1 were added onto a layer of human umbilical vein endothelial cells (HUVEC) in a transwell over agarose-coated wells (12 wells/assay), stimulated (superantigen + PR3-ANCA-IgG) and incubated at 37°C for four days. Viability of the granuloma-like spheroids was characterized using fluoresceindiacetate and propidium iodide staining. Phenotype (CD3, CD20, CD26, CD28, WGM2, CD164) and functional features (Osteopontin, TNFα) were determined using immunohistochemistry and ELISA.

Results: Our human in vitro granuloma model for WG proved to be stable and reproducible. Viability of the granuloma-like spheroids was higher than 95%, except for the PMN. The mean number for granuloma-like three-dimensional spheroids was 12/12 for the WG cases, but only 5/12 for healthy controls (n=4; p<0.05). At the two-dimensional level only 1/5 healthy controls exhibited granulomatous structures, whereas 5/5 WG cases displayed such structures. Further, the more active the disease, the more differentiated monocytes (CD164+) and CD26+ T cells were found. On the other hand, in vitro granulomas from inactive disease displayed mainly CD3+ and CD28+ cells, but only few if any CD164+ or CD26+ cells.

Conclusion: Employing a combination of transendothelial migration and spheroid in vitro assay, WG-specific three-dimensional granuloma-like spheroids were formed, which can be used as a tool to better analyze the nature and/or cause of granulomas in WG.

36-068

CIRCULATING ANTI-INFLAMMATORY CYTOKINES (IL-10, IL-13 AND TGF-β IN GIANT CELL ARTERITIS (GCA)

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Background: GCA is characterized by an intense systemic inflammatory response although remarkable differences may be observed among patients. We have previously reported a correlation between the intensity of the acute phase response and circulating levels of TNF and IL-6 in GCA patients (Hernández-Rodríguez et al, *Arthritis Rheum* (AC&R) 2002, in press). IL-10, IL-13 and TGFβ have been considered to have anti-inflammatory properties because they inhibit the synthesis of pro-inflammatory cytokines by T cells and macrophages. The putative role of these cytokines in regulating the intensity of the inflammatory response in GCA has not been investigated.

Objective: To determine plasma concentrations of IL-10, IL-13 and TGFβ and their relationship with the intensity of the systemic inflammatory response in patients with GCA.

Patients and Methods: Circulating levels of TGFβ, IL-10 and IL-13 were determined in 56 untreated patients with biopsy proven GCA and in 15 healthy controls. Four parameters were used to evaluate the intensity of the systemic inflammatory response (fever, weight loss, ESR $\geq$ 85 mm, and Hb < 11 gm/dL). Patients were considered to have a weak inflammatory response when had 2 or less inflammatory parameters (group 1) and a strong inflammatory response when 3 or 4 parameters were present (group 2).

Results: Twenty-three patients had a weak (group 1) and 23 a strong (group 2) initial systemic inflammatory response. No differences in IL-10 levels among GCA patients and controls were observed, but IL-10 concentrations were high-

er in group 2 (4.2 ± 3.1 pg/mL) compared with group 1 (1.4 ± 2.5 pg/mL), p=0.002. Circulating TGFβ levels were significantly higher in GCA patients (962 ± 589 pg/mL) than in controls (744 ± 791 pg/mL) (p=0.04). Although TGFβ levels in group 1 were lower (866 ± 574 pg/mL) than in group 2 (1100 ± 596 pg/mL), differences were not significant (p=0.1). Circulating IL-13 levels were undetectable in most patients and controls.

Conclusions: GCA patients with a weak systemic inflammatory response do not have higher concentrations of anti-inflammatory cytokines TGFβ, IL-10 and IL-13 than patients with a strong acute phase reaction. The limited increase in TGFβ and IL-10 levels observed in GCA patients suggests that these cytokines do not significantly down-regulate the intensity of the systemic inflammatory response in this disease.

FIS 98/0443, FIS 00/0689, Fundació Pedro Pons

37-069

TISSUE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES (IL-1β, IL-6 AND TNFα) IN GIANT CELL ARTERITIS (GCA) PATIENTS: CORRELATION WITH THE INTENSITY OF THE SYSTEMIC INFLAMMATORY RESPONSE

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Background: The systemic inflammatory response is mediated by pro-inflammatory cytokines, mainly IL-1β, IL-6 and TNFα which are synthesized mostly by activated macrophages. TNFα, IL-1β and IL-6 mRNAs have been detected in temporal arteries from patients with GCA, a disease characterized by a remarkable acute phase reaction.

Objective: To assess the relationship between tissue expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) in temporal artery biopsies from GCA patients and the intensity of the systemic inflammatory response.

Patients and Methods: Temporal artery sections from 50 GCA patients with a similar degree of histologic involvement were immunostained with antibodies against IL-1β, IL-6 and TNFα. Ten normal temporal arteries from patients in whom a surrogate diagnosis was obtained were also studied. Four inflammatory parameters were considered to evaluate the intensity of the systemic inflammatory response (fever, weight loss, ESR ≥ 85 mm, and Hb < 11 gm/dL). Immunostaining was blindly quantitated using a predefined score considering the percentage of cell staining at the intima/media junction (1: <25%, 2: 26-50%, 3: 51-75% and 4: 76-100%).

Results: Tissue expression of IL-1β, IL-6 and TNFα was intense and occurred mainly within the granulomatous reaction at the intima/media junction. Cytokine expression was variable among patients even displaying a comparable degree of inflammatory changes. No cytokine expression was observed in control samples. Patients with a strong systemic inflammatory response (4 parameters) exhibited significantly higher scores for IL-6 (5 vs 8 patients, p=0.034) and for TNFα (7 vs 12 patients, p=0.025) than patients with a weak systemic inflammatory reaction (0 parameters). IL-1β expression also tended to be stronger in patients with a strong inflammatory response but the difference was not statistically significant.

Conclusions: Tissue expression of pro-inflammatory cytokines IL-1β, IL-6 and TNFα is prominent in full-blown giant-cell arteritis lesions. IL-6 and TNFα expression correlates with the intensity of the systemic inflammatory response.

FIS 98/0443, FIS 00/0689, DAKO, Fundació Pedro Pons, Hospital Clinic Research Award

38-129

DOES ANCA FORMATION RESULT FROM AN ANTIGEN-TRIGGERED IMMUNE RESPONSE IN WEGENER'S ENDONASAL INFLAMED TISSUE?

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Purpose: PR3-specific antibodies (PR3-ANCA) occupy a pathogenetic role in Wegener's granulomatosis (WG). The detection of germinal center-like B lymphoid infiltrates in association to endonasal WG granuloma raised the question, whether an antigen-driven immune response which finally leads to the ANCA associated generalized vasculitis is initiated in the upper respiratory tract. B lymphocytes from nasal tissue of patients with localized and generalized WG were analyzed for distribution and mutational pattern of antibody-encoding genes in order to draw nearer the suspected antigens.

Materials and methods: Cryosections from immediately snap-frozen endonasal biopsies were screened for B lymphocytes by CD20-staining (APAAP). Tissue was protein-digested, DNA was extracted and subjected to a polymerase chain reaction (PCR) of 35 cycles using six VH- and a mix of JH-family-specific oligonucleotides as primers. PCR products were cloned and sequenced. Nucleotide and amino acid sequences were analyzed for mutations and compared to all accessible sequences from gene databases.

Results: Rearranged immunoglobulin genes were detected for all VH families (VH1-6) indicating a polyclonal repertoire. By sequence analysis of a hundred bacterial colonies derived from the cloned PCR products, 66 individual rearrangements of V-D-J segments could be determined. The sequence analysis revealed a high frequency of mutations with amino acid substitutions and a biased repertoire of represented genes indicating selection by an antigen. Three particular VH genes were overrepresented that had been found in PR3-ANCA producing cells. Furthermore, amino acid replacement often led to negatively charged residues characteristic for the binding-site to PR3.

Conclusions: Besides the immunopathogenetic role of neutrophils, T-lymphocytes and monocytes in WG these findings indicate an involvement of B-lymphocytes in the autoimmune mechanism comparable to other rheumatic diseases like RA and SLE. In WG this probably happens through the generation of high-affinity ANCAs by contact to PR3 or a cross-reacting microbial epitope in the inflamed endonasal tissue.

Pathogenesis—Possible Role of ANCA and AECA in Selected Forms of Systemic Vasculitis

39-017

ABNORMAL GALACTOSYLATION OF POLYCLONAL IgG IN ANCA-ASSOCIATED SYSTEMIC VASCULITIS PATIENTS

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IgG-anti-neutrophil cytoplasmic antibodies (ANCA) are implicated in the pathogenesis of small vessel vasculitides, such as Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA). We have analyzed the oligosaccharide profiles of the intact polyclonal IgG, isolated from the serum of 20 ANCA positive patients at the time of acute presentation. For patient samples 40-75% of released oligosaccharides were devoid of galactose (G0-IgG), compared to values of 23-30% for age and sex matched controls. In the absence of galactose the terminal sugar residues are N-acetylglucosamine. Increased levels of G0 IgG have been reported for several inflammatory diseases (rheumatoid arthritis, juvenile arthritis, etc) and have been associated with disease progression/outcome. Possible contributing mechanisms are suggested by the demonstration that G0-IgG can activate the complement cascade through activation of mannan binding lectin and can enhance antigen presentation by uptake through the mannose receptor on dendritic cells.

Thus hypogalactosylation could impact on the inflammatory response and immune regulation of autoantibody production.

40-018

CONTRIBUTION OF ABNORMAL DIFFERENTIATION AND FUNCTION OF NEUTROPHILS TO MPO-ANCA PRODUCTION: ANALYSIS OF ICSBP-KO MICE

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Half populations of splenocytes were morphologically observed as neutrophils, because abnormalities in the maturation of myeloid cells have been shown in interferon consensus sequence-binding protein (ICSBP)-/- mice. We measured dysfunction of granulocytes in spleens of these mice. Higher production of an auto-antibody MPO-ANCA (MPO specific anti-neutrophil cytoplasmic antibody) was accompanied by aging of the mice, whereas anti-double-stranded DNA antibody titer was not detected. Release of myeloperoxidase (MPO) from the purified neutrophils from splenocytes was 64% of that from neutrophils from peripheral blood, and O2- production was 53%. In addition, cells adhering to a slide glass were round by microscopic examination, and white granules were seen in the cells by transmission electron microscopy. Interestingly, ICSBP-/- mice showed defective eosinophils in their peripheral blood due to suppression of mRNA of eosinophil peroxidase in bone marrow. These results suggest that dysfunction of neutrophils in spleens of ICSBP-/- mice relates to an increase of MPO-ANCA titer with aging, and that severe suppression of eosinophil peroxidase mRNA expression in the bone marrow of ICSBP/IRF-8 permits circulation of abnormal eosinophils.

41-025

NEUTROPHIL ACTIVATION AND LEVELS OF PROTEINASE 3 IN PATIENTS WITH ANCA-ASSOCIATED SYSTEMIC VASCULITIS

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Background: In the ANCA-associated systemic vasculitides, patients form autoantibodies against neutrophil granular proteins. Some correlation is seen between ANCA titer and disease activity, but whether this is cause or consequence is still unknown. Our theory is that dysfunctional leukocytes, e.g., constantly somewhat activated neutrophils with increased production and/or leakage of granular proteins, lead to an increased amount of circulating antigen and hence predisposition to autoimmunity. In order to address this, proteinase 3 (PR3), one of the main ANCA antigens, stored in azurophil granules, and NGAL (neutrophil gelatinase-associated lipocalin), a specific marker of neutrophil degranulation, localized in secretory granules, were measured. CRP and Cystatin C were measured as markers of inflammation and renal function, respectively.

Methods: Both PR3 and NGAL were measured in plasma by means of ELISA technique. The NGAL ELISA was a sandwich method using affinity purified rabbit-anti-NGAL. In the PR3 ELISA we used anti-PR3 monoclonals as capture-antibodies and affinity purified rabbit-anti-PR3 for detection. PR3-ANCA, MPO-ANCA and capture-PR3-ANCA were measured by Wieslab AB.

Results: PR3 was significantly raised ($p<0.0001$) in ANCA patients (690±470, n=59) compared to healthy blood donors (350±110, n=30) as well as disease controls (422±200, n=46). The patients had a tendency to divide into two groups, one with normal PR3 levels (410±130, n=32) and one with raised levels (1050±660, n=27). No correlation was seen with disease activity, inflammation or renal function. Nor did we see any correlation between capture-PR3- or MPO-ANCA and PR3. Negative correlation was, however, seen with PR3-ANCA ($r=-0.4$, $p=0.01$). The raised NGAL levels correlated

strongly with decreased renal function (r=0.8, p<0.001). After correction for this, slightly increased levels (120±55, n=59) were seen compared to the healthy blood donors (92±56, n=26), but not compared to the disease controls (128±38, n=48). In the disease controls there was a significant correlation between NGAL and PR3 (r=0.3, p<0.05), but this was not the case in the ANCA patients. Whether patients had PR3- or MPO-ANCA was of no significance.

Conclusions: In our measurements, we found significantly raised levels of proteinase 3 in plasma from patients with ANCA-associated vasculitis, regardless of ANCA specificity. This was due to neither decreased renal function, nor ongoing inflammation, nor neutrophil activation. Plausible mechanisms, in demand of further research, include defects in the reticuloendothelial system, genetic factors and selective neutrophil degranulation or leakage.

42-026

PHOSPHATIDYLINOSITOL-3-KINASE CONTROLS ANCA-INDUCED RESPIRATORY BURST IN HUMAN NEUTROPHILS

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ANCA activates human PMN primed with TNF-α in vitro. PI-3 Kinase (PI3-K) and the protein Akt have been implicated in the control of the phagocyte respiratory burst. We tested the hypothesis that PI-3 Kinase controls the ANCA-induced respiratory burst in human PMN. TNF-α-primed PMN were stimulated with a monoclonal antibody (mab) to MPO, and with human PR3- and MPO-ANCA, respectively. Activation of Akt was assessed by Western blotting with phospho-specific antibodies. Superoxide release was measured by the ferricytochrome assay, and translocation of ANCA antigens by FACS. The effect of TNF-α and MPO-ANCA on the composition of the Akt signaling complex was studied using immunoprecipitation and GST pull-down assays. Western blotting revealed a rapid, but transient, Akt phosphorylation during TNF-α priming and a second phosphorylation after addition of ANCA. Inhibition of PI3-K by LY294002 blocked both Akt phosphorylation and superoxide generation. 20.2±3.4 nmol O_2^-/0.75x10^6 PMN/45 min were released after stimulation with PR3 ANCA, and 5 μM LY294002 decreased this amount to 0.3±2.6 nmol (n=10, p<0.05); these values were 23.3±2.9 versus 1.6±3.6 for MPO-ANCA (n=10, p<0.05). Interestingly, p38 MAPK inhibition with 10 μM SB202190 that also decreases ANCA-induced superoxide generation, prevented S473 phosphorylation of Akt in response to TNF-α and to ANCA. However, SB202190, but not LY294002 abrogated TNF-α-mediated surface translocation of ANCA antigens demonstrating that superoxide generation and ANCA antigen translocation proceed by disparate mechanisms. Characterization of the Akt signaling module showed that Akt, PAK1 and Rac1 exist in complex in resting PMN cytosol. TNF-α stimulation caused increased association of PAK1 with Akt. Consecutive stimulation with a mab to MPO did not cause additional change in the Akt signaling complex. Our data demonstrate the importance of PI3-K for the ANCA-induced oxidant production by human PMN. Pharmacological inhibition of this kinase may control ANCA-induced inflammation.

43-043

ANTI-PR3-ANTIBODIES INDUCE THE RELEASE OF PROCOAGULATORY FACTORS FROM ISOLATED MONOCYTES—ROLE OF NF KAPPA B ACTIVATION

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Capillary thrombosis is one of the early pathologic features of ANCA-associated vasculitis. As ANCAs are capable of activating neutrophils and monocytes in vitro, we investigated the effect of these autoantibodies on the release of activators and inhibitors of the fibrinolytic system from isolated human monocytes. Human monocytes were purified by countercurrent centrifugal elutriation, and were stained for PR3 surface expression after the isolation procedure. Incubation of monocytes with murine monoclonal anti-PR3-antibodies, but not with mouse control IgG, resulted in a time- and dose-dependent release of plasminogen-activator-inhibitor type 2 (PAI-2) into the cell supernatant. In contrast, release of tissue-plasminogen-activator (TPA) was diminished in the presence of anti-PR3 antibodies. These responses were equally observed upon monocyte incubation with c-ANCA-IgG, but not with normal human IgG. In the presence of caffeic acid phenylethyl ester (CAPE), an inhibitor of the activation of the transcription factor NF-kappaB, the anti-PR3-induced release of PAI-2 was dramatically reduced. When analyzed by EMSA gel shift assay, nuclear translocation of NF-kappaB was observed in monocytes stimulated with anti-PR3, but not with control antibodies. We conclude that ANCA activate the secretion of the main inhibitor of fibrinolysis, PAI-2, and inhibit the release of the anticoagulatory factor TPA from human monocytes. Activation of NF-kappaB dependent signaling pathways seems to be centrally involved in these processes. The ANCA-induced alterations of coagulatory activity may contribute to the development of capillary thrombosis in ANCA-associated small vessel vasculitis.

44-046

THE PROTEIN C PATHWAY AND VASCULAR INFLAMMATION

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The endothelial protein C receptor (EPCR) is a member of the protein C pathway that accelerates protein C activation on the surface of endothelial cells. This provides an on-demand source of activated protein C, shown by multiple clinical studies to be required for coagulation control and the host response to inflammation. A soluble form of EPCR (sEPCR) is released from the endothelium as a result of thrombin-induced metal-

loproteinase activity. sEPCR normally circulates in plasma at about 100 ng/ml and at much higher levels in patients with sepsis, systemic lupus erythematosus or Wegener's granulomatosis. Recent biochemical studies demonstrated that sEPCR binds to the surface of PMA-activated neutrophils (*J Immunol* 2001, 165:4697-4703). sEPCR binding to neutrophils is supported by proteinase-3 (PR3), the Wegener's autoantigen, and by a beta-2 integrin, probably CD11b/CD18. Less sEPCR binds if neutrophils are pre-incubated with PR3-ANCA from some, but not all, of a small cohort of Wegener's patients. However, the nature of neutrophil PR3 expression and support of sEPCR binding to neutrophils is not well understood.

Results: PR3 purified from neutrophils was labeled in the active site with a chloromethylketone derivative of fluorescein (FITCcmk-PR3). Efficient labeling was judged by the >99% loss of activity. Neutrophils activated with phorbol myristate acetate bound FITCcmk-PR3, suggesting that PR3 released as a consequence of neutrophil granule mobilization can return to the neutrophil and bind to the membrane. Flow cytometry data demonstrate that TNF/fMLP-treated neutrophils also bind PR3-ANCA and an anti-PR3 monoclonal antibody, consistent with the presence of surface PR3 antigen. The importance of the membrane surface in PR3 expression was supported by studies using synthetic phospholipid vesicles. The amidolytic activity of neutrophil PR3 was determined in the presence of sEPCR and liposomes of varying phospholipids and molar ratio compositions. The nature of the anionic phospholipid head group (sigmoidal rates with phosphatidylcholine:phosphatidylserine vesicles) and bilayer organization (effect of phosphatidylethanolamine) was an important influence on PR3 substrate recognition (Km') and sEPCR effects.

Conclusions: The results suggest that the neutrophil membrane environment is an important contribution to PR3 accessibility, either through endogenous expression or by local rebinding events. This is dependent on neutrophil activation and does not require the PR3 active site. Furthermore, the neutrophil lipid microenvironment effects PR3 activity and sEPCR interactions, thus potentially modulating subsequent PR3-ANCA binding and inflammatory events.

45-074

REDUCTION OF MONOCYTE TRANSENDOTHELIAL MIGRATION BY C-ANCA

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Background: While the interaction of anti-PR3-antibodies with neutrophils has been extensively studied in vitro, their interaction with monocytes is less characterized. In the present study, we investigated the influence of anti-PR3-antibodies on monocyte adhesion and transendothelial migration.

Methods: Monocytes were isolated by counterflow centrifugal elutriation. For transmigration studies monocytes were allowed to migrate across endothelial cells, grown to confluence on transwell inserts, in response to the chemoattractant MCP-1 (monocyte chemoattractant protein-1). Monocyte adherence was analyzed by coincubating fluorescence labeled monocytes with endothelial cells in a microplate assay.

Results: Incubation of monocytes with monoclonal anti-PR3-antibodies in the transwell chamber assay caused a significant reduction of transendothelial monocyte migration. This effect could be reproduced by IgG fractions from patients with active Wegener's granulomatosis, whereas an isotype matched control IgG or IgG fractions of healthy controls were ineffective. On the other hand, monocytes adherence to unstimulated or TNF-α stimulated endothelial cells was not altered in the presence of anti-PR3-antibodies. As it has been reported that c-ANCA inhibit the proteolytic activity of PR3, we then investigated whether proteolytic active PR3 is required for successful monocyte transmigration. Serine protease inhibitors with activity against PR3, like alpha-1-antitrypsin or α-ketooxadiazole-inhibitor, caused a distinct reduction in monocyte transmigration similar to the reduction caused by anti-PR3-antibodies. On the contrary, secretory leukocyte protease inhibitor (SLPI) with activity against cathepsin G and elastase, but not against PR3, did not modify monocyte transendothelial migration.

Conclusion: We conclude that anti-PR3-antibodies reduce monocyte transendothelial migration by interaction with the proteolytic activity of PR3. The retention of monocytes in the lumen of microvessels could contribute not only to the development of vascular lesions, but also to granuloma formation in Wegener's granulomatosis.

46-075

INTERFERENCE OF PR3-ANCA WITH THE ENZYMATIC ACTIVITY OF PR3

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Introduction: Anti-neutrophil cytoplasmic antibodies (ANCA) against proteinase 3 (PR3) are strongly associated with Wegener's granulomatosis (WG) and are thought to be involved in its pathogenesis. In vitro functional effects of these antibodies have been suggested to correspond better to disease activity than levels of PR3-ANCA.

Methods: To further investigate the relation between functional effects of PR3-ANCA and disease activity, we tested IgG samples from sera of 43 WG patients and four controls for their capacity to interfere with the proteolytic activity of PR3. Blood was drawn either during active disease or during remission of WG. Moreover, sera of seven patients were analyzed before, during and after relapse. The enzymatic activity of PR3 was determined using a small synthetic substrate (MeSuc-AAPV-pNA), casein, and by complexation of PR3 with its natural inhibitor alpha-1-antitrypsin (alpha-1-AT).

Results: Most of the IgG samples from WG patients inhibited the enzymatic activity of PR3 and the complexation of PR3 with alpha-1-AT. A difference in the capacity to interfere with the proteolysis of casein and with the complexation of PR3 with alpha-1-AT was observed between samples taken during active disease and during remission of WG, but this was not observed for the hydrolysis of MeSuc-AAPV-pNA. How-

ever, PR3-ANCA titers giving fifty percent inhibition of the PR3/alpha-1-AT complexation and the proteolytic activity of PR3 for the hydrolysis of MeSuc-AAPV-pNA were lower for remission samples compared to samples during active disease, indicating a relatively higher inhibitory activity in the former samples. PR3-ANCA titers correlated with the inhibitory activity both for patients with active disease and for patients during remission.

Conclusion: With a fixed amount of IgG, PR3-ANCA-containing IgG from patients with active disease had a higher inhibitory capacity towards the proteolytic activity of PR3 than did PR3-ANCA-containing IgG from patients during remission of WG. However, when correcting the results for the PR3-ANCA titer, PR3-ANCA of patients during remission had a relatively higher inhibitory capacity towards the proteolytic activity of PR3 than did PR3-ANCA of patients during an active phase. These results may indicate that PR3-ANCA of patients with active disease recognize different epitopes on PR3 than do PR3-ANCA of patients during remission of WG. These findings may have relevance for the pathogenicity of the antibodies.

47-077

IgG-MEDIATED ACTIVATION OF LEUKOCYTES IS INDEPENDENT OF Fc-GAMMA RECEPTOR POLYMORPHISM

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Introduction: Ligation of Fc-gamma receptors for IgG (FcgR) can trigger potent effector cell responses. Genetic polymorphisms of these receptors have been shown to modify IgG binding and influence internalization of immune complexes. Indeed, in patients with infectious or autoimmune diseases, skewing towards low-binding FcgR alleles has been demonstrated. The objective of this study was to investigate the influence of FcgR polymorphism on leukocyte activation.

Methods: We analyzed activation of neutrophils and monocytes stimulated by aggregated or solid phase-coated IgG1, IgG2, and total IgG. Neutrophil donors were selected based on their FcgR genotype and homozygous for either FcgRIIa-H131/FcgRIIIb-NA1 (HH-NA1/1) or FcgRIIa-R131/FcgRIIIb-NA2 (RR-NA2/2). Monocyte donors were homozygous for either FcgRIIa-H131/FcgRIIIa-V158 (HH-VV) or FcgRIIa-R131/FcgRIIIa-F158 (RR-FF). Binding of immunoglobulins to lymphocytes was determined by flow cytometry. Activation of neutrophils was measured as the production of reactive oxygen intermediates (ferricytochrome c reduction), degranulation (lactoferrin release), and cytokine production (IL-8). TNF-alpha secretion was used as a measure of monocyte activation.

Results: As determined by flow cytometry, IgG1 aggregates firmly bound to neutrophils of both types of donors, albeit more avidly to donors expressing HH-NA1/1 alleles. In contrast, IgG2 aggregates firmly bound to HH-NA1/1 FcgR neutrophils only. This binding could be blocked by pre-incubation of neutrophils with FcgRIIa and FcgRIIIb blocking antibodies. Despite the differences in binding of IgG subclasses to HH-NA1/1 and RR-NA2/2 neutrophils, we observed no differences in their activation as measured by oxygen radicals production, lactoferrin release and IL-8 production. Activation of both types of neutrophils with IgG1 or IgG2 aggregates could be at least partially blocked by the addition of FcgR blocking antibodies. Similar to neutrophils, HH-VV and RR-FF monocytes were not distinguishable in their response to IgG, IgG1, and IgG2 as measured by TNF-alpha release, although RR-FF monocytes do not bind IgG2 complexes.

Conclusion: We conclude that although IgG-mediated activation of leukocytes is dependent on FcgR, it does not appear to be influenced by FcgR polymorphisms. These results are in favor of a new mechanism for IgG-mediated leukocyte activation, in which even a short interaction between IgG and FcgR is sufficient to generate an appropriate inflammatory response. This may have important implications for inflammatory responses in infectious and autoimmune diseases.

48-080

EFFECT OF TNF-α ON Fcγ RECEPTOR IIA (FcγRIIA) AND β_2-INTEGRIN DISTRIBUTION ON NEUTROPHIL SURFACE ANALYZED BY CONFOCAL LASER SCANNING MICROSCOPY

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Background: Tumor necrosis factor-α (TNF-α) is essential for the induction of the neutrophil activation induced by anti-PR3 or anti-MPO antibodies. We found that Fcγ receptor IIa (FcγRIIa) and β_2 integrins are involved in this reaction (1). Additionally, we suggested that the requirement of TNF-α is probably not only due to an effect of TNF-α on the surface expression of antigens, and another or additional role of TNF-α should be considered (2).

Aim of the study: To assess a possible effect of TNF-α on FcγRIIa and β_2-integrin distribution on neutrophil surface analyzed by confocal laser scanning microscopy.

Results and Discussion: The confocal results exactly match our previous activation results. The experiments showed that TNF-α (2 ng/ml) induced clustering (but not increased surface expression) of FcγRIIa, indicating that FcγRIIa signaling might be enhanced, and induced colocalization of FcγRIIa with β_2 integrins. Moreover, the blocking CD18 mAb MHM23 prevented the ANCA-induced respiratory burst as well as the FcγRIIa clustering. Thus, the FcγRIIa clustering seems to be essential for the induction of the burst, and the colocalization of FcγRIIa with β_2 integrins is probably involved in this process. In conclusion, TNF-α exerts a direct effect on neutrophil signal transduction induced by ANCA by inducing FcγRIIa clustering and possibly by colocalizing the relevant receptors for this process.

(1) Reumaux et al, *Blood* 1995.
(2) Reumaux et al, submitted.

49-084

THE ANCA TARGET ANTIGEN BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN (BPI) IS EXPRESSED IN HUMAN DERMAL FIBROBLASTS

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The ANCA target antigen BPI is an antibiotic, endotoxin-neutralizing and antiangiogenetic protein found in granules of neutrophil granulocytes. Since small molecular neutrophil proteins like defensins were recently detected in epithelial cells, the aim of our study was to determine expression of BPI in non-hematopoietic cells. Cell cultures of human dermal fibroblasts were examined for BPI expression on mRNA and protein level using a BPI-specific RT-PCR, capture-ELISA with murine BPI-specific monoclonal antibodies and IIF after fixation. Cells were stimulated with TNFα, IL4 the active metabolite of cyclophosphamide, dexamethasone and microbes (*S. aureus, P. aeruginosa, C. albicans*). BPI is constitutively expressed on mRNA and protein level in fibroblasts. The expression of BPI is upregulated by proinflammatory cytokines like TNFα and IL4 ranging from 2 to 20 ng/10^6 cells. Immunosuppressive drugs like cyclophosphamide, but not steroids, and *Staphylococcus aureus* down-regulate BPI expression. The ubiquitous presence of BPI outside neutrophil granulocytes indicates an important function in first-line defense against microbes and suggests a role in the local limitation of endotoxin-triggered inflammation. Interaction with BPI-ANCA may impair these functions and facilitate an increased inflammatory response. Moreover, downregulation by immunosuppressive drugs or *S. aureus* may cause a gap in the local spectrum of innate antibiotics making vasculitis patients prone to infections with gram-negative bacteria.

50-085

ANCA SPECIFICITY OF PROTEINASE-3 IN WEGENER'S GRANULOMATOSIS

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The overwhelming percentage of Wegener's granulomatosis (WG) patients with antineutrophil cytoplasmic antibodies (ANCA) suggests that these autoantibodies may play a significant role in this vasculitic disease. ANCA titers often modulate with disease activity indicating that these antibodies might have a direct causative role in the systemic damage observed in patients. One of the major antigenic targets of ANCA in WG is the proteinase-3 (PR-3) protein; however, little is known about how the relationship between PR-3 and ANCA could induce or perpetuate disease. Currently, it is believed that ANCA may induce the release of cytokines from neutrophils and monocytes resulting in hyper-activation and subsequent over-expression of PR-3 which, if not properly inhibited, could cause injury to surrounding tissues. Recently, PR-3 has been shown to bind the soluble endothelial protein C receptor (s-EPCR) suggesting that these proteins are possibly involved with coagulation and inflammatory responses. ANCA could further cause damage in WG patients by binding with PR-3 thus interfering with its interaction with s-EPCR.

Thus, the binding relationship between ANCA and PR-3 should be exactly mapped in order to analyze whether or not ANCA can actually interfere with PR-3 and its natural functions in vivo. This study seeks to determine the common antigenic targets of ANCA on proteinase-3 through sequential epitope-mapping. Overlapping octapeptides of PR-3 were synthesized on derivatized, polyethylene solid phase supports. Ten WG patients, previously determined to have ANCA by immunofluorescence and anti-PR-3 by ELISA, were tested for reactivity with the PR-3 octapeptides. The average binding of all ten patients to the proteinase-3 protein revealed that ANCA reactivity to the proteinase-3 protein occurred at six commonly bound epitopes. Seven out of the ten patients bound epitope 1 (MAHRPPSPAL), seven out of ten patients bound epitope 2 (AQPHSRPYMAS), five out of ten patients bound epitope 3 (SLQMRGNPGSHF), seven out of ten patients bound epitope 4 (VLGAHNVRTQ), five out of ten patients bound epitope 5 (AMGWGRVGA), and five out of ten patients bound epitope 6 (TLRRVEAKGRP). The results of these experiments show that ANCA do in fact bind to linear portions of the proteinase-3 protein which might lead to the disruption of in vivo binding between proteinase-3 and its natural substrates.

51-105

ANCA BINDING TO MONOCYTES ACTIVATES COMPLEMENT

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Antineutrophil cytoplasmic antibodies (ANCA) found in the sera of patients with vasculitis are known to crosslink ANCA antigen to Fcγ receptors on the surface of monocytes and neutrophils with consequent activation of inflammatory effecter mechanisms. We hypothesized that complement and complement receptors may also be targets for activation. We investigated this possibility in vitro, using human monocytes from healthy donors, isolated by centrifugation on ficoll, followed by adherence on plastic. 5 x 10^6 monocytes were incubated for 20 minutes at 37°C with 2 ng/ml TNFα. Subsequently 200 µg of isolated human IgG containing anti-myeloperoxidase (MPO) or anti-proteinase 3 (PR3) antibodies or normal human IgG (nhIgG) were added together with 50 µl of normal human serum (nhs) for 45 minutes at 37°C. Saturating doses of FITC labeled rabbit anti-human C3d or anti-C4c or an irrelevant antibody anti-IgE was added to the monocytes and incubated in the dark for 45 minutes at 4°C. The cells were then washed twice in PBS and resuspended in PBS/1% BSA/1% formaldehyde and stored at 4°C in the dark until FACS analysis. FACS analysis was performed using a flow

cytometer using an argon laser at excitation wavelength of 488 nm and emission wavelength of 530 (+15) nm. Additional experiments were performed with heat inactivated nhs (heated at 56°C for 30 minutes to inactivate complement), EGTA treated nhs (20 mM EGTA/0.8 mM Mg2+) to inhibit the calcium dependent classical pathway in the presence of the magnesium dependent alternative pathway, and C1q deficient serum. Incubation of normal human monocytes with nhs and anti-MPO or anti-PR3 IgG led to a dose and time dependent deposition of the complement breakdown products C3d and C4c. Both anti-MPO and anti-PR3 IgG led to significantly higher C3d deposition (median: range log fluorescence intensity 213:208-217 and 215:209-220, respectively) than incubation with nhIgG (152:142-158) ($p<0.05$). Similarly monocyte C4c deposition following anti-MPO or anti-PR3 IgG (214:209-223 and 210:205-213, respectively) was significantly higher than with nhIgG (157:154-161) ($p<0.05$). ANCA IgG-induced monocyte C3d and C4c deposition was completely abolished by decomplementation of nhs (by heat inactivation); following calcium depletion with EGTA; and following incubation with C1q deficient serum. Incubation of TNFα primed human monocytes with anti-MPO or anti-PR3 IgG followed by nhs leads to complement activation as determined by monocyte deposition of C3d and C4c. This complement activation occurs via the classical pathway as it is abrogated by C1q deficient serum, calcium depletion and heat inactivation of nhs. Deposition of complement components on monocytes or bystander endothelium may augment inflammation in ANCA positive vasculitis.

52-114

ANTI-ENDOTHELIAL CELL ANTIBODIES (AECA) RECOGNIZE A 100 kDa ANTIGEN IN MICROSCOPIC POLYANGIITIS (MPA) BUT NOT IN WEGENER'S GRANULOMATOSIS (WG), CHURG-STRAUSS SYNDROME (CSS) OR POLYARTERITIS NODOSA (PAN)

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Objective: To analyze the repertoire of reactivities of AECA in small and medium sized artery vasculitis.

Methods: Using a quantitative immunoblotting technique on extracts of cultured human umbilical vein endothelial cells (HUVEC), we analyzed the reactivities of serum IgM and IgG from patients fulfilling the ARA and Chapel Hill criteria for the diagnosis of PAN related or not to hepatitis B virus (HBV), WG, MPA, or CSS. Blood samples were obtained from 20 patients with non-HBV cPAN and 10 patients in each other group at the time of diagnosis and before treatment, 10 patients with chronic active hepatitis B without PAN and 60 age- and sex-matched healthy controls. Their sera were tested at the same IgG (200 µg/ml) and IgM (20 µg/ml) concentrations.

Results: MPA patients' IgM reacted with numerous HUVEC extract protein bands, with the two most important being of 100 and 65 kDa. In contrast, IgM from healthy controls and the other patients bound predominantly to one 65-kDa band and a few other minor bands. MPA patients' IgG reacted with 6-8 protein bands, mainly of 100 and 65 kDa, whereas IgG from healthy controls and the other patients reacted with 3-4 protein bands (including the 65-kDa band).

Conclusion: These results provide evidence that a specific 100 kDa antigen is recognized by AECA from MPA patients.

Disclosure: This work has been supported by Université Paris XIII and INSERM (CreS N°4CR08).

53-120

HUMAN AND MURINE PR3: FUNCTIONAL AND ANTIGENIC DIFFERENCES WITH POTENTIAL RELEVANCE FOR THE STUDY OF ANCA-ASSOCIATED VASCULITIS

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Rationale: PR3 is the target antigen for C-ANCA in ANCA-associated vasculitis (AAV) and an azurophil granule constituent also expressed on the PMN surface under inflammatory conditions. PR3-ANCA are thought to be pathogenic in AAV. In preparation of a murine model for PR3-ANCA associated vasculitis (PR3-AAV), we compared enzymatic activity, inhibitor spectrum and antigenicity of human PR3 (hPR3) and its murine homolog (mPR3).

Methods/Results: Recombinant hPR3 and mPR3 were expressed in HMC-1 cells which process granule serine proteases. HrPR3 was purified from HMC-1 by sequential anion- and cation-exchange chromatography. MrPR3 requires ethanol precipitation and subsequent binding of the 80% ETOH precipitate to phenyl-superose followed by elution with 2-propanol. Catalytic activity of mrPR3 per unit (t-boc-Ala) for substrate N-MeO-succ-AAPV-pNa is 6-fold higher than that of human PR3. a1-PI inhibits hrPR3 and mrPR3, but eglin C only inhibits mrPR3. These data indicate that mPR3 is more human elastase-like than hPR3. Polyclonal rabbit antibodies raised against hrPR3 and mrPR3 don't crossreact by immunoprecipitation (IP), Western blot or ELISA. Less than 10% of high-titer PR3-ANCA positive sera from AAV patients showed very weak crossreactivity with mrPR3 by IP or ELISA.

Conclusions: Functional similarities and differences in substrate and inhibitor spectrum as well as antigenic differences of human and murine PR3 exist which need to be understood for an appropriate interpretation of murine models of PR3-AAV. The differences can also be exploited for PR3-ANCA-epitope mapping as well as for structure-function analysis of PR3.

Epidemiology of Vasculitis

54-006

DRUG ALLERGY IS ASSOCIATED WITH PRIMARY SYSTEMIC VASCULITIS (PSV)

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Background: Allergy has been associated with PSV (Wegener's granulomatosis [WG] and Churg Strauss Syndrome [CSS]) and is one of the classification criteria for CSS. This is supported by reports of raised IgG levels and Th2 predominant cytokine profiles in CSS and active WG. We examined the evidence for allergy in a case-control study.

Methods: Detailed histories (including a validated questionnaire[1] were taken from 75 adult PSV patients, 220 age/sex matched non-disease hospital controls, 19 systemic rheumatoid vasculitis and 34 age/sex matched asthma controls. Details included: type (skin, drug, insect, plant, food), date and cause of allergy; allergic rhinitis; asthma; family history of allergies/asthma; vaccination or steroid withdrawal in the preceding 6 months; smoking history; TB exposure; hepatitis and blood transfusion. Odds ratios (OR) and 95% confidence intervals (C.I.) were calculated by conditional logistic regression. Total PSV and subgroups (47 WG, 26 CSS, 12 microscopic polyangiitis (mPA), 30 cANCA/PR3 positive, 19 pANCA/MPO positive) were compared to non-disease controls. PSV and CSS were also compared to disease controls.

Results: ORs (95% C.I.) were significantly raised for combined allergy [2.21 (1.30-3.77)], drug allergy [3.38 (1.81-6.29)] and asthma [4.96 (2.49-9.88)] but not other allergy types or rhinitis. Significant ORs (95% C.I.) were found for drug allergy in WG [3.46 (1.63-7.12)], mPA [3.70 (1.02-13.45)] and cANCA [4.60 (1.99-10.61)] but no other groups. Antibiotic allergies (predominantly penicillin) gave significant ORs for PSV [4.15 (1.99-8.65)], WG [4.42 (1.92-10.18)], CSS [4.02 (1.16-14.01)] and cANCA [5.89 (2.28-15.19)] in contrast to other drug allergies. As expected, steroid withdrawal, asthma and rhinitis was higher for CSS vs non-disease but not asthma controls, and PSV had fewer blood transfusions [0.23 (0.08-0.69)] than SRV. Other allergies, family history, smoking, TB exposure, hepatitis and vaccinations were not associated with total PSV or any subgroup.

Conclusions: PSV (especially WG and mPA) was associated with antibiotic allergy. Drug allergies are heterogeneous but, in penicillin allergy, beta-lactam specific T-cells are reported to have a Th2 skewed cytokine profile. One results support the potential role of this type of allergic response in the pathogenesis of PSV.

1. Cuadrado et al, BJR, 1994, 33: 749-753

55-007

PRIMARY SYSTEMIC VASCULITIS—MORTALITY IN A POPULATION-BASED COHORT

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Background: Immunosuppression has greatly reduced mortality in PSV but poorer prognosis has been reported in patients with microscopic polyangiitis (mPA), renal disease, radiographic pulmonary infiltrates and increasing age. Most reports, from tertiary referral centers, are likely to be affected by selection bias. We investigated mortality in an unselected population-based cohort.

Methods: 97 PSV patients, resident in the Norwich Health Authority (NHA) were identified by a prospective vasculitis register (50 Wegener's Granulomatosis-WG, 28 mPA, 19 Churg Strauss Syndrome-CSS). Age at diagnosis, sex, ANCA type, presence or absence of renal/respiratory disease, cause of death and comorbidity were obtained by case note review. For each year incident and prevalent cases and deaths were recorded. Norfolk City Council data were used to obtain population and mortality figures for the 1994 NHA population. Standardized mortality ratios (SMR) were calculated by indirect standardization for 90 PSV patients, diagnosed January 1989-December 1998, compared to the NHA population. A poisson distribution was assumed. SMR's were compared between age-groups, sex and diagnoses using *z*-values. A Cox proportional hazards model compared survival by diagnosis, sex, age, ANCA type and presence/absence of comorbidity and renal or respiratory disease for all cases (May 1988-May 2000).

Results: The SMR (95% C.I.) for PSV was 4.78 (2.98-6.59), higher for men than women [5.94 (3.11-8.76) vs 3.05 (1.16-6.59) p=0.09]. Differences were not significant between age groups or diagnoses. 1 year survival was similar for WG, mPA and CSS (85.5%, 82.7% and 83.2%) but 5 year survival differed: WG=75.9%, mPA=45.1%, CSS=68.1%. Mean survival for PSV was 51.5 months (1-144 months). Survival was less for >65 vs <65 year olds (Log rank, p=0.009) and mPA compared to other diagnoses (Log rank, p=0.07). Hazard ratios [HR (95% C.I.) showed significantly increased risk with age [>61 years, HR=9.22 (2.02-42.0), p=0.03] and mPA [vs CSS, HR=2.52 (0.89-7.15), p=0.077] but no significant differences with ANCA type, comorbidity, renal or respiratory involvement.

Conclusion: The association of increased mortality with age in PSV is due to the expected difference in mortality between age-groups rather than more severe disease as previously suggested.[1] mPA has poorer late prognosis than other diagnoses.

1. Vassallo M et al, JRCP, 1997, 31(4): 396-400

56-008

ARE ENVIRONMENTAL FACTORS IMPORTANT IN SYSTEMIC VASCULITIS?

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Background: The aetiology of Primary Systemic Vasculitis (PSV) is unknown. Potential risk factors include infection, silica, solvents, metal fumes and rural residence.[1] We carried out a case-control study to further explore these and other environmental factors.

Methods: 75 PSV patients (from a prospective vasculitis register), 220 age/sex matched non-disease hospital controls,

19 systemic rheumatoid vasculitis and 34 age-sex matched asthma controls were interviewed using a modified version of a previously used questionnaire.[2] Details included: social class, occupational and residential history, silica, smoking, pets and detailed farm exposure in the year prior to symptom onset (Index Year). Jobs were coded by the Standard Occupational Classification 2000. Job exposure matrices were used to assess levels and duration of silica, solvent and metals exposure. Odds ratios (OR) and 95% confidence intervals (C.I.) were calculated by conditional logistic regression. Total PSV and subgroups (47 Wegener's (WG), 12 microscopic polyangiitis (mPA), 16 Churg-Strauss syndrome (CSS), 19 pANCA/MPO & 30 cANCA/PR3 positive) were compared to controls.

Results: Significantly raised ORs (95% C.I.) were found for a number of factors including farm exposure in the Index Year in PSV [3.15 (1.70-5.83)] and WG [3.59 (1.83-7.03)]. Exposure to livestock (cows, sheep, chickens) was significantly associated with PSV [3.78 (1.17-12.22)]. Working in high silica exposure jobs in the Index Year gave raised ORs for PSV [3.62 (1.41-9.31)], WG [3.45 (1.16-10.25)] and CSS [5.6 (1.34-23.46)]. A history of a high solvent exposure occupation was significantly associated with PSV [2.35 (1.03-5.37)], WG [3.69 (1.54-8.85)] and cANCA [3.43 (1.22-9.68)]. There was no trend to increasing PSV risk with duration of exposure to silica or solvents. There were no significant differences for other items investigated.

Conclusions: This is the first study to report an association between farm exposure and PSV. The association with exposure to livestock may suggest an infectious aetiology, but no single animal is implicated. Results also support a role for silica and solvent exposure in PSV.

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2. Duna GF, Clin Exp Rheum, 1998; 16: 669-674

57-009

SEASONAL AND PERIODIC VARIATION IN PRIMARY SYSTEMIC VASCULITIS (PSV)

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Background: Some previous studies report that PSV is more common in the winter and may show a periodic fluctuation over many years.[1,2] This suggests that an infectious trigger may be important. We studied seasonal and annual fluctuations in PSV over a ten-year period in an unselected, U.K. population and compared annual fluctuation with common infections.

Methods: All PSV cases diagnosed in the Norwich Health Authority (NHA) between Jan 1989-July 2000 were identified by a prospective vasculitis register. The date of first symptom of PSV (Index date), date of diagnosis, ANCA type and disease classification (Wegener's Granulomatosis-WG, microscopic polyangiitis-mPA, Churg-Strauss Syndrome-CSS) were determined by case note review. Details of the annual fluctuation in mycoplasma pneumonia, parvovirus and chlamydia for the Eastern region (U.K.) were obtained from the Public Health Laboratory Services, London. Annual fluctuations were compared using the poisson distribution and seasonal differences by the chi-squared test.

Results: Of 96 NHA residents diagnosed with PSV between Jan 1989-July 2000, 88 had an Index date between Jan 1989-Dec 1998. There was a trend towards higher onset of PSV in winter and lower in summer, especially in WG and cANCA positive patients (table). There were no significant annual peaks and troughs in the onset of PSV. Annual peaks of infections did not correspond to non-significant fluctuations in PSV.

TABLE 1.
SEASONAL VARIATION IN PSV AND SUBGROUPS (%)

	PSV	WG	mPA	CSS	cANCA	pANCA
Winter (Dec-Feb)	29.9	25.5	42.9	27.8	29	35
Spring (Mar-May)	25.3	23.5	17.1	38.9	19.4	30
Summer (Jun-Aug)	17.2	15.7	17.1	16.7	9.7	15
Autumn (Sept-Nov)	27.6	35.3	22.9	16.7	41.9	20

Conclusions: Data weakly support an autumn/winter peak and summer dip in WG and cANCA. There was no evidence for a cyclical fluctuation in PSV over 10 years or an association of peaks of influenza, mycoplasma, parvovirus or chlamydia.

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2. Tidman M, J Intern Med, 1998, 244(2): 133-41

58-014

CLINICAL AND EPIDEMIOLOGICAL ANALYSIS OF GIANT CELL (TEMPORAL) ARTERITIS FROM A NATIONWIDE SURVEY IN 1997 IN JAPAN: THE FIRST GOVERNMENT SUPPORTED NATIONWIDE SURVEY

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Objective: To elucidate epidemiological and clinical manifestations of Japanese patients with giant cell arteritis (GCA), the first nationwide survey for GCA was performed in 1997 in Japan.

Methods: The questionnaire on the patients with GCA who had been seen in 1997 was sent to 10,717 departments in Japan. One hundred seventy-seven patients were reported from 6,835 divisions. The answers to the questionnaires detailed in the clinico-epidemiological features on 77 patients were obtained and analysis was conducted on 71 GCA patients.

Results: Prevalence in patients 50 years of age and older in 1997 was 1.47 per million population in Japan. The averaged age at onset was 71.5 years old. The male: female ratio was 1:1.7. The association with visual loss (6.5%), jaw claudication (14.7%), and polymyalgia rheumatica (PMR) (28.2%) were low in frequency compared to those reported from other countries. More than half of the patients were treated with prednisolone less than 40 mg/day with the efficacy of 90.2%. Only three (4.5%) patients were reported as deceased due to other causes.

Conclusion: It revealed that the prevalence of GCA in Japan is extremely low compared to other countries. The clinical findings of visual loss, jaw claudication, and PMR were low in frequency among Japanese patients with GCA. We assumed that the low prevalence of GCA in Japan is due to low frequency in HLA-DNA typing of DRB1*0401,0404 among the Japanese population.

59-024

EXPOSURE TO SILICA AND ANCA-ASSOCIATED VASCULITIS

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Introduction: Exposure to silica is considered among etiological factors of antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV). A study is carried out to investigate the association between ANCA and occupational exposure to silica dust.

Methods: 123 patients (122 men and one woman) exposed to silica were examined (mean age 67.6 y, mean exposure 20.0 y). ANCA were tested by immunofluorescence, its specificity by ELISA for proteinase 3 (PR3), lactoferrin, bactericidal-permeability increasing protein (BPI), and myeloperoxidase (MPO). Laboratory and clinical data were collected and analyzed. 27 men represented age-matched control group.

Results: ANCA among silica-exposed persons was detected 21x (17.1%), 2x anti-MPO, 4x anti-BPI, 3x anti-PR3, 1x anti-LTF. No patient suffered from AAV. ANCA were found significantly less frequently (4.9%) in the group of persons with history of SiO_2 exposure without signs of silicosis (risk of silica, RS) than in the group with simple silicosis (SS) (28.6%) or complicated silicosis (CS) (29.6%). Frequency of ANCA+ in controls was 3.6%. Kidney function impairment was found more frequently in ANCA+ patients. Odds ratio for ANCA positivity and the relative risk estimate for patients with both forms of silicosis was highly significant. Predictor factor for ANCA positivity was silicosis, history of tuberculosis, and higher serum creatinine level.

Conclusion: Exposition to silica itself is not associated with increased frequency of ANCA. Risk factors for ANCA positivity are silicosis, tuberculosis, and kidney function impairment. However, the presence of ANCA is not associated with vasculitis in silicotic patients. Other factors must be involved in the triggering of vasculitis in ANCA-positive patients.

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60-036

GERMAN VASCULITIS REGISTER: RESULTS OVER THE FIRST THREE YEARS

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Background: Little has been published on the epidemiology of primary systemic vasculitis (PSV). Much of the data comes from referral centers or covers only small areas, leading to referral or selection bias. This prompted us to establish a Vasculitis Register for North and South Germany to determine the incidence of PSV in a population-based study in a large region (nearly 5 million habitants) at 1/1/1998.

Methods: Data on all new cases of PSV (as defined by the CHCC) are obtained from the following sources: (1) all departments of all hospitals, including their outpatient clinics, (2) all departments of pathology, and (3) reference immunological labs. At three-month intervals all sources were asked by mail (up to three times) to screen for newly diagnosed cases of PSV.

Results: Over the first three years (1998 - 2000) 597 PSV patients were identified. The incidence of PSV was 45 to 54 cases/million/year without differences between north and south Germany. The incidence of ANCA-associated PSV (WG, CSS, MPA) was 9-12/mio/year. The most frequent ANCA-associated PSV was the WG with an incidence of 7 new cases/mio/year. Over the whole period 84 patients with newly diagnosed WG were registered. Their median age at diagnosis was 60 years, conspicuously higher than described in large WG cohorts. On the other hand, the time between the first WG symptoms and diagnosis was only 3 months.

Conclusion: Compared to other countries, in Germany the incidence rate of WG was similar to that in Norway (8) and US (8), but higher than in Spain (5), and lower than in UK (11). If these results reflect a real north-south difference similar to that found for the GCA or whether be caused by differences in the case finding methods (population based study in a large region vs. studies from referral centers or small regions) remains unclear.

61-039

POLYARTERITIS NODOSA IN CHILDHOOD

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Introduction: This study describes the clinical, histological, and angiographic features of polyarteritis nodosa (PAN) presenting in childhood.

Methods: Retrospective review of case notes of patients diagnosed with PAN. Only patients who satisfied 3 or more of 10 classification criteria as defined by the American College of Rheumatology (ACR) were included. Angiography was reviewed independently by 2 blinded radiologists.

Results: Between 1971 and 1998, 38 children satisfied 3 or more of 10 ACR classification criteria for PAN. There was a male preponderance of 1.9:1. Mean age was 7.9 years (range

0.3-14.4 years). All had fever and elevation of acute phase reactants. Additional clinical features included rash (61%), renal impairment (24%), hypertension (34%), myalgia (79%), weight loss (79%), testicular pain (20% of males), peripheral neuropathy (13%), cerebral involvement (8%), and sub-arachnoid hemorrhage (3%). No patient had evidence of hepatitis B infection. 9/12 skin biopsies revealed vasculitis. Renal biopsy was performed in 9 patients and revealed crescentic glomerulonephritis (GN) (4/9), mesangio-proliferative GN (3/9), and focal segmental sclerosis (1/9). Vasculitis was also demonstrated on biopsy of the liver, temporal artery, and gut. 35/38 patients had abnormal visceral angiography. A spectrum of angiographic findings was documented and included aneurysms, renal perfusion defects, collateral renal arteries, arterial cut-off, and pruning of the renal arteries. Overall, the mortality for PAN was 8%.

Conclusion: PAN has a wide spectrum of presentation and is a great imitator of many pediatric conditions. Often the diagnosis remains elusive unless specifically sought, and visceral angiography and tissue biopsy play a key diagnostic role.

62-040

SYSTEMIC NECROTIZING VASCULITIS AND INFLAMMATORY BOWEL DISEASE OF CHILDHOOD

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Systemic necrotizing vasculitis (SNV) can mimic inflammatory bowel disease (IBD). The differentiation between primary SNV and IBD can be clinically testing, however it is important to distinguish these disorders since their treatment and outcomes are different. The aims of this study were therefore to describe a series of children with SNV who initially presented with clinical features suggestive of IBD. 7 children (5 boys, mean age 8.6 years, 2.5-14 years) presenting between 1993-98 satisfied inclusion criteria. All had abdominal pain, failure to thrive, diarrhea (4/7 bloody), and laboratory evidence of a severe acute phase response. Mean colitis score was 4.7 (3-7). Other clinical features included renal impairment (1/7), vasculitic rash (5/7), myalgia (6/7), testicular pain (1/5), and polyarthritis (3/7). pANCA was present in 3/7. Anti-enterocyte antibodies were present in 2/5 patients. Labelled white cell scan showed increased gut uptake in 5/6 patients. Visceral angiography was suggestive of vasculitis in 6/6 studies performed, with renal (5/6), and mesenteric or hepatic (6/6) vascular bed involvement. Endoscopy was abnormal in 6/7, with patchy loss of normal mucosal vascular patterns and areas of sharply demarcated disease activity at watershed areas as noted features. Gut histology revealed indeterminate chronic inflammatory changes in all 7 patients. Treatment comprised systemic steroid (7/7), cyclophosphamide (4/7), azathioprine (7/7), mycophenolate (2/7), cyclosporin (1/7), ASA derivatives (4/7), colchicine (1/7), and plasma exchange (1/7). At mean follow-up of 4 years, all patients are currently in remission although have had a relapsing clinical course, and one patient is off all treatment. Primary SNV can mimic IBD in its clinical presentation. Serology including ANCA and anti-gut antibodies do not help to discriminate between the two groups of diseases. Extra-intestinal manifestations, and acute phase responses, which are disproportionate to the degree of intestinal inflammation, may provide clues to the presence of a primary SNV.

63-042

INCIDENCE AND CLINICAL FEATURES OF WEGENER'S GRANULOMATOSIS IN OLMSTED COUNTY, MINNESOTA, 1990-1999

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Objective: To assess the incidence and clinical features of Wegener's granulomatosis (WG) in a population based cohort of patients since the introduction of antineutrophil cytoplasmic antibody (ANCA) testing.

Methods: Case ascertainment of patients with WG was performed by retrospective medical history review of 231 patients with the diagnosis of systemic vasculitis in Olmsted County, during the years 1990-1999. For completeness, the medical histories of all 699 patients from Olmsted County undergoing ANCA testing in this period were also reviewed to ensure that no cases of WG were missed by the medical record review.

Results: ANCA testing was performed in 49 patients with a diagnosis of vasculitis. Of these, 6 had a positive c-ANCA (all of whom had WG), 11 had a positive p-ANCA, and in the remaining 32, ANCA was negative. A total of 8 incident cases of WG occurred (3 men, 5 women); median age 60.5 yrs. (range 40-81). The overall age and sex adjusted annual incidence of WG was 0.83 cases/100,000 population (95% CI 0.25-1.42). For the population age ≥18 years, the age and sex adjusted annual incidence was 1.1 per 100,000 (95% CI 0.33-1.9). Two patients died during the follow-up period. C-ANCA was positive in 6, and p-ANCA (myeloperoxidase) was positive in the other. Half (4) of the patients were diagnosed with WG prior to obtaining ANCA results; in the other 4 ANCA results were available prior to a final diagnosis of WG and were useful in disease classification.

At the time of diagnosis, the median Birmingham vasculitis activity score (BVAS) was 23.5 (range 18-34, and the median BVAS/WG was 10 (range 5-15); activity scores correlated well between these scales. The frequency of organ involvement in these patients (n,%) was: ears/nose/throat 5 (62.5); lung 6 (75); kidney 6 (75); muscle/joint 6 (75); eye 1 (12.5); peripheral nervous system 3 (37.5); central nervous system 0 (0); gastrointestinal 2 (25); heart 2 (25); skin 5 (62.5); malaise 5 (62.5).

Conclusions: Since the advent of widespread ANCA testing, WG continues to be rare. The incidence of WG as seen in this population based study performed in Olmsted County is similar to that seen in older hospital based studies, suggesting that availability of ANCA testing has not lead to a marked increase in the numbers of patients diagnosed with this disease. The organ involvement in this study is similar to that of older hospital based series. A positive c-ANCA was 100% specific for the diagnosis of WG in this population.

64-060

CLINICAL FEATURES OF SYSTEMIC VASCULITIS IN SANTIAGO, CHILE: A TEN-YEAR STUDY

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Objective: To describe the clinical features of microscopic polyangiitis (MPA), polyarteritis nodosa (PAN), and Wegener's granulomatosis (WG) in a Chilean cohort of patients.

Methods: Case ascertainment was performed by retrospective review of medical records, of 173 patients with the diagnosis of systemic vasculitis from 1990 to 2001. The diagnoses were made according with the ACR and Chapel Hill criteria. Thirty-two patients were excluded because they did not fulfill these criteria. Therefore, we included 65 MPA, 18 PAN, and 58 WG patients.

Results: The mean follow-up (months) for MPA was 15 (1-120), PAN 24 (2-60), and WG 20 (1-120). The median age (years) at diagnosis for MPA was 61 (19-82), PAN 44 (17-83), and WG 50 (20-82). Gender distribution was similar among the three groups (male: 68%, 67%, and 57% respectively). The main clinical features for MPA were renal involvement (68%) (characterized by elevated plasmatic creatinine levels and inflammatory urinalysis), peripheral nervous system involvement (57%), pulmonary hemorrhage (28%), and skin disease (32%). For PAN were cutaneous involvement (45%), peripheral nervous system involvement (39%), hypertension (22%), abdominal pain (22%), myopathy (28%), and renal disease (17%). For WG were alveolar hemorrhage (62%), renal involvement (78%), ENT compromise (65%), and ocular disease (26%). In both, MPA and WG, creatinine levels above 2.0 mg/dl were associated with higher mortality ($p < 0.01$). MPA patients with pulmonary hemorrhage had significantly higher levels of creatinine compared to those without it (6.8 vs 3.4 mg/dl, $p < 0.01$). ANCA by immunofluorescence was performed in 56 MPA (77% ANCAp, 3% ANCAc, 20% negative) and in 55 WG patients (17% ANCAp, 76% ANCAc, 7% negative). The majority of PAN patients were ANCA negative (88%). Global mortality for each group was 18%, 14%, and 17%, and the major causes of death were infections.

Conclusion: The clinical features of our patients are similar to other published data. In our WG and MPA patients the main predictor for death was renal disease with a creatinine above 2 mg/dl. In the MPA cohort the presence of pulmonary hemorrhage is also a significant predictor of death.

65-067

ANCA NEGATIVE POLYARTERITIS NODOSA IN LUND 1990-2001

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The aim of this study was to characterize all patients with polyarteritis nodosa (PAN) seen at our units during the last twelve years and to analyze their outcome. Patients with signs of small vessel vasculitis, such as presence of ANCA or crescentic glomerulonephritis, were excluded. Nine patients (five male and four female) were found to meet the criteria of PAN and were studied retrospectively in detail. Five were diagnosed by angiography and four by biopsy. The median age at diagnosis was 45 years (range 8-77). The time from first symptom to diagnosis varied from 2 weeks to 38 months (median 4 months). Only one patient was found to have hepatitis that could have contributed to the development of PAN.

Organ involvement at diagnosis:

abdominal	6	muscle	3
renal	5	joint	2
skin	5	testicle	2
hypertension	5	eye	1
peripheral nerve	3	lung	1

The patients were followed for 0.5-17 years (median 3 years). Five patients had altogether seven relapses. The median time from diagnosis until the first relapse was 3 years (range 1-6). One patient died after five months. Two patients developed end stage renal failure and started treatment with hemodialysis seven and eight years after diagnosis. Both had malignant hypertension at diagnosis.

During the twelve year period five new cases were diagnosed among patients living in our local catchment area of 300,000 inhabitants, which gives an annual incidence of 0.5 per million. PAN is a rare disease in Sweden, but should not be forgotten in patients with systemic symptoms without ANCA.

66-115

PREVALENCE OF POLYARTERITIS NODOSA (PAN), MICROSCOPIC POLYANGIITIS (MPA), WEGENER'S GRANULOMATOSIS (WG) AND CHURG-STRAUSS SYNDROME (CSS) IN A FRENCH URBAN POPULATION IN 2000: A CAPTURE-RECAPTURE ESTIMATE

Mahr, A[1,2], Guillevin L[2], Poissonnet M[2], Aymé S[1]. [1]Paris, [2]Bobigny, France.

Objective: To estimate the prevalences of PAN, MPA, WG and CSS in an urban multiethnic population.

Methods: Cases were collected in Seine–Saint-Denis *Département*, a northeastern suburb of Paris, which has 1,093,515 adults (≥15 yr), 28% of whom are of non-European ancestry. The study period encompassed the entire calendar year 2000. Cases were identified by general practitioners, the departments of all the public hospitals and 2 large private clinics, and the Public Health Insurance System. The Chapel Hill nomenclature was used to define MPA, and ACR criteria to define WG and CSS; PAN was diagnosed based on clinical, laboratory, histological and/or angiographic findings. Only histologically and/or angiographically documented cases were retained. Three-source capture–recapture analysis (CRA) was performed to correct for incomplete case ascertainment.

Results: A total of 65 confirmed cases were identified; among 18 non-verifiable cases, 7 additional cases were estimated to be true cases. CRA estimated that 21 cases had been missed by any

1 of the 3 sources. Accordingly, prevalences per 1,000,000 adults (CI 95%) was estimated to be 31.8 (23-41) for PAN, 18.9 (11-27) for MPA, 23.0 (16-30) for WG and 10.2 (5-16) for CSS. The overall prevalence was 1.9 times higher for subjects of European ancestry than for non-Europeans (p = 0.02).

Conclusion: This study provides the first prevalence estimates for these 4 vasculitides for a multiethnic and urban population. The significantly higher prevalence observed for Europeans may infer a genetic susceptibility of Caucasians. Compared to previous estimates based mostly on rural populations, the higher frequency of PAN and the lower frequency of WG might suggest specific environmental etiological factors.

67-127

HIGH-DOSE INTRAVENOUS IMMUNOGLOBULINS IN ANCA-ASSOCIATED SYSTEMIC VASCULITIS (AASV)

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New drugs are regularly tested in AASV to reduce disease activity and adverse events associated with corticosteroids and immunosuppressives. Intravenous immunoglobulins (IVIg) are mainly used in patients who are resistant to usual therapeutic regimen. We report on the results of IVIg therapy in patients with AASV who have relapsed.

Five relapses occurring in 4 patients (Wegener's disease: 2 patients, microscopic polyangiitis: 2 patients) were treated by 6 monthly courses of IVIg (0.5 mg/kg/d during 4 days), and prednisone (0.5 g/kg/d). One patient was on azathioprine (50 mg/d). Disease activity was assessed by the Birmingham vasculitis activity score (BVAS). ANCA levels (IF, ELISA) were tested before each course of IVIg and at the end of the study.

At the time of the relapse, the median BVAS was 11 (3-15). Two out of 5 relapses were major (cerebral and renal angiitis). ANCA were positive in each relapse (MPO-ANCA n=4, PR3-ANCA n=1). At the end of the study, complete remission was achieved in every case (BVAS=0). ANCA levels decreased in 2 cases (1/1 PR3-ANCA, 1/4 MPO-ANCA). Acute renal failure due to osmotic tubular injury occurred during one course of treatment, followed by complete recovery without dialysis. Other side effects were minor (n=7). Three new relapses occurred from 2 to 18 months after the last course of IVIg.

We conclude that IVIg may be an alternative to conventional immunosuppressive drugs for inducing remission in relapsing AASV. However, a high rate of relapses occurred after IVIg withdrawal, underscoring the need for additional maintenance therapy.

68-128

VASCULITIS: ARE CURRENT CLASSIFICATIONS USEFUL IN CLINICAL PRACTICE?

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Introduction: Systemic vasculitis is a heterogeneous group of clinical manifestations of unspecific etiology, with or without cutaneous compromise. The gold standard test is the histopathological study. In this descriptive study we reviewed the histological studies with confirmed vasculitis diagnosis and made a correlation with the current classifications.

Materials and Methods: In a retrospective way, we studied all the confirmatory histological studies processed between 1953 and 1990 and prospectively from 1991 to 1997 at Hospital San Juan de Dios (Bogota, Colombia) that had a confirmatory result for vasculitis. Then, we classified them using both Chapel Hill and Lie's classifications in order to determine their accuracy and usefulness in clinical practice.

Results: In this descriptive trial, we found 304 histopathological studies with documented vasculitis of 140,717 that were made in this period. We found an annual incidence of 22 per 10,000 in this population group; the mean age of presentation was 36 (range: 10 to 82); the female:male ratio was 2:1; skin (69%) and muscle (15%) were the most important organs processed. It was possible to classify only 40 histological studies (13%) using the Chapel Hill consensus and its different subsets. The most frequent vasculitis found was that of median vessels (24 of 40, 60%), and all of them corresponded to nodosum poliarteritis. With Lie's classification we could find a correlation in 121 plaques (40%). In this case, primary vasculitis was the most frequent diagnosis (78 of 121, 64%), the miscellaneous group being the most common (38 of 78, 49%) represented by Bnerguer disease. Secondary vasculitis occupied the second place (43 of 121, 35%), represented by connective tissue diseases (27 of 43, 63%), mainly SLE and dermatopolimyositis (14 of 43 in each case). Leucocitoclastic (67 of 183, 37%) and linfomonocitic (49 of 183, 27%) vasculitis conformed the most frequent unclassified groups.

Conclusions: Considering that the histopathologic study is the gold standard for diagnosis of vasculitis, we found that current vasculitis classifications are incomplete and let many pathologies out of these categories. Therefore, further efforts should be made in order to create more complete and comprehensive tools to classify these diseases tending to facilitate clinicians practice and scientific trials.

Diagnostic Modalities, Surrogate Markers of Disease Activity, and Tools for Outcome Measurements

69-002

PROTEINASE 3 IS THE MAJOR AUTOANTIGEN IN HEPATITIS C VIRUS INFECTION

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Hepatitis C virus (HCV) infection has been found to be

TABLE

	CHOP patients	Literature patients	Summary
Number of subjects (females, males)	6 (5, 1)	136 (107, 29)	142 (112, 30)
Mean age, yrs (range)	8.6 (1.6-17)	11.5 (3-17)	11.4 (1.6-17)
Reported signs and symptoms	Fever (4/6), stroke (1/5), arthralgias (2/5), fatigue (2/5), skin nodules (3/6), anorexia/wt loss (3/6), claudication (2/5), chest pain (2/5), back pain (2/5), palpitations (2/5), headache (1/5), vomiting (1/6)	Fever (20/54), stroke (4/24), arthralgias (5/30), fatigue (2/5), skin nodules (5/26), abd pain (18/97), vomiting (30/120), claudication (17/107), chest pain (7/75), palpitations (25/104), anorexia/wt loss (15/99)	Fever (24/60), stroke (5/29), arthralgias (7/35), fatigue (4/10), skin nodules (8/32), abd pain (18/97), vomiting (31/126), claudication (19/112), chest pain (9/80), palpitations (27/109), anorexia/wt loss (18/105)
% with HTN	67% (4/6)	89% (121/136)	88% (125/142)
% with ESR ≥20	83% (5/6)	59% (72/121)	61% (77/127)
% with HTN and ESR ≥20	67% (4/6)	70% (48/69)	69% (52/75)
% with cardiomegaly on CXR	50% (2/4)	55% (30/55)	74% (25/34)
Imaging results	Angiography in 5 pts; aorta, subclavian, vertebral, carotid, cerebral, celiac, splenic, renal arteries involved; MR in 4 pts (2 with gadolinium); thickened aortic, carotid, renal, celiac, subclavian walls or pseudoaneurysm of the iliac artery	Angiography in 118 pts; involvement of the aorta, subclavian, carotid, superior mesenteric, celiac, pulmonary, coronary, splenic, hepatic, vertebral, brachiocephalic, renal, and cerebral arteries	100% of patients who had imaging done showed abnormalities of the aorta and vessels of the thorax, abdomen, or head

strikingly associated with autoimmune phenomena. The aim of the present study was to investigate the presence of various autoantibodies in patients with HCV infection.

ANCA, anti-E3 antibody, and RF were positive in 278/516 (55.6%), 276/516 (53.3%), and 288/516 (56%) patients with HCV infection, respectively. Positivity for ANA was present in 15.8%, anti-ssDNA in 15.6%, anti-dsDNA in 8.5%, aCL in 5%, anti-SS-B/La in 4.1%, anti-SS-A/Ro (60 kD) in 3.9%, anti-E2 in 3.3% and anti-SSA/Ro (52 kD) in 1.2 %, anti-MPO in 4.8%, anti-Topo II and anti-actinin in 0%. All sera with ANCA showed c-ANCA pattern and contained anti-PR3 specificity. HCV patients with ANCA showed a higher prevalence of skin involvement, anemia, abnormal liver functions and α-Fetoprotein (α-FP). The prevalence of autoantibodies was not affected by the treatment of interferon-alpha (IFN-α)

In conclusion, autoantibodies are commonly found in patients with HCV infection. There is a high prevalence of anti-E3, ANCA, and RF in these patients. Proteinase 3 is the major target antigen in HCV infection.

70-021

TAKAYASU ARTERITIS IN CHILDREN

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Takayasu arteritis (TA) is a rare, chronic idiopathic granulomatous vasculitis of the aorta and its branches, predominantly affecting young women (<40 yrs old).

Objective: To determine the diagnostic features of children diagnosed with TA.

Methods: We identified 136 patients <18 yrs old from published reports on TA using the National Library of Medicine PubMed system. We systematically analyzed demographic and clinical data at presentation. We then identified 6 patients with TA cared for at CHOP in the last 10 years and compared them to published cases.

Results: See Table at top of page.

Discussion: Failure to recognize the early signs of TA in children leads to a delayed diagnosis (19 mos vs. 10 mos for adults), more severe hypertension, more congestive heart failure (66%), and higher mortality (30-35% vs. 5-15% in adults). Hypertension and elevated ESR are found in most patients with TA and should merit further screening for TA. MRI/MRA of the thoracic and abdominal aorta and great vessels, using gadolinium contrast to image stenotic arteries with thickened enhanced vessel walls is emerging as a non-invasive tool to diagnose TA.

71-032

ARE C-ANCA ALWAYS USEFUL IN LONG-TERM FOLLOW-UP OF WEGENER'S GRANULOMATOSIS?

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Wegener's granulomatosis (WG) is a necrotizing vasculitis seriously involving mostly upper airways, lungs and kidneys, requiring a long-term therapy with several side effects. It is well established that c-ANCA are very useful in diagnosing the disease.

In order to evaluate the reliability of c-ANCA not only in the diagnosis, but also in the long-term follow-up of WG we considered eight patients (seven males and one female), aged at diagnosis from 20 to 65, followed up at our Clinic for a period of time of five to ten years. The patients were evaluated at least twice per year with clinical examination and laboratory and radiological tests. All patients had their diagnosis made at least five years ago by histological examination of a bioptic tissue specimen (from nose or lung or kidney) and all of them had highest titers of c-ANCA (1:640 to 1:10,240), always confirmed by ELISA test.

All patients were treated with steroids and cyclophosphamide with good response. In particular, c-ANCA became negative in six months-one year. Two patients have done very well even after therapy had been discontinued. Their c-ANCA are always negative. Two other patients are still on therapy: they presented three and four relapses in five and eight years, respectively, but their c-ANCA titer raised only minimally and the autoantibodies rapidly disappeared. The other four patients had a clinical relapse (especially with pulmonary nodules and cavitations), with increase of C reactive protein, but absence of c-ANCA, and rapid response to immunosuppressive therapy.

It is noteworthy that c-ANCA titer is likely to follow the severity of the disease, especially in those patients for whom recovery seems harder. On the other hand c-ANCA do not seem highly reliable in detecting a relapse of WG and probably in the long-term follow-up of patients with WG the suspicion of a relapse should be based mostly on the clinical setting because the reappearance of c-ANCA, even after reduction of doses or discontinuation of therapy, is not constant.

72-034

INCREASED SPECIFICITY FOR SYSTEMIC VASCULITIS WITH CAPTURE ELISA FOR MPO?

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Background: High levels of MPO-ANCA are usually found only in patients with systemic vasculitis. Low levels are less specific and can be found also in many non-vasculitic conditions. This pilot study investigates two different capture-MPO-ANCA assays concerning their specificity for the diagnosis of small vessel vasculitis compared to standard ELISA.

Methods: Patients whose first test exhibited a low or moderately elevated value in standard MPO-ANCA ELISA were included in this study. Patient records were reviewed and a diagnosis was established using the Chapel Hill nomenclature. Sera were tested using two different capture assays based on the monoclonal antibodies 2B11 and 099. These antibodies are known to react with different non-overlapping epitopes on the MPO molecule. If the result in the capture assay yielded a value that was less than 30% of the value in the standard assay, it was considered a significant reduction.

Results: Out of 36 patients with low MPO-ANCA, 27 were diagnosed as having small vessel vasculitis and 9 patients were found to have other diagnoses. For patients with other diagnoses than vasculitis the result of the 2B11 assay was significantly reduced in 55% (5/9) of the cases. Only 7% (2/27) of the vasculitis patients showed reduced results with the 2B11 assay. With the 099 assay corresponding figures were 45% (4/9) and 37% (10/27).

Conclusion: A capture assay based on Mab 2B11 seems to be more specific for the diagnosis of small vessel vasculitis as compared to standard ELISA and the other capture ELISA. Antibodies against the 2B11 epitope may be irrelevant for the diagnosis of small vessel vasculitis.

73-050

FIVE DISTINCT CLINICAL SUBSETS AND THEIR PROGNOSTIC IMPLICATIONS IN MPO-ANCA ASSOCIATED VASCULITIS

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Aim: Clinical subsets in MPO-ANCA associated vasculitis were classified and were examined on the association of these subsets to prognosis.

Method: 50 patients were studied on the clinical subsets and prognosis. Five clinical subsets, which were renal limited type, pulmorenal type, systemic vasculitis type, pulmonary type, and non-pulmorenal type, were identified. The prognosis of life and renal function in these types was studied more than 3 years.

Results: 50 patients were classified into 24 cases with renal limited type, 13 cases with pulmorenal type, 7 cases with systemic vasculitis type, 2 cases with pulmonary type, and 4 cases with non-pulmorenal type. The death cases over 3 years observation period occurred in 8% with renal limited type, in 31% with pulmorenal type, in 57% with systemic vasculitis type, in 50% with pulmonary type, and in 0% with non-pulmorenal type. Hemodialysis was performed in 50%, 77%, 100%, 0% and 0% in each group, respectively. The main causes of death were GI tract lesions, respiratory failure, or infections.

Conclusion: Clinical subset identification is very useful to presume the prognosis of survival and kidney function in MPO-ANCA associated patients.

74-056

IS THE CHURG-STRAUSS SYNDROME (CSS) AN ANCA-ASSOCIATED VASCULITIS?

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Background: ANCA (antineutrophil cytoplasmatic autoantibody) is reported to be present in about 10-80% of

patients with CSS and to be useful as a diagnostic tool. This is in contrast to Wegener's granulomatosis and microscopic polyangiitis, where ANCA is reported to be present in much more consistently high percentage. Therefore the clinical value of ANCA in CSS is questionable. Because of the relative rarity of CSS many reports about ANCA in CSS are based on small numbers of patients. This together with different methods applied for ANCA detection in the respective investigations may contribute to these variable results concerning the prevalence of ANCA in CSS.

Objective: To evaluate the prevalence of ANCA in Churg-Strauss syndrome (CSS) using different methods in well-characterized patients.

Patients and methods: We performed a prospective study on sera of 75 patients with CSS. Diagnosis was made according to the ACR and CHC criteria. We used the first sera of 75 patients after the diagnosis was established. If the first presentation was during inactive disease, we additionally screened a second serum at the time of active disease. 24 patients never had active disease. Screening of ANCA was done using an established indirect immunofluorescence technique (IFT). All sera were investigated serially by direct ELISA for common antigen specificities such as PR3, MPO, CG, Lactoferrin and BPI. In addition to these immunoassays we also used new established capture ELISAs for detection of PR3- and MPO-ANCA.

Results: In IFT only six patients were positive (2 x cANCA, 4 x pANCA). In direct ELISA, two had a PR3-ANCA, four had an MPO-ANCA. In capture ELISA we found 8 patients with ANCA, one patient had a PR3 antigen, seven others had an MPO antigen. Of patients with MPO-ANCA positive capture ELISA, four were IFT-negative. In total 10 patients (13.3%) with CSS were ANCA-positive at one point of disease, regardless of the antigen and of the method applied for screening.

Conclusion: Compared with the literature, we found a lower association of ANCA with CSS. Our data do not support the notion that ANCA is of immuno-diagnostic value in CSS.

75-057

ASSESSMENT OF ACTIVITY AND DAMAGE IN ANCA-ASSOCIATED VASCULITIS IN INDIA

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Background: Wegener's granulomatosis, once thought to be uncommon in India, is being recognized with increasing frequency in Indians. In the present study, the assessment of the primary systemic necrotizing vasculitis was done using the Birmingham Vasculitis Activity Score (BVAS) and Vasculitis Damage Index (VDI).

Method: 76 patients with ANCA-associated vasculitis were evaluated using the BVAS and VDI, between January 1990 and June 2001. The diagnosis of Wegener's granulomatosis and Churg Strauss disease were made by 1990 ACR criteria and that of microscopic polyangiitis by Chapel Hill Consensus. ANCA, ANA and anti-DNA were estimated by indirect immunofluorescence & antibodies to PR3 and MPO by ELISA. All other causes for secondary vasculitis and infections were excluded.

Results: There were 40 males and 36 females. The mean age at diagnosis was 43.4. The mean disease duration prior to diagnosis was 3.4 months. The distribution of vasculitis were: Wegener's granulomatosis - 48, microscopic polyangiitis - 10, Churg Strauss - 6 and crescentic glomerulonephritis - 12. cANCA was positive in 48 (63.15%) and pANCA in 21 (27.63%). ANA and anti-DNA were negative in all the patients. The mean BVAS score at baseline was 16.4. The mean VDI system score was 3 and the mean total VDI score was 4.6. Using the Vasculitis Damage Index, the following items of damage were seen: musculoskeletal damage-12 (15.8%); skin damage-16 (21%); ENT damage-28 (36.8%); pulmonary damage-42 (55.3%); cardiovascular damage-34 (44.7%); renal damage-51 (67.1%); peripheral vascular damage-26 (34.2%); ocular damage-21 (27.6%); neuropsychiatric damage-48 (63.2%); and other damage & drug toxicity-14 (18.4%).

Conclusion: 1. ANCA-associated vasculitis was rare in India, present in only 0.001% of hospital admissions. 2. Neuropsychiatric manifestations were common (63%). 3. The BVAS and VDI offer a comprehensive and cumulative measure of disease activity and damage in the serial assessment of vasculitis patients.

76-058

HEPATITIS C VIRUS RELATED CRYOGLOBULINEMIC VASCULITIS IN EGYPT

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Introduction: Cryoglobulinemic vasculitis is strongly associated with hepatitis C virus (HCV), whose prevalence in Egypt is 10-25%. The aim of this study was to determine the clinical and serological features of HCV-cryo patients presenting with vasculitis.

Methods: We identified all cases of HCV vasculitis referred to two rheumatology units in Cairo between 1998 and 2001. Patients underwent standardized clinical evaluation using the Birmingham Vasculitis Activity Score (BVAS), a physician's global assessment (10 cm horizontal line), PCR for hepatitis C, and serology for rheumatoid factor, complement and cryoglobulins.

Results: We identified 28 patients (M 8; F 20; median age 51 years; range 37 - 70, disease duration range 1 month to 15 years) with cryoglobulinemic vasculitis. All cases were HCV +ve by PCR, 19/28 had cryoglobulinemia detected at the onset, whilst a further 2 cases developed cryoglobulins during disease flares (sensory neuropathy in 1 case, nephritis and retinal vasculitis in 1 case). All patients had skin lesions (5 with ulcers). 17/28 had one or more features of neurological involvement (sensory neuropathy in 11; motor neuropathy in 2; mixed sensory/motor neuropathy in 4, seizures in 2 and sensorineural deafness in 1). Renal disease was present in 8 cases. Constitutional symptoms were common (11/28). Four patients

developed deep vein thromboses. Hypocomplementemia was present in 21 cases and RF was +ve in 17/21 tested. PGA and BVAS had a linear correlation (r = 0.73). The absence of cryoglobulins in 7 cases was associated with less severe organ involvement, using PGA (P<0.05), although BVAS levels were not statistically different between the cryo +ve and the cryo −ve group.

Conclusion: Cryoglobulinemic vasculitis associated with HCV in Egypt has a high propensity to cause multi-organ damage. BVAS is a valuable instrument in assessing activity in these patients. Interestingly, a minority of patients without detectable cryoglobulins have similar clinical features to cryo +ve patients. This raises the possibility that HCV may induce a vasculitis independently of the presence of circulating cryoglobulins.

77-059

A CRITICAL EVALUATION OF COMMERCIAL IMMUNOASSAYS FOR ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES DIRECTED AGAINST PROTEINASE 3 AND MYELOPEROXIDASE IN WEGENER'S GRANULOMATOSIS AND MICROSCOPIC POLYANGIITIS

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Objective: To determine the performance characteristics of 11 commercial enzyme-linked immunoassay (ELISA) kits for the detection of antineutrophil cytoplasmic antibodies (ANCA) directed against proteinase 3 (PR3) and myeloperoxidase (MPO) in defined patient groups (Wegener's granulomatosis=WG and microscopic polyangiitis=MPA)

Patients and Methods: Serum samples were derived from patients with histological and clinical diagnosis of WG (n=50), MPA (n=42), SLE (n=15), RA (n=15) and healthy controls (n=30). Each of these sera was tested for the presence of ANCA by indirect immunofluorescence technique (IFT) and PR3- and MPO-ANCA by 11 commercially available ELISA kits. In addition, in-house PR3- and MPO-ANCA capture ELISAs were performed.

Results: Using PR3-ANCA as a diagnostic test for WG there were considerable differences in sensitivity (from 22% to 70%) and negative predictive values (NPV) (from 43% to 70%) among the different ELISA kits, while specificity (from 93% to 100%) and positive predictive values (PPV) (from 93% to 100%) varied only modestly. The highest sensitivity (74%) and specificity (100%) for PR3-ANCA were obtained with the in-house capture ELISA. Similar differences and trends were observed for MPO-ANCA assays. Diagnostic sensitivity was more than 60% in 4 ELISA kits and at least 50% in 6 of 10 kits. The PPV varied from 84% to 100% and the NPV varied from 58% to 70%. Only one MPO-ANCA ELISA kit and in-house capture ELISA were the best assays for detecting MPA (sensitivity 62% and specificity 100%). In WG and MPA, maximum sensitivity for ANCA was obtained with IFT (80% and 70%, respectively).

Conclusion: PR3-ANCA and MPO-ANCA determined with commercial available direct ELISA kits were of poor sensitivity for WG and MPA and the immunofluorescence remains the superior method for ANCA detection in these diseases. The in-house PR3 and MPO-ANCA capture ELISAs perform better than direct ELISAs because combine a higher specificity with a comparable sensitivity.

78-076

A POSITIVE PR3-ANCA TITER AT SWITCH TO AZATHIOPRINE THERAPY IS ASSOCIATED WITH A DISQUIETING RELAPSE RATE IN ANCA-RELATED VASCULITIS

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The CYCAZAREM study showed that switching cyclophosphamide to azathioprine after 3 months of remission does not lead to more relapses within 18 months after diagnosis compared to continued cyclophosphamide therapy in patients with ANCA-related vasculitis. Although long-term data are not available, this regimen is widely adopted in the treatment of these patients, also at our center. We had the impression, however, that ANCA titers in patients switched to azathioprine rose early and that they relapsed more frequently during longer follow-up. We, therefore, analyzed patients diagnosed with ANCA-related small-vessel vasculitis between 1990 and 2000 at our center, ≥1 year follow-up, and treated with cyclophosphamide only (1990-1996) or switched to azathioprine after 3 months of remission (1997-2000).

Included were 128 patients of whom 44 (34%) switched to azathioprine. Fifty-three patients (41%) relapsed. Actuarial disease-free survival at 2 and 4 years was 76% and 65% in the cyclophosphamide group compared to 76% and 51% in the azathioprine group (log-rank test: RR 1.4, 95% CI 0.8-2.7; p=0.20). Relapses were more frequent in patients with PR3 (n=93) as compared to MPO-ANCA (n=35) specificity (RR 3.2, 95% CI 1.4 - 4.4). In PR3-ANCA associated vasculitis a positive as compared to a negative ANCA titer at 12 months tended to be associated with relapse (RR 1.7, 95% CI 0.9-3.0). In patients with PR3-ANCA associated vasculitis switched to azathioprine (n=33) a positive PR3-ANCA titer at the moment of treatment switch was significantly associated with relapse (RR 2.6, 95% CI 1.1 - 8.0). In patients with a negative ANCA titer at treatment switch disease-free survival at 2 and 4 years was 80% and 62%, and nearly identical to patients treated with cyclophosphamide only. In patients ANCA positive at switch disease-free survival was only 58% and 17%.

We conclude that our data, although retrospective and not from a controlled randomized trial, seriously question the safety of switching cyclophosphamide to azathioprine after 3 months of remission in patients with PR3-ANCA associated vasculitis who are still ANCA positive at treatment switch.

79-098

PATIENTS WITH P-ANCA/ANTI-MPO POSITIVE MICROPOLYANGIITIS (MPA) ARE AT HIGHER RISK TO DEVELOP HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

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In our center 2/5 patients newly exposed to heparin because of hemodialysis in year 2000 who develop HIT had p-ANCA/anti-MPO positive MPA. Moreover among the few published cases with HIT because of heparin exposure due to hemodialysis 5 had ANCA-positive vasculitis (Roe SD, NDT, 1998: 3226. Burdese M, Giornale Italiano di Nefrologia, 2001; S18: S25). HIT clinical diagnosis can be confirmed with the positive test for antibodies against the PF4/heparin antigenic complex. We tested sera from 41 pts with p-ANCA/anti-MPO positive MPA. 12 pts with C-ANCA positive vasculitis were also tested. Tests were performed by ELISA (HPIA-Diagnostica Stago, France). To exclude interferences on the test by high titers of ANCA, tests were performed either in pts not exposed to heparin or in pts in the inactive phase of the vasculitic disease. 26/41 pts with p-ANCA/anti-MPO positive MPA were exposed to heparin because of hemodialysis and/or plasmafiltration, 9 (35%) had a positive test for anti-PF4/heparin antibodies, all in the group with active disease (19 pts). None of 15 pts with p-ANCA/anti-MPO positive MPA not exposed to heparin had a positive test. None of 12 C-ANCA/anti PR3 positive pts (8 exposed and 4 unexposed to heparin) had a positive test. The 9 positive pts (3 males and 6 females, age 15-84) were all exposed to heparin because of hemodialysis.Two pts were also exposed because of plasmafiltration. Thrombocytopenia (< 50% of the initial platelet count) developed in all pts, 6-30 days after the first exposure and was generally not severe. Mild to moderate thrombocytopenia was the only manifestation in 4 pts. 3/ 9 pts developed repeated clot formation in the dialyser and extracorporeal circuit despite adequate doses of heparin. Two pts died, one because of severe pulmonary embolism complicating iliac-femoral thrombosis of the leg where the catheter for hemodialysis was placed; the other pt had subarachnoid hemorrhage and died because of stroke complicating severe cerebral vasospasm. The 2 pts who died had very high titers of anti-PF4/heparin antibodies.

In conclusion, pts with p-ANCA/anti-MPO positive MPA, in the acute phase of disease, when exposed to heparin for hemodialysis and/or plasmafiltration, are at risk to develop HIT. The risk seems to be higher than in non-vasculitic uremic pts. In some of these pts, HIT can be responsible or contribute to substantial morbidity.

80-099

THREE PATIENTS WITH SPLEEN NODULES AND NEGATIVE ANCA TEST: ATYPICAL MANIFESTATION OF WEGENER'S GRANULOMATOSIS?

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Wegener's granulomatosis (WG) is a systemic vasculitic disease potentially affecting any organ system, therefore its clinical presentation is highly variable. The presence of positive ANCA test is of great value for the diagnosis, especially in atypical presentations. Spleen involvement has been described in WG, usually in patients with typical generalized disease. We report three patients (pts), two female and one male, 32, 31 and 19 years old, all ANCA negative, who presented spleen nodules as main manifestation. Spleen appeared slightly enlarged with several, hypoechogenic areas, 1 to 3.5 cm in diameter, solid or colliquated. The nodules tended to increase in number and size during time. Besides spleen involvement, patients presented painful subcutaneous nodules on legs and arms (3 pts), vulva (1 pt), and scrotum (1 pt). Lymph node involvement was present in two pts (mediastinal associated with peritoneal in 1 pt and retroperitoneal in 1 pt) and rapidly evolved in colliquated masses. A single liver nodule, 3.5 cm in diameter, with central necrosis, was found in 2 pts. Two pts showed lesions in sites typically involved in WG, represented by multiple cavitating lung nodules in 1 pt and by transient rhinitis with crusting in the other. All three patients presented high grade fever with remittent course, profuse night sweats, weight loss and increase of inflammation indexes. Multiple biopsies from the organs involved were taken in all three patients. Tissue necrosis was a primary component of the disease. The necrosis had geographic pattern with large, irregular areas extensively replacing the parenchyma. The necrotic centre varied from caseation-like to suppurative. Epithelioid histiocytes and rare multinucleated giant cells were present at the periphery of necrotic foci, with a palisading arrangement. Histological criteria diagnostic for WG (vasculitis, microabscesses and scattered multinucleated giant cells in a highly inflammatory background) were only identified in the nasal biopsy from a single patient. Stains for micro-organisms and search for mycobacterium using PCR resulted invariably negative. Extensive serological test for infectious diseases were all negative. Pts recovered after combination treatment with corticosteroids and cyclophosphamide. Two pts with a follow-up of 11 and 13 years had several relapses at time of tapering or stopping treatment and currently are on continuous low-dose therapy. ANCA persisted always negative. An extensive work-up, developed in many years, couldn't identify a definitive diagnosis in all these cases. Could they represent an atypical form of WG?

81-100

HISTOPATHOLOGICAL FEATURES OF TRACHEAL BIOPSIES IN SUBGLOTTIC STENOSIS

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Subglottic stenosis can be the early or the only manifestation of Wegener granulomatosis (WG). As in other forms of localized WG disease ANCA test can be negative and only histology can provide the diagnosis of WG. According to literature data, only rarely can tracheal biopsies be truly diagnostic. We reviewed the tracheal biopsies related to 33 patients with symptomatic subglottic stenosis admitted to the Center of Respiratory Endoscopy and Laser Therapy. All the biopsies were obtained at the time of

mechanical assisted laser resections. Patients were classified as Wegener granulomatosis (WG)(14 pts) on the basis of ANCA-positive test and/or typical clinical manifestations of WG other than subglottic stenosis such as rhinitis with crusting, orbital disease, bronchial stenosis, with/without histological confirmation. All other pts were classified as idiopathic stenosis (IS) (19 pts). A total of 76 biopsies stained with hematoxylin-eosin, elastic stain, and special stains for microorganisms were analyzed. Histological features were classified as to "diagnostic" for WG (dWG) (microabscesses/necrosis/palisated granulomata; giant cells unassociated with granulation tissue or foreign bodies, and vasculitis), or "non-specific" (NS) (inflammatory infiltrate, microthrombi, granulation tissue with/without giant cells, and fibrosis).

Within the WG group, 8 out of 14 pts (57%) showed at least one dWG feature, and only two of them showed all dWG features. Within the IS group, 4 out of 19 IS pts (21%) showed at least one major feature and a single pt presented all dWG features in his biopsies. Among other histological changes, tissue fibrosis was observed with similar frequency in the two groups (11/14 and 15/19 in WG and IS, respectively); the fibrosis occasionally involved small vessels (vascular "scars") (1/14 in WG and 4/19 in IS).

Conclusions: (1) In WG pts, although tracheal biopsies are less informative than nasal biopsies and rarely show features fully diagnostic for WG, they show in a considerable number of cases at least one diagnostic feature. (2) The majority of IS pts show non-specific changes in their biopsies and remain completely undefined both clinically and pathologically. The occurrence of fibrosis involving the small vessels in a minority of pts suggests that possible vascular damages may occur during the course of the disease, but definite proof of this hypothesis is totally lacking. (3) The small group of IS pts showing one or more dWG features may represent truly real cases of WG with a localized form of the disease.

82-101

RECOMBINANT PROTEINS TO ANALYZE AUTOANTIBODIES TO PROTEINASE 3 IN SYSTEMIC VASCULITIS

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Introduction: Anti-neutrophil cytoplasmic autoantibodies (ANCA) directed to proteinase 3 (PR3-ANCA) are closely associated with the systemic necrotizing vasculitides, in particular Wegener's granulomatosis (WG). The presence of PR3-ANCA in serum is usually detected by ELISA with native PR3 as a substrate. As the isolation of native PR3 from neutrophils is laborious and expensive, development of ELISA with recombinant PR3 might be a good alternative. The aim of our study was to test the usefulness of recombinant PR3 ELISA for the detection of PR3-ANCA in systemic vasculitis.

Methods: We analyzed sera of 114 patients with ANCA-associated vasculitis, including 90 with WG, 12 with microscopic polyangiitis (MPA), 6 with Churg-Strauss syndrome (CSS), and 6 with necrotizing crescentic glomerulonephritis (NCGN). All samples were collected at the moment of diagnosis. Together with sera of 20 healthy controls and 59 disease controls (20 with systemic lupus erythematosus, 20 with ulcerative colitis, 19 with autoimmune hepatitis) they were tested for PR3-ANCA by direct ELISA using native PR3 and two recombinant forms of PR3, and by capture ELISA using native PR3, two recombinant forms of PR3, and a crude extract of azurophilic granules. Recombinant antigens were expressed in the baculovirus system and one of them (recPR3-1) was an enzymatically inactive mutant.

Results: 4-6 of 79 and 3-4 of 79 control sera tested positive in direct and capture ELISA, respectively. Most of the sera positive in direct ELISA were derived from patients suffering from autoimmune hepatitis. In the patient group, we observed a correlation between the results of direct and capture ELISA, which was the case for all antigens used. However, capture ELISA was a slightly more sensitive test for PR3-ANCA than the direct assay (see table).

TABLE.
POSITIVE SAMPLES. CUT-OFF VALUE WAS DEFINED AS MEAN + 2 SD OF DISEASE CONTROL GROUP (N=59)

	Direct ELISA			Capture ELISA			
	Native PR3	RecPR3-1	RecPR3-2	Granule extract	Native PR3	RecPR3-1	RecPR3-2
WG n=90	67	53	47	69	71	57	51
MPA n=12	3	3	2	4	5	4	4
CSS n=6	0	0	0	2	2	2	2
NCGN n=6	1	1	0	0	1	1	1

RecPR3-1 and recPR3-2 performed less well than native PR3 in both direct and capture assay. Despite being an enzymatically inactive mutant, recPR3-1 was more efficient in detecting PR3-ANCA than recPR3-2. The results of capture ELISA obtained with two different monoclonal anti-PR3 antibodies were similar.

Conclusions: In this study, we show that ELISA with native PR3 is more sensitive assay for PR3-ANCA in systemic vasculitis than the same test using recombinant forms of PR3. Irrespective of the antigen used, capture ELISA gives more positive results than direct assay. These results suggest that ELISA with native antigen still remains a method of choice for the detection of PR3-ANCA. However, the further optimization of these assays, especially with recombinant antigens, might lead to improvement of their sensitivity and specificity.

83-103

PERFORMANCE OF THE BIRMINGHAM VASCULITIS ACTIVITY SCORE FOR WEGENER'S GRANULOMATOSIS (BVAS/WG) IN A RANDOMIZED CLINICAL TRIAL

The Wegener's Granulomatosis Etanercept Trial (WGET) Research Group

Objective: To evaluate the performance of a disease-specific activity score for WG in patients enrolled to date in a randomized, double-masked, placebo-controlled clinical trial. To preserve the masking in this ongoing trial, the analyses reported include only comparisons of patients with severe versus limited disease.

Methods: The BVAS/WG is scored by physician-investigators at the baseline, 6-week, and 3-month visits, and then at every 3 months until the common trial closeout. Disease activity of WG is scored in 9 separate organ systems, with additional space for adding other items not listed on the form. Only items attributed to active WG are scored in BVAS/WG (damage, adverse effects of treatment, and intercurrent medical problems are recorded elsewhere). Items scored are categorized as either new/worse or persistent. The BVAS/WG also provides a framework for categorizing patients with WG as "severe" or "limited" based on the presence of organ-threatening disease and the need for cyclophosphamide therapy. A physician global assessment (PGA) of disease activity, scored on a 10 cm visual analog scale, is also performed at each visit.

Results: The mean BVAS/WG at entry among the first 116 patients enrolled was 7.0 (median: 6; Q1-Q3: 4,9). Patients whose disease was classified as severe at trial entry (N = 85) had a mean BVAS/WG of 8.0 (median: 7; Q1-Q3: 6,10), compared with 4.2 (median: 4; Q1-Q3: 3-5) for those with limited disease (N = 31)(P = 0.0001). The differences between the severe and limited patients were reflected in higher PGA scores at baseline. The mean PGA among the severe WG patients was 6.1 cm (median: 6.6; Q1-Q3: 4.8-7.8), compared with 4.3 cm (median: 4.1; Q1-Q3: 2.7-5.6) among those with limited disease. For BVAS/WG and PGA at baseline, the correlation coefficient was 0.60 (P < 0.0001). Mean duration of disease since diagnosis was substantially longer among patients with limited WG (22 months), compared to those with severe disease (2.7 months).

Conclusions: Correlation between the BVAS/WG and PGA of disease activity is high. Patients with limited WG have substantially lower BVAS/WG scores at baseline. This may correlate inversely with disease damage, which likely reflects duration of disease.

84-109

DOES CRYOGLOBULINEMIA REPRESENT AN EXCLUSION CRITERION FOR THE DIAGNOSIS OF POLYARTERITIS NODOSA (PAN)?

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Objective: To analyze the significance of mixed cryoglobulins (MC) detection in patients with PAN.

Method: We retrospectively analyzed 580 patients from the French Vasculitis Study Group. Eight patients who met the ARA criteria for the diagnosis of PAN had histologically documented fibrinoid necrosis of small- and/or medium-sized arteries and type II/III MC; one also had mesenteric and renal arteries microaneurysms.

Results: Three patients had transient low-level (< 100 mg/l) MC III with normal C4 levels and negative rheumatoid factor; 2 patients had PAN with multiple relapses and chronic high-level (≥100 mg/ml) type II/III MC, with low C4 levels and rheumatoid factor; 1 had high-level MC II and lymphoplasmacytic lymphoma; 1 had MC II and was co-infected with hepatitis B and hepatitis C viruses; 1 had HIV-related PAN, low-level MC III and normal C4.

Conclusion: Distinguishing essential MC from classic PAN may be difficult. Fibrinoid necrotic lesions may occur in cryoglobulinemia-related vasculitis and are not specific to PAN. The detection of cryoglobulins during the course of PAN must lead to a search for B-cell lymphoproliferation or chronic viral infection, which can be the cause of vasculitis. We propose that MC > 100 mg/l associated with a low C4 level could represent, in the absence of microaneurysms and viral infection, an exclusion criterion for the diagnosis of PAN.

85-110

CLINICAL STUDY OF PATIENTS WITH SYSTEMIC VASCULITIS ADMITTED TO INTENSIVE CARE UNIT

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Objective: To evaluate presenting features and the abilities of disease-activity scores to predict outcome of patients with systemic vasculitis (SV) admitted to intensive care unit (ICU).

Methods: The medical charts of all patients with SV followed in a University Hospital (Hôpital Avicenne) and admitted to ICU between 1982 and 2001 were retrospectively reviewed for clinical presentation, intensive care severity scores (APACHE II, SAPS II), Birmingham Vasculitis Activity Score (BVAS) and outcome.

Results: Twenty-six patients (M/F: 1.6; age: 45.2 ± 16.2 yr; mean duration of SV: 28.4 ± 74.9 mo) with SV (Wegener's granulomatosis n=4; microscopic polyangiitis n=4; polyarteritis nodosa n=3; HBV-related polyarteritis nodosa n=3; Churg-Strauss syndrome n=7; cryoglobulinemic vasculitis n=2; and others n=3) were admitted to ICU for active vasculitis (n=20; 77%) with predominantly pulmonary (50%) and/or renal (40%) involvement; infection (n=3; 12%) and miscellaneous (n=3; 12%). SV was diagnosed in 11 (42%) patients in the ICU. Four (15%) patients died in the ICU and the total mortality rate was 38% after a follow-up of 30.7 ± 29.9 mo. Mean disease-severity scores at ICU admission calculated for early (ICU) and late (end of follow-up) survivors and non-survivors are reported in the table.

Score at ICU admission	ICU			End of follow-up		
	Survivors	Non-survivors	p[a]	Survivors	Non-survivors	p[a]
APACHE II	15.7±6.7	28.0±9.27	0.01	16.4±7.37	19.89±9.93	0.55
SAPS II	26.0±10.95	50.0±22.05	0.02	25.87±11.82	36.89±19.40	0.21
BVAS[b]	18.0±9.67	31.0±14.85	0.13	14.67±4.62	26.87±12.96	0.02

[a] Non-parametric Kruskal-Wallis test.
[b] Only for patients admitted to the ICU for active vasculitis.

Conclusion: The main reason for ICU admission was active

vasculitis, often the first disease manifestation, leading to its diagnosis. The standard intensive care severity scores were able to predict in-hospital mortality, but not overall mortality at the end of follow-up. For patients admitted to the ICU for active vasculitis, BVAS was associated with long-term outcome.

86-111

MICROSCOPIC POLYANGIITIS (MPA) AND POLYARTERITIS NODOSA (PAN): HOW AND WHEN DO THEY START?

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Objectives: To describe the first clinical symptoms attributable to MPA or PAN, and to determine the time to diagnosis and its impact on outcome.

Methods: We retrospectively reviewed the medical files of 75 patients (mean follow-up: 6.9 yr) with biopsy-proven MPA (n = 37) or PAN (n = 38, including 26 related to hepatitis B virus infection). The first clinical signs attributable to vasculitis, the clinical signs at the time of diagnosis, time to diagnosis, subsequent relapse(s) and survival were recorded. The relapse and mortality rates were also analyzed as a function of the median time to diagnosis. Statistical analyses were performed, when appropriate, with chi-square, Student's t- and log-rank tests.

Results: General symptoms (fever ≥38°C, weight loss, fatigue) were the most common findings at disease onset (71%), followed by myalgias/arthralgias (61%), and neurological (24%), cutaneous (13%) and gastrointestinal manifestations (13%); 8% had only general symptoms. Initial manifestations were similar in both entities except for gastrointestinal symptoms and peripheral neuropathy which were more frequent in PAN ($p = 0.01$ and $p = 0.03$, respectively). The mean time to diagnosis was 269 d (median: 90; range: 7-2550). Overall mortality and relapse rates were 33 and 39%; these rates did not differ significantly between MPA and PAN. Time to diagnosis ≥90 d was not associated with different clinical features at the time of diagnosis or with an increased risk of mortality, but tended to predict a greater risk of subsequent relapses ($p = 0.05$).

Conclusion: It appears that MPA and PAN initially start with non-specific symptoms that frequently last for several months before the diagnosis is made. That a longer time to diagnosis tended to predict a higher relapse rate suggests the existence of a subgroup of patients with a less acute but more refractory disease.

87-122

ANCA STATUS, LEUKOTRIENE RECEPTOR ANTAGONIST USE, AND CHURG-STRAUSS SYNDROME

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Rationale: To review the clinical course of Churg-Strauss Syndrome (CSS) and correlate ANCA status and leukotriene receptor antagonist use with the disease process.

Methods: A retrospective chart review was performed of all patients seen at our institution since 1990, who carried or were assigned the diagnosis of CSS.

Results: 96 patients had symptoms suggestive of CSS. 82 met either the American College of Rheumatology's (ACR) criteria (78/82, 95%), or Lanham's criteria (61/82, 74%), or the Chapel Hill classification scheme (58/82, 71%). Of the 82 patients, 39 were female and 43 male. The average age at diagnosis was 49 years (range 10-77). ANCA testing was performed in 66 patients. 25/66 (38%) were P-ANCA positive and 1/66 was C-ANCA positive. Of patients who were tested for ANCA at the time of initial diagnosis 17/25 (68%) patients were ANCA positive. 11/23 (48%) tested during a vasculitic flare were positive, and 1/40 (3%) tested during remission were ANCA positive. Leukotriene receptor antagonists (LRA) were used by 21/82 (26%) of patients. In 13 they were started before the diagnosis of CSS. Of the 7 cases started after diagnosis, 2 had a vasculitic relapse, and 5 remained in remission. In 1 patient the time course of LRA usage was unclear. All patients received corticosteroids with a further 42/82 (51%) requiring other immunosuppressants, most commonly cyclophosphamide.

Conclusions: The ACR were the most inclusive criteria for the diagnosis of CSS. ANCA if present appears to correlate with disease activity, but it doesn't appear to correlate with specific disease manifestations. The pathogenic role of leukotriene inhibitors in CSS also remains unclear, with almost one quarter of the patients receiving it, in this study, apparently suffering no ill effect.

Treatment and Outcomes

88-004

IS NAILFOLD VASCULITIS (NFV) IN RHEUMATOID ARTHRITIS BENIGN?

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Background: Vasculitis is a well-recognized and potentially serious complication of rheumatoid arthritis. Vessels of all sizes can be affected and as a consequence the clinical manifestations may vary, from isolated NFV due to digital endarteritis through to a necrotizing arteritis with internal organ and peripheral nerve involvement—systemic rheumatoid vasculitis (SRV). NFV is generally considered to be benign, requiring simply observation. We have previously reported a favorable prognosis in a cohort of patients after a mean follow-up of 22 months. The aims of this study were to re-evaluate this cohort of patients after a longer follow-up.

Methods: Clinical details were obtained by retrospective case note review of NFV and SRV patients, previously identified prospectively between 1988-94. SRV was diagnosed using the Scott and Bacon criteria. NFV was not treated with cytotoxic agents.

Results: 29 patients (16 males) with NFV were followed

for a median of 86.5 months from diagnosis of NFV (Table). One patient developed SRV with mononeuritis multiplex 65 months after NFV was diagnosed and died 5 months later. One patient developed a vasculitic leg ulcer and died after 120 months. In neither case was a cause of death available. 14 patients developed extra-articular manifestations (including Sjögren's syndrome - 8, bronchiectasis - 2). There was significant mortality in both groups, but in the NFV group this was not due to active RA.

Conclusions: This study confirms that isolated NFV can be regarded as a benign condition with a low long-term risk of developing SRV. Observation of NFV does not warrant introduction of cytotoxic therapy. Extra-articular disease occurs frequently in this group of patients. The high mortality was not attributable to active RA, but this was an elderly cohort of patients.

TABLE

	NFV	SRV
No. of patients	29	47
Sex (M/F)	16/13	25/22
Median duration of RA (yrs)	10 (<1-40)	12 (<1-40)
Seropositive (%)	97	89
Nodules (%)	73	57
Mortality M/F (%)	56/46	80/57
Median interval diagnosis NFV/SRV to death (mos)	67	45

89-005

PREDICTORS OF REMISSION AND RELAPSE IN WEGENER'S GRANULOMATOSIS

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Aim: To study the course of disease activity in a population-based cohort of Wegener's granulomatosis (WG) patients and describe predictors for complete remission and relapse.

Methods: Retrospective study of 56 WG patients (62.5% males, median age 50 years) of whom 52 survived 3 months and were followed for 45.5 months (6-173). Disease activity was assessed by Birmingham Vasculitis Activity Score (BVAS-1) and permanent organ damage by Vasculitis Damage Index (VDI). Induction therapy consisted of prednisolone (Pred) 0.5-1 mg/kg and cyclophosphamide (CYC) daily orally 2mg/kg (19 patients) or IV pulses 15mg/kg every 2nd week (32 patients). Baseline clinical and laboratory features and cumulative treatment during the first 6 months were recorded. Simple and multiple regression analyses were used to find risk factors (hazard ratio [HR] or odds ratio [OR] with [95% confidence interval] for remission and relapse by Cox proportional hazards model or logistic regression analyses). Data are given as median (range).

Results: There was no baseline difference between the two CYC-treatment groups, except that pulse treated patients had higher BVAS-1 scores (27 vs 23, P=0.02). All patients achieved either complete (85%) or partial remission (15%). Higher baseline BVAS-1 increased the chance of complete remission (BVAS-1 increase by 5 points, HR=1.22 [1.05-1.42]), while cumulative dose of CYC during the first 6 months was associated with increased chance of sustained complete remission (dose increase by 5 gram, OR=1.76 [1.10-2.82]). Relapse occurred in 31 patients (59.6%) after 18 months (4-108). The risk of relapse did not decline over time, but the risk was reduced with longer time on Pred >20mg/day during the first 6 months (increase by 1 month, HR = 0.78 [0.62-0.98]) and increased in patients with heart involvement (HR = 2.87 [1.09-7.58]). Therapy resistance, defined as death within 3 months or never achieving complete remission, was associated with baseline organ damage (VDI increase by 1 point, OR = 1.53 [1.03-2.27]).

Conclusion: Initial high disease activity increased, and the presence of baseline organ damage reduced the chance for complete remission in WG. Sustained remission was associated with more intensive initial treatment in terms of higher CYC doses and longer time on Pred >20mg/day.

90-011

LONG-TERM OUTCOME OF PATIENTS WITH ANCA-POSITIVE VASCULITIS (WEGENER'S GRANULOMATOSIS [WG], MICROSCOPIC POLYANGIITIS [MPA]) AND DOCUMENTED RENAL INVOLVEMENT

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Objectives: Comparison of renal outcome and survival in a large cohort of patients (pat) with WG and MPA during the last 20 yrs: 92 pat with WG (53 m, 39 f; median age 54 yrs [11-79]) and 34 pat with MPA (16 m, 18 f; median age 60 yrs [17-81]). Follow-up: median of 71 months (mo). Initial s-crea ≥3 mg: 38/92 pat with WG and 15/34 pat with MPA. Treatment: (modified) Fauci scheme; median cyclophosphamide (cyc) dosage 36.5 g (0.75-412) within a median of 17.7 mo in 91/92 pat with WG and in 33/34 pat with MPA, followed by azathioprine in 34/91 pat with WG and in 11/33 pat with MPA. Steroid doses: median dosage of 9.7 g (0.75-111.9) for a median of 28 mo (0.2-141).

Results: Clinical remission was obtained in 83/92 pat with WG and 30/34 pat with MPA after a median of 5 mo. Initial dialysis (D) was required in 23/92 pat with WG and in 6/34 pat with MPA. Recovery of renal function was obtained in 22/23 pat with WG and in 3/6 pat with MPA; both groups had initial D during a median of 30 days (1-480). Plasmapheresis was performed in 5/92 pat with WG. End-stage renal failure was observed in 19/92 pat with WG (initial D: 10/19) and in 9/34 pat with MPA (initial D: 5/6). Renal relapse (reappearance of nephritic sediment) within 5 years was seen in 60% of pat with WG and in 56% of pat with MPA. Repeat renal biopsy was performed in 13/91 pat with WG (10/13 de novo IgA, 1/13 de novo M Goodpasture) and in 7/34 pat with MPA. Death due to disease activity was observed in 3/16 pat with WG and 1/6

pat with MPA, due to infection in 2/16 pat with WG and 1/6 pat with MPA. Malignancies were observed in 8/92 pat with WG (1 leukemia, 4 bladder-carcinoma (ca) in pat with cyc treatment >2 yrs (3 cured), 1 colon-ca, 2 prostate-ca) and in 2/34 pat with MPA(both squamous-cell ca [both cured]). Side effects of steroid therapy were: cataracts in 14% of pat with WG and 8.8% with MPA; osteoporosis and/or aseptic osteonecrosis in 14.1% of pat with WG and 11.8% with MPA; diabetes mellitus (inherited in 9/25 pat with WG and in 6/10 pat with MPA) in 27.2% of pat with WG and 29.4% with MPA.

Conclusion: The renal relapse rate of patients with ANCA-positive vasculitis was high. Reappearance of nephritic sediment in WG was not always indicative for relapse of ANCA-positive vasculitis. Mortality in the elderly population was mainly related to non-vasculitic causes. Renal outcome in patients with MPA was not different from that of WG.

91-020

PAUCI-IMMUNE NECROTIZING CRESCENTIC GLOMERULONEPHRITIS IN THE ELDERLY

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Aim of the study: to determine clinical, biological and histological characteristics of pauci-immune glomerulonephritis in patients older than 70 years.

Methods: Retrospective study including 46 patients from 5 nephrologic centers suffering from microscopic polyangiitis (39), Wegener's granulomatosis (6) and Churg and Strauss syndrome (1). Statistical analysis was performed using a chi-square test of Pearson or Fisher exact model to test the association of categorical variable, whereas a t-test was used for continuous variables.

Results: Mean age was 76 +/- 4.6, sex ratio was 1.2 (F/M). Interval between onset of symptoms and diagnosis was 10.7 +/- 18.5 months. Number of organs involved (kidney included) was 2.3 +/- 1.1. Constitutional symptoms (fatigue, weight loss and fever) were common. Extra-renal symptoms were less frequent: lung involvement (39.1%), purpura (23.9%) and arthralgias (13.4%). Laboratory signs of inflammation were constant (CRP = 68.8 +/- 55.92 g/l, leukocytosis = 11481 +/- 5886/mm^3, Hb = 8.5 +/- 1.56 g/l). ANCA were found in 20/21 patients. Hematuria and proteinuria (1.6 +/- 1.5 g/24h) were always detected. Mean serum creatinine level at the time of diagnosis was 525.1 +/- 303.6 µmol/l. Renal biopsies usually showed irregular glomerular lesions of various ages. Necrotic lesions were more often segmental than global. Percentage of normal glomeruli was 18.3 +/- 18.7%. Percentage of global sclerotic glomeruli was 44.7 +/- 25.8%. Interstitial fibrosis (scored 0 to 2) was 1.3 +/- 0.77. Treatment included corticosteroid therapy (40/46) and cyclophosphamide (30/46). Six patients under dialysis and without extra-renal symptoms were not treated.

Twelve months after the diagnosis, outcome was chronic renal failure (45.7% of patients; mean creatinine clearance: 32.73 ml/min), chronic dialysis (23.9%), and death (30.4%). Causes of early death were infection (6 patients), cardiovascular complications (4), neoplasm (1) and interruption of renal replacement therapy (2). The predictors of renal survival were entry serum creatinine value (>500 µmol/l; P=0.01) and proteinuria (>1.5 g/24h; P=0.01). The predictor of patient survival was albuminemia (<28 g/l; P=0.01).

Conclusion: Pauci-immune necrotizing crescentic glomerulonephritis in the elderly is a slowly progressive disease, more often restricted to the kidney, with delay in diagnosis and therefore delay in instituting effective treatment. Early diagnosis (detection of hematuria, ANCA and renal biopsy) and early treatment may improve the disastrous outcome of this disease.

92-033

EFFECTIVE THERAPY FOR SUBGLOTTIC STENOSIS IN WEGENER'S GRANULOMATOSIS

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Background: Subglottic stenosis (SGS) occurs in ~20% of pts with WG. In general, systemic therapy for SGS has been ineffective. Tracheostomy has been required in ~50% of cases. One previous study (Langford et al, A&R 1996) demonstrated utility of intralesional depocorticosteroid injection and dilation (ILGCS-D) for WG-SGS lesions. Until now, other studies have not been performed to confirm or refute the utility of ILGCS-D.

Patients and Methods: 14 patients with WG and critical SGS (<3-4 mm patency), dyspnea or stridor, received ILGCS-D by the same surgeon (IE). SGS was visualized under general anesthesia (jet ventilation, suspension laryngoscopy). Aliquots of 40-80 mg depomethylprednisolone were injected into the stenotic lesion in each of 4 quadrants, followed by progressive mechanical dilation. Repeat ILGCS-D was provided if symptoms recurred or severe "silent" restenosis was noted on follow-up examination.

Results: In 8 patients in whom ILGCS-D was the first intervention, tracheal patency was maintained after 1 or 2 dilatations (mean F/U=24.5 months, range = 8 months-6 years). In 6 patients who had prior procedures, including 3 tracheostomies, extensive scar formation led to less satisfactory results. Tracheostomy closure not was achieved in the 3 patients and 3 required multiple (up to 3) dilations before patency was maintained. There were no complications associated with therapy. Hospital discharge was possible on the day of the procedure.

Conclusions: SGS intralesional depo-GCS injection and dilation are effective combined therapies in WG, especially when applied to newly recognized lesions. Although results are less encouraging when applied in the setting of prior scarifying therapies, treatment may be useful in select cases. Our experience is the first to confirm that previously reported in a similar

cohort. We propose that intralesional depo-GCS and dilation be considered as 1st line therapy for WG-SGS.

93-035

AN AUDIT OF INITIAL ASSESSMENT IN WEGENER'S GRANULOMATOSIS

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Background: Accurate initial assessment of organ involvement in Wegener's granulomatosis (WG) is essential to determine immediate and future management.

Objectives: We audited the initial assessment of patients referred to the Rheumatic Diseases Unit (RDU) subsequently diagnosed with WG. As no published gold standards for assessment exist, the following essential items were chosen following consensus discussion: blood pressure (BP), urinalysis (and microscopy if hematuria present), serum creatinine, chest radiograph (CXR) and tissue biopsy.

Methods: A search of the RDU computer database identified 35 patients with WG excluding those with incomplete medical records and re-referrals. Medical records and CXRs of these patients were reviewed to establish if basic investigations had been performed and, if so, their results.

Results: 19 F:16 M. Mean age 53, range 13-85. Features at presentation (%): Ear, nose and throat (94), arthralgia (66), cutaneous (51), renal (40), ocular (29), pulmonary (20) and neurological (17). Results of basic investigations are presented in the table. In 29% (10/35) of patients at least one test was not performed. 11% (4/35) of patients had hypertension (diastolic BP > 99) and in none were recommendations made for BP to be rechecked or treatment to be commenced. 6% (2/35) of patients with microscopic hematuria did not have urine microscopy or a renal biopsy performed. All patients with an elevated creatinine were further investigated. 9 patients had an abnormal CXR: 1 had evidence of previous tuberculosis, 4 had active pulmonary disease and 4 were further investigated.

Conclusion: 71% of patients referred to a tertiary rheumatology centre and later confirmed to have WG received adequate basic investigation at presentation. Patients found to be hypertensive should have their BP repeated and those with hematuria should have urine microscopy performed. These results indicate that we should be more vigilant in performing basic tests in patients with suspected WG. In this regard, these data have been presented to our department and the audit will be repeated in 2005 to reassess the recording of basic investigations in patients with WG.

	BP	Urine	Creatinine	CXR	Biopsy
Test performed (no. of patients)	33/35	33/35	31/35	31/35	30/35
Test performed (% of patients)	94	94	89	89	86
Test abnormal (% of patients)	11	46	23	26	87

94-044

THE USE OF INTERFERON-ALFA-2B/PEGYLATED INTERFERON AND RIBAVIRIN TREATMENT IN RENAL DISEASE AND CHRONIC HEPATITIS C INFECTION WITH OR WITHOUT CRYOGLOBULINEMIA – A CASE SERIES

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Background: Hepatitis C virus (HCV) infection is associated with extrahepatic manifestations as membranoproliferative glomerulonephritis (MPGN) with or without cryoglobulinemia, membranous glomerulonephritis (MGN) and FSGS. Standard treatment for HCV is interferon and ribavirin, but in renal insufficiency ribavirin has been contraindicated due to fear of side effects.

Patients and methods: 7 patients, 2 with cryoglobulinemia and vasculitic manifestations as skin and oral ulcers, neuropathy, cerebral vasculitis, arthralgia, fever and glomerulonephritis (GN), 3 with MPGN, 1 with MGN and 1 with FSGS were treated with a combination of interferon and ribavirin. 2 patients were given pegylated interferon (PEG) and ribavirin. Most patients had renal insufficiency at presentation, with GFR between 10-75 mL/min. One patient had HCV genotype 1, the remainder 2 and 3. Duration of therapy was according to genotype (6-12 months). Ribavirin in plasma was monitored by HPLC throughout the treatment to avoid overdosing, aiming at a target concentration of 10-15 µmol/L. The main side effect of ribavirin, hemolytic anemia, was monitored closely with Hb controls.

Results: 7/7 patients became HCV-RNA-PCR negative and 3/7 (MPGN and FSGS) have maintained both renal and virological remission. One vasculitis patient responded with complete remission, but relapsed virologically and has had a minor vasculitic flare after 9 months. The other, who recently received PEG, improved with regard to vasculitic symptoms and proteinuria, but still has low-grade CRP of unclear significance. The MGN patient currently treated with PEG is in virological remission and improving. Finally, one MPGN patient didn't tolerate interferon, but is in renal remission with low-dose ribavirin. Only one vasculitis patient had low-dose immunosuppression in addition to anti-viral therapy. Average daily ribavirin dose was 200-800 mg. Hb was maintained in all patients with adequate iron stores and erythropoietin up to 20,000 IU/week.

Conclusions: Interferon and ribavirin can, with ribavirin monitoring, safely be used in HCV-related vasculitis and glomerulonephritis irrespective of renal function. Patients with cryoglobulinemia and vasculitis might benefit from longer treatment than indicated by HCV genotype only.

95-045

ANTI-TUMOR NECROSIS FACTOR (ANTI-TNF) THERAPY IN TAKAYASU'S ARTERITIS (TA) RESISTANT TO CONVENTIONAL THERAPY

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TA may cause stenosis, dilatation or aneurysms of large vessels. Many patients (pts) fail to sustain remission despite glucocorticoid (GCS) and cytotoxic therapy.

Goal: Assess anti-TNF therapy in TA.

Methods: Pilot study in 5 pts. Treatment: Etanercept 25-50 mg BIW. Infliximab: 1 pt, up to 7 mg/kg Q8wks.

Results: 1M, 4F, 20-38 y.o. TA duration pre-anti-TNF = 5.2 yrs (M). Prior relapses: 1-12 (M=5.5). Before study, relapses when prednisone <21 mg QD (M). Previously also failed methotrexate, azathioprine, cyclosporin, tacrolimus or mycophenolate. After anti-TNF, time to unprecedented improvement <2mos. Follow-up: anti-TNF therapy = 23.6 (M, 18-27) mos. 1 pt refused GCS re-treatment. 3 others tapered and discontinued GCS. 5th in remission on 5 mg of prednisone QD. Duration remission (M) = 13.4 mos. Remission off GCS (4pts) = 6.8 mos.

Conclusion: Anti-TNF therapy appears to be a useful adjunct to GCS in TA.

96-054

BLOCKING TNF-ALPHA WITH INFLIXIMAB RESULTS IN SUCCESSFUL TREATMENT OF CYCLOPHOSPHAMIDE-REFRACTORY WEGENER'S GRANULOMATOSIS

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Objective: To study the effect of the chimeric monoclonal anti-TNF-α antibody infliximab in Wegener's granulomatosis (WG) refractory to standard treatment with cyclophosphamide and high-dose corticosteroids for the induction of remission.

Patients and Methods: 6 patients with active, generalized, biopsy-proven WG refractory to standard treatment were followed prospectively. Infliximab was administered in addition to standard therapy with cyclophosphamide and high-dose corticosteroids at a dosage of 3 mg/kg in two patients and at a dosage of 5 mg/kg in four patients with a 2-week interval after the first administration and a 4-week interval for the consecutive infusions.

Results: Disease manifestations refractory to standard cyclophosphamide and corticosteroids were: imminent visual loss due to progressive retroorbital granulomas in 3 patients, rapidly progressive glomerulonephritis in 1 patient, pulmonary-renal syndrome in 1 patient, and progressive cavitating pulmonary granulomas (also biopsy-proven) in 1 patient. Addition of infliximab resulted in a rapid and significant improvement of vasculitis activity in 5 patients. Corticosteroid doses could be tapered in these 5 patients. Acute-phase responses (e.g., CRP) normalized. C-ANCA titers were no longer detectable. The BVAS was reduced to zero in these patients. 1 patient was withdrawn because of a suspected systemic infection. The higher dosage (5 mg/kg) was more effective. One patient continues on TNF-α blockade. One patient relapsed after 10 months and remission was reinduced by addition of infliximab. Three patients have remained in remission during follow-up for 12 to 18 months.

Conclusion: TNF-α blockade successfully induces remissions in refractory WG. Infliximab appeared effective and safe. Close monitoring for side effects such as infections is mandatory. Infliximab may appear as a more specific treatment in WG, where TNF-α has been demonstrated to play an important role in the induction of vasculitis and granuloma formation. Maintenance of remission after infliximab treatment still means another challenge to our therapeutic efforts.

97-061

SAFETY AND EFFICACY OF TNFα BLOCKADE IN RELAPSING VASCULITIS

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Blockade of tumour necrosis factor alpha (TNFα) using infliximab, a chimeric monoclonal antibody against TNFα, is an effective treatment in rheumatoid arthritis and Crohn's disease. Sight-threatening Behçet's disease has also been successfully treated with infliximab. A preliminary study has also reported clinical improvements in the primary systemic vasculitis, Wegener's granulomatosis, with the soluble TNFα receptor etanercept.

We report the compassionate treatment of six patients with refractory vasculitis using infliximab. Diagnoses were: Wegener's granulomatosis (3), microscopic polyangiitis (3). Three were PR3-ANCA and 1 MPO-ANCA positive. Four were female, mean age was 58 years (range 23-77 yrs) and mean disease duration was 3.5 yrs. All had suffered at least three clinical relapses and had received prolonged corticosteroids and at least four immunosuppressive drugs. Vital organ involvement at the time of infliximab included eye (4) and lung (3); in addition, five had profound constitutional symptoms. Mean prednisolone dose was 17 mg.

Three intravenous doses of infliximab 200 mg were administered at monthly intervals for three months. One patient complained of fatigue, myalgia and blurred vision 24 hours after the first infusion which did not recur on rechallenge. Infliximab was otherwise well tolerated. Five patients had remission of their disease, four within two weeks of treatment. This allowed steroid withdrawal in three and reduction by more than 50% in two. Disease activity assessed by the Birmingham Vasculitis Activity Scores (BVAS) improved from a mean of 6.3 to 0.8 at three months (**Figure 1**). One receiving continued infliximab for six months relapsed when the treatment interval was extended to two months. Mean falls in ESR and CRP were 17 mm/hr and 13, respectively. ANCA status was unchanged.

Anti-TNFα therapy heralds a new wave of specifically targeted biological interventions of potential value in the treatment of vasculitis. It offers the hope of improved therapeutic efficacy over current agents and the possibility of reducing exposure to steroids and immunosuppressives.

Further studies are warranted to confirm these observations and explore the role of infliximab as a component of initial protocols.

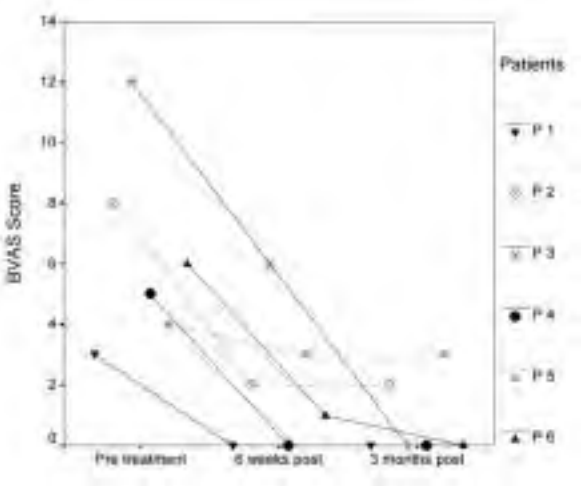

Figure 1. BVAS scores for the six infliximab-treated patients (P).

98-062

RENAL RELAPSE IS AN IMPORTANT DETERMINANT OF RENAL SURVIVAL IN PATIENTS WITH PR3-ANCA ASSOCIATED VASCULITIS WITH RENAL INVOLVEMENT

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Severe renal disease is a feature of ANCA-associated small-vessel vasculitis. We evaluated patient and renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement at diagnosis during long-term follow-up.

Seventy-three patients were diagnosed between 1982 and 1995 and followed until 2000 allowing > 5 years of follow-up. All patients were treated with prednisolone, cyclophosphamide and, if necessary, plasmapheresis. Survival curves were estimated, and univariate (log-rank test) and multivariate (Cox proportional hazards) analysis with patient and renal survival as dependent variables was performed.

Of 73 patients included, 16 (22%) died within 1 year after diagnosis. Of 24 patients (33%) dialysis dependent at diagnosis, 2 remained and 2 again became dialysis dependent < 1 year; 9 died early without renal recovery. Risk factors for death occurring within one year in univariate analysis (RR, 95% CI) were age > 65 years (4.4, 2.0-16.7) and dialysis dependency at diagnosis (2.9, 1.2-10). In multivariate analysis CRP level (1.02, 1.01-1.04) was also associated with worse prognosis. Eighteen patients died > 1 year, with male gender (5.0, 1.1-23) and developing dialysis dependency during follow-up (4.0, 1.3-13) associated with this outcome.

Risk factor for renal failure within one year was dialysis dependency at diagnosis (30, 8.4-101). Of 53 patients dialysis independent 1 year after diagnosis, 9 patients became dialysis dependent during follow-up. A renal relapse was strongly associated with development of renal failure in long-term follow-up (29, 3.2-260).

In conclusion, early death and failure to recover renal function in PR3-ANCA associated vasculitis is associated with age > 65 years and dialysis dependency at diagnosis. Long-term renal survival is determined by renal relapses during follow-up only. In contrast to MPO-ANCA related vasculitis slow, progressive renal failure without relapses is rarely observed in this group.

99-063

OUTCOME OF RENAL VASCULITIS IN LONDON: 1995-2000

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The outcome of renal vasculitis in 313 new patients diagnosed 1995-2000 was determined. Diagnoses were: ANCA-associated systemic vasculitis (AASV) (246), Henoch-Schönlein purpura (25), cryoglobulinemic vasculitis (7), polyarteritis nodosa (17) and anti-GBM disease (18).

Of those with AASV, diagnoses: 38% microscopic polyangiitis (MPA), 27% Wegener's granulomatosis (WG), 11% renal-limited vasculitis (RLV) and 4% Churg-Strauss (CSA). Mean length of prodromal symptoms, 4 months; median age 66. 57% were male and 83% Caucasian. ANCA were present in 92%. RLV/MPA: 65% P-ANCA/MPO, 25% C-ANCA/PR3; WG: 83% C-ANCA/PR3, 12% P-ANCA/MPO. Initial creatinine was higher in RLV and MPA ($p=0.002$).

Survival at 1 and 5 yrs was 82% and 76%, respectively (standardized mortality rate of 242%). Mortality was associated with age >60 ($p<0.001$), end stage renal failure (ESRF) ($p<0.001$), initial creatinine >200 mmol/l ($p=0.01$) and sepsis ($p=0.048$). 28% developed ESRF, of whom 47% died. 54 patients presented with creatinine >500 mmol/l; 29 achieved dialysis independence. There was no association of death or ESRF with gender, diagnosis or ANCA status. Relapse was most common in WG ($p=0.048$) and C-ANCA/PR3 specificity, and was not associated with age or creatinine. Leukopenia occurred in 41% and was associated with sepsis ($p<0.001$). Other major adverse events included cardiovascular (9.5%), bone (4.5%) and malignancy (4.5%). Functional status as assessed by Karnofsky score was low (mean 60). Treatment regimens varied with respect to the dose and route of administration of cyclophosphamide, cumulative steroid exposure and duration of remission therapy with azathioprine.

Within AASV, diagnosis and ANCA subgroup is unimportant in terms of major outcomes. ESRF and death in renal vasculitis are closely related to creatinine at presentation, thus diagnostic delay may have a major influence on outcome. Leukopenia should be avoided due to the close association with sepsis and death. Future therapeutic regimens should address the toxicity and partial efficacy of current treatment of particular importance in the elderly.

100-065

15-DEOXYSPERGUALIN IN PATIENTS WITH INTRACTABLE ANCA-ASSOCIATED SYSTEMIC VASCULITIS

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The combination of cyclophosphamide (CYC) and oral corticosteroids (OCS) is effective in the majority of patients with ANCA-associated vasculitis. However, it carries substantial risk of drug-related morbidity and mortality. New regimens are desired especially in refractory cases. The immunosuppressant 15-deoxyspergualin (DSG) is effective in autoimmune disease and transplantation in animal models as well as in acute kidney transplant rejection in humans. To assess the efficacy and tolerability of DSG we conducted an open label multicenter pilot trial in patients with intractable ANCA associated systemic vasculitis who were either unresponsive or had contraindications for standard immunosuppressants. The patients included 19 cases of Wegener's granulomatosis (WG) and one case of microscopic polyangiitis (MPA). Eighteen of 20 patients had received CYC before and 8 of them had received CYC immediately before study entry. During the study only concomitant steroid usage was allowed. DSG (0.5 mg/kg/day) was given s.c. for 2 to 3 weeks until the WBC count dropped to 3,000/ml followed by a rest until at least a count of 4,000/ml WBC was recovered. This was repeated up to 6 cycles. Remission rate by DSG treatment was 70% (6 cases of complete remission and 8 cases of partial remission). The therapeutically prospective leukopenia occurred in each patient in a regular pattern during the cycles and was transient without exception. No mortality or septicemia was observed. Mild to moderate bacterial infections mainly in the respiratory tract, mucosal candida infections and one herpes zoster infection were observed but resolved under adequate treatment without sequel. We conclude that DSG can successfully treat patients with refractory WG under careful monitoring of WBC count.

101-066

15-DEOXYSPERGUALIN AND CYCLOPHOSPHAMIDE, BUT NOT MYCOPHENOLATE MOFETIL, PROLONG SURVIVAL AND ATTENUATE RENAL DISEASE IN SCG/KJ MICE

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15-Deoxyspergualin (DSG) is an immunosuppressant with a unique mode of action currently undergoing a phase II trial for treatment of patients with Wegener's granulomatosis. We compared here the efficacy of DSG to cyclophosphamide (CYC) and mycophenolate mofetil (MMF) in scg/kj mice, an inbred mouse strain, that develops crescentic nephritis, systemic necrotizing vasculitis and pANCA spontaneously. Mice were randomly assigned to i.p. treatment with either DSG (2 mg/kg/day, n=25), CYC (1.8 mg/mouse/week, n=25), MMF (60 mg/kg/day, n=12 or 100 mg/kg/day, n=15) or vehicle (VEH, glucose 5% 0.3 ml/day, n=25) beginning at disease onset at the 9 week of life and lasting until the death of the animals. ANCA, BUN, proteinuria and hematuria were determined in all animals every 14 days. Sera were analyzed for the presence of pANCA by IIF, proteinuria was determined quantitatively and hematuria semi-quantitatively. Survival was calculated using the Kaplan-Meier method analyzing differences with log-rank testing. 50% survival in VEH treated animals was 123 days. At that point survival was 100% in CYC or DSG treated animals (log-rank $p<0.001$). However, mean survival in both MMF groups was not significantly different from VEH treated animals (MMF60: 117 days [95% CI 108 to 127], MMF100: 117 days [95% CI 110 to 124]). Proteinuria remained on baseline levels in the CYC and DSG groups and was significantly reduced when compared to controls (8 week: CYC 0.4±0.12 mg, DSG 0.5±0.21 mg, VEH 0.4±0.12 mg; 18 week: CYC 0.5±0.21 mg, DSG 0.5±0.33 mg, VEH 3.9±2.38, $p<0.05$ each). However, MMF did not reduce proteinuria significantly (18 week: MMF60 6.87±10.8 mg, MMF100 6.51±12.9 mg, VEH 3.9±2.38). Hematuria, BUN and ANCA titers were significantly decreased in CYC and DSG treated mice when compared to controls, however MMF showed no effect. Thus DSG and CYC, but not MMF, prolong life, limit renal damage and prevent autoantibody formation in scg/kj mice.

102-073

ANTI-THYMOCYTE GLOBULIN (ATG): A THERAPEUTIC OPTION FOR WEGENER'S GRANULOMATOSIS (WG) UNTREATABLE WITH CONVENTIONAL THERAPIES

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Introduction: A subset of WG patients does not respond to daily oral cyclophosphamide (oCyc) and glucocorticosteroids (GC) or suffers of intolerable side effects. Anecdotal data suggest that ATG may be a treatment option for these patients. We now describe the follow-up of 12 patients treated with ATG for refractory WG.

Patients and Methods: 12 patients with histologically proven active WG (7 unresponsive to oCyc, 5 intolerant of oCyc) were treated with ATG Merieux within or according to the SOLUTION protocol designed by the European Vasculitis Study Group (EUVAS) for refractory systemic vasculitis.

Results: Before ATG administration, patients had received a mean of 4 (2 to 6) different therapeutic approaches including oCyc in all and experimental therapies in 5, without control of disease activity (3.5 +/- 2 relapses during a disease duration of 72 +/- 52 months). 11 of 12 patients showed a favorable response to ATG with partial (9) or complete (2) remission of disease activity. During a follow-up of 20 +/- 12 months, 4

patients relapsed after 12 (2-29) months. Seven patients are free of relapse for 12 (3 - 31) months. Although further immunosuppressive treatment was required in all, a dose reduction or a change to a less aggressive regimen could be achieved in 10 cases. One patient died due to active WG 3 days after ATG administration. Side effects of the ATG treatment were mild with fever and chills during the first administration, serum sickness (1 case) and infections (3 cases, not life threatening).

Conclusion: ATG seems to be a treatment option for severe WG refractory to cyclophosphamide and glucocorticosteroids.

103-086

CONTENT AND EVALUATION OF AN INTERDISCIPLINARY PATIENT EDUCATION PROGRAM IN SYSTEMIC VASCULITIS (PEPVAS)

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Background: Standardized patient education programs are effective as an additional therapy in chronic diseases, eg, rheumatoid arthritis, systemic lupus erythematodes, and are able to reduce disease activity and depression. Most patient education programs are based on cognitive behavioral interventions and are supposed to improve the patient's self-efficacy.

Purpose: (1) To develop, establish and standardize an inter-disciplinary patient education program (PEPVAS) for patients with primary systemic vasculitides (PSV). (2) To evaluate the therapeutic effect of the program with a prospective study in a pre/post design.

Methods: In past years, interdisciplinary seminars on disease, therapies, side effects, coping strategies, nutrition and physiotherapy were developed in our center for patients with PSV. This unstandardized approach was revised according to the guidelines of the German Rheumatology Society and the new version was implemented.

Results: Our newly designed patient education program comprises 5 modules each conceived for 90 minutes interactive training based on information presented on transparencies by the participating disciplines (physicians, psychologists, nurses, dieticians, physiotherapists). To evaluate the program and measure the therapeutic effect, a documentation system with physician- and patient-administered questionnaires assessing different aspects of health status as health-related quality of life (SF-36), disease extent (DEI) and activity (BVAS), laboratory parameters (ESR, CRP, ANCA), employment status, disability, knowledge and self efficacy was developed and completed before and 1 and 6 months after participating in the program. Knowledge and all components of health-related quality of life improved.

Conclusions: To our knowledge this is the first standardized patient education program for PSV. We present the content of five modules and first results of the evaluation of 20 participating patients. The practicability and acceptance of the program were high. These early results indicate an effect of PEPVAS.

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104-087

PATIENT OUTCOME IN WEGENER'S GRANULOMATOSIS AND MICROSCOPIC POLYANGIITIS WITH RENAL INVOLVEMENT

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Patients with ANCA-associated small vessel vasculitides such as Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) did suffer from an extremely bad prognosis until the introduction of cyclophosphamide and corticosteroids according to the scheme introduced by Fauci. However, during the last decade some reports have focused on the problems with cancer and other side effects of therapy in these patients. We have previously published data on a five-year follow-up on 123 consecutive patients with WG or MPA with renal involvement, and documented an increased risk for malignancy, particularly after one year of cyclophosphamide treatment. We therefore aimed at a longer follow-up time on this cohort of patients, investigating the patient survival data and frequency of cancer.

This study comprised 117 consecutive patients, 43 women and 74 men, with a Wegener's granulomatosis or microscopic polyangiitis. All patients had a biopsy-proven renal involvement and were followed up for 8.4 years (range 0.1-336 months). Six patients out of the 123 were thus lost to follow-up. The cumulative relative survival was analyzed according to Hakulinen, comparing the present cohort to the general Swedish population, matched for age and gender. Cancer incidence data were obtained from the South Swedish Regional Tumor Registry.

The cumulative relative survival 10 years after diagnosis of the vasculitis was 73% for the whole group. Analyzing men and women separately revealed a lowered cumulative relative survival rate for men. This was not the case for women who survived the first year after the diagnosis of vasculitis with renal involvement. One new case with cancer was registered since last follow-up; in total there were 16 cases with cancer during the whole follow-up period. Urinary bladder cancer was registered in four patients, all men, with a standardized morbidity ratio of 7.1, 95% CI 1.9-18.2.

In conclusion, men surviving the first year after the diagnosis of a WG or MPA with renal involvement have a lowered cumulative relative survival, compared to the general male population.

105-088

ANTI-β-GLUCAN ANTIBODY IS USEFUL AS AN INDICATOR OF IMMUNOLOGICAL COMPETENCE AND A PREDICTOR OF THE OCCURRENCE OF DEEP MYCOSES DURING IMMUNOSUPPRESSIVE THERAPY FOR ANCA-ASSOCIATED VASCULITIS

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Aim: Patients with antineutrophil cytoplasmic antibody-associated (ANCA) vasculitis (AAV) sometimes recover after treatment with potent steroids and immunosuppressive agents. However, deaths do occur as a result of opportunistic infections. β-Glucans are present diffusely in nature among fungi, bacteria and plants. Since β-glucans are not immunogenic, the ability of the human body to produce antibodies against β-glucans in the cell wall has not been sufficiently clarified. The present study was performed to establish a method to analyze serum titers of anti-β-glucan antibodies to predict the occurrence of infectious diseases supervening AAV and also to identify an indicator of immunological competence of the host. We also examined the clinical significance of the method.

Methods: Serum antibody titers induced against β-glucan in solubilized *Candida* cell wall (anti-CSBG antibody) were analyzed by ELISA. Subjects were 22 healthy adults, 77 patients with RA and 35 patients with AAV.

Results: Anti-CSBG antibody recognized the β-1,6 structure of normal chains of β-glucans. Mean antibody titers were 5,527 ± 1,686 U and 838 ± 546 U in the healthy and RA groups, respectively. Mean antibody titer was 687 ± 543 U in the AAV group before treatment (in the active phase), but this value decreased significantly to 533 ± 432 U after immunosuppressive treatment ($P < 0.01$). Serial analyses of anti-CSBG antibody titers in individual patients showed that whereas the titer increased in cases with remission of AAV, it decreased in those with deep mycoses. Analysis of anti-CSBG antibody in cases of AAV is useful to estimate immunological competence during nonspecific immunosuppressive therapy and to predict the occurrence of deep mycoses.

106-089

HEALTH-RELATED QUALITY OF LIFE IN SYSTEMIC VASCULITIS: VALIDATION OF THE GENERIC INSTRUMENT SHORT FORM 36 (SF-36)

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Purpose: Measurement of physical, mental and social function is essential for the evaluation of patients (pts) with rheumatic diseases. The purpose of this study is the assessment of health-related quality of life (HRQL) with the multi-dimensional generic questionnaire Short Form 36 (SF-36) in a university-based rheumatology department, the evaluation of its psychometric properties, acceptance and practicability in daily use and the comparison of pts with primary systemic vasculitides (PSV), connective tissue diseases (CTD) and fibromyalgia (FM) with a healthy population.

Methods: The 36-item questionnaire measures the dimensions physical function (PF), role physical (RP), pain (P), vitality (V), mental health (MH), role emotional (RE), social function (SF), and general health (GH). Scales range from 0-100 with high figures indicating high HRQL. Laboratory parameters were examined to assess convergent validity.

Results: In this cross-sectional study 279 consecutive patients admitted to the department of rheumatology with PSV (n=172), connective tissue diseases (n=96) and fibromyalgia (n=11) completed the SF-36 within three days of admission. The mean age was 57.2, 51 and 58.8 years, respectively. All patient groups estimated their HRQL lower than an age-adjusted reference population. Pts with PSV estimated their HRQL in all aspects higher than pts with CTD or FM. Values between .84 and .94 for Cronbach's alpha indicate high internal consistency. CRP and HRQL correlate statistically significantly.

Conclusions: Pts with PSV estimate their HRQL significantly higher than pts with CTD in the physical dimensions (PF, V, P) despite older age. Reasons, which will be elucidated in longitudinal studies, may be slightly lower disease activity and effects of patient education in our PSV cohort. The SF-36 represents a valid and reliable instrument that shows high acceptance and practicability in daily clinical use. Further studies with patient focus groups may be useful to identify disease-specific aspects of health-related quality of life in PSV that are not covered by the SF-36.

107-094

TRIMETHOPRIM-SULFAMETHOXAZOLE MONOTHERAPY FOR ACTIVE LOCO-REGIONAL OR LIMITED WEGENER'S GRANULOMATOSIS

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Standard therapy for Wegener's granulomatosis (WG) consists of cyclophosphamide and corticosteroids. At the costs of significant morbidity and even mortality, this therapy leads to remission of disease activity in most patients. Incidental reports suggest that WG limited to the upper and lower airways can be treated with trimethoprim-sulfamethoxazole (TS).

Since 1993 we have performed a cohort study with TS monotherapy 2 × 160/800 mg in untreated patients presenting with biopsy-proven active WG limited to the upper and lower airways. Complete remission (CR) was defined as the total absence of symptoms or signs attributable to active WG (BVAS 0) in combination with a normal serum CRP level, partial remission (PR) as an improvement in disease activity score and CRP without fulfilling the criteria for CR. Treatment failure was the need for alternative or additional treatment to control WG, or relapse of disease activity during TS.

Included were 31 patients (age 29-86 years; 10M/21F), 25 at diagnosis, 6 at relapse. In 14 patients disease activity was confined to the ENT region (loco-regional WG), 17 patients had in addition to ENT activity arthralgias, episcleritis or pulmonary lesions (limited WG). All patients had nasal mucosal abnormalities with necrotizing granulomatous inflammation with or without vasculitis on nasal biopsy. ANCA were detected in 26 patients (84%; PR3-ANCA 20, MPO-ANCA 6). Treatment with TS was successful in 27 patients (87%, 95% CI 70-96%), with CR in 18, and PR in 9 patients. Time to maximal treatment response was 3 months (1 to 15). TS was stopped in 2 patients due to side effects, 2 patients had disease progression after 1-2 months of therapy. Eleven patients relapsed 14 months (2 to 32) after start of TS treatment, 5 of 9 (56%) after partial and 6 of 18 (33%) after complete remission (RR 5.8; 95% CI 4.6-21.3). All were treated with

cyclophosphamide and prednisolone. Disease-controlled survival with TS monotherapy was 70% (95% CI 53-87%), 60% (95% CI 41-79%), and 36% (95% CI 14-58%) at 12, 24, and 36 months.

Given the observed response rate, initial therapy with TS may obviate the need for more toxic conventional treatment for prolonged periods in patients presenting with loco-regional or limited Wegener's granulomatosis. Especially in patients with initially a complete response on trimethoprim-sulfamethoxazole, long-term control of the disease seems possible.

108-095

INTRAVENOUS IMMUNOGLOBULIN (IVIG) TREATMENT OF MPO-ANCA-RELATED MICROSCOPIC POLYANGIITIS

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Anti-neutrophil cytoplasmic antibody (ANCA)-related microscopic polyangiitis (MPA) requires strong immunosuppressive treatment including steroid and cyclophosphamide; however, such treatment is not always applicable to elderly or immunocompromised patients. For these patients, intravenous immunoglobulin (IVIG) treatment is an alternative because IVIG can modulate the immune system without severe side effects. Eight patients with myeloperoxidase (MPO)-ANCA positive MPA (age, 61-84 y.o.; average, 73 y.o.), histopathologically proven by renal biopsy, received IVIG treatment in our hospitals. In seven of the eight patients, the IVIG treatment has provided significant amelioration of the following ANCA-related symptoms and signs: high fever (3 cases), appetite loss (3 cases), elevated CRP values (6 cases), and progressive renal dysfunction (3 cases). In only one case was steroid pulse therapy required, but in other cases relatively low dose of oral steriod with minimal cyclophosphamide was enough to suppress exacerbation of glomerulonephritis (GN) as the maintenance therapy. IVIG seems to exert a convincing effect for patients with MPO-ANCA-related MPA, especially those who cannot endure strong immunosuppressive treatment.

109-097

CLINICAL STATUS OF 897 PATIENTS WITH TAKAYASU ARTERITIS IN JAPAN

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Patients with Takayasu arteritis (TA) are still increasing in Japan. However, progress of medical tools and social care permit early diagnosis and therapy, thereby improving prognosis.

We surveyed the clinical status of 897 patients with TA. 30% of patients (Group I) no longer require steroid therapy. 40% (Group II) are in a stable clinical state on a small dose of steroid therapy with or without immunosuppressant therapy. 5% (Group III) are in an unstable state with recurring relapses despite medical and/or surgical treatment.

20% (Group IV) are suffering from various complications, such as aortic regurgitation, hypertension, ischemic heart disease, pulmonary infarction, strokes and cataracts, all of which are adequately manageable. 5% (Group V) require strict medical attention for serious complications, such as congestive heart failure, arrhythmia, heart attack and/or renal failure.

These data suggest that diagnosis of TA at an early stage is essential for improved prognosis.

110-106

OUTCOMES OF VASCULAR INTERVENTION (SURGERY AND ANGIOPLASTY) IN PATIENTS WITH TAKAYASU ARTERITIS

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Background: Takayasu arteritis (TA) can be disabling or life-threatening. Stenoses are the most frequently encountered lesions. Symptoms usually result from ischemia to organs supplied by diseased vessels. Optimal management of TA involves appropriate medical therapy for inflammatory aspects of large-vessel disease on one hand, and revascularization procedures (surgical or endovascular) for anatomic lesions, before irreversible damage is sustained. Despite advances in angioplasty and surgery, significant morbidity continues to occur because of limitations in treatment of established anatomic lesions.

Objective: To provide an analysis of outcomes of vascular interventions in 18 TA patients who received care at the Cleveland Clinic Foundation between 1979 to 2001.

Patients and Methods: Retrospective chart review. Coronary artery revascularization procedures were excluded from the review for patients older than 35 years old since it was felt that ability to discriminate between atherosclerosis and TA might not be possible beyond this (granted arbitrary) age. The primary outcome measure was patency of vessels treated by angioplasty, bypass or intravascular stent placement. Patency was assessed by repeat invasive angiography or magnetic resonance angiography. Secondary outcomes included complications from the procedure, morbidity and mortality.

Setting: Tertiary care referral center.

Intervention: Revascularization procedures included balloon angioplasty, stent placement, or vascular surgery with graft anastomosis.

Results: Data are available for 18 patients. Mean age at first revascularization procedure was 34.1 years. A total of ***48*** revascularization procedures were performed in 18 patients. 33 bypass procedures were performed. Thirty percent (10/33) of all grafts restenosed or reoccluded over 2 to 194 months after surgery. Eight percutaneous balloon angioplasties were performed, with a 50% (4/8) rate of restenosis/occlusions at 2-22 months; 7 stents were installed, of which 3 reoccluded (42.9%) at 2-22 months. There were no deaths associated with revascularization procedures.

Conclusion: Despite providing short-term benefit, revascularization procedures adapted for atherosclerotic disease are associated with a higher failure rate in patients with TA.

111-107

LONG-TERM REMISSION OF POLYARTERITIS NODOSA (PAN) ASSOCIATED WITH MYELODYSPLASTIC SYNDROME AFTER INTRAFAMILIAL ALLOGENEIC BONE-MARROW (BM) TRANSPLANTATION

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Objective: To test the efficacy of bone marrow allograft in a patient with PAN refractory to treatment and myelodysplastic syndrome.

Patient: A 33-year-old man presented in 1993 with fever, weight loss, polyarthritis, abdominal pain, digital ischemia and low neutrophil count at 1,000/mm^3. Retinal angiography detected vasculitic lesions; renal and mesenteric artery arteriographies revealed multiple microaneurysms. ANCA were negative. PAN was diagnosed and the disease worsened despite prednisone (1 mg/kg/day). Methylprednisolone, cyclophosphamide, intravenous immunoglobulin, methotrexate and cyclosporine were prescribed successively, without prolonged efficacy. In 1995, large granular lymphocytes, corresponding to polyclonal T lymphocytes, were identified and, in 1996, sideroblastic anemia was diagnosed. Blood transfusions were frequently required and systemic vasculitis remained uncontrolled despite prednisone (30 mg/day) and methotrexate (20 mg/wk). In February 1998, an intra-familial bone marrow allograft was performed. Conditioning regimen combined total body irradiation and melphalan (140 mg/m^2).

Result: Four years post-transplant, complete remission of systemic vasculitis was obtained and karyotypic analysis of BM showed complete chimerism, with no evidence of myelodysplastic syndrome. However, persistent skin and liver graft-vs-host disease is treated with mycophenolate mofetil.

Conclusion: Bone marrow allograft may cure PAN in patients with associated or premalignant or malignant hemopathy.

112-113

TREATMENT OF POLYARTERITIS NODOSA (PAN) AND MICROSCOPIC POLYANGIITIS (MPA) WITH POOR PROGNOSIS FACTORS: A PROSPECTIVE TRIAL COMPARING STEROIDS (CS) AND 6 OR 12 CYCLOPHOSPHAMIDE (CYC) PULSES IN 65 PATIENTS

Guillevin L, Cohen P, Mahr A, Arène JP, Mouthon L, Puechal X, Pertuiset E, Gilson B, Hamidou M, Lanoux P, Bruet A, Ruivard M, Vanhille Ph, Cordier JF, French Vasculitis Study Group (FVSG). Bobigny, France.

Background: The reference treatment for severe PAN without virus infection and MPA comprises CS and pulse CY, but optimal CY treatment duration has not been established. We conducted a trial to determine whether 6 or 12 CY pulses given in combination with CS could cure the disease.

Methods: Upon inclusion in this trial, organized by FVSG, 65 (18 PAN, 47 MPA) patients were randomized to receive 12 (n=34) or 6 (n=31) CY pulses combined with CS. None had received prior treatment for vasculitis. PAN and MPA were histologically proven or met ACR criteria. CS were administered as follows: a daily 15 mg/kg pulse for 3 days, then 1 mg/kg/d orally for 3 weeks. CS were then progressively tapered and definitively stopped after 1 year. CY pulses were administered every 2 weeks for 1 month, then every 4 weeks. No maintenance treatment was given after stopping CY. The endpoint of the study was the number of events (relapses and/or deaths) occurring in each group, analyzed according to an intention-to-treat strategy. The outcome was evaluated by Cox proportional hazards analysis.

Results: The main baseline clinical manifestations—poor condition 60/65, arthralgias and/or myalgias 38, peripheral neuropathy 37, glomerulonephritis 34, vascular nephropathy 12, renal insufficiency 30, gastrointestinal involvement 26, and cardiomyopathy 7—were similar for both groups. Mean five factor score (FFS) at entry was 1.8 ± 0.8; mean BVAS was 21.8 ± 7.7; mean follow-up was 32 ± 21 months. Comparing the 12- and 6-pulse groups, respectively, complete remissions were obtained in 88% and 84%; relapses occurred in 24% and 54%, and 18% and 26% died; 35% and 65% experienced an event during follow-up. Survival analysis showed a significantly lower relapse probability ($P = 0.02$; hazards ratio [HR] = 0.34) and higher event-free survival ($P = 0.02$; HR = 0.44) for the 12 CY-pulse group while the mortality rates were similar for both groups ($P = 0.47$).

Conclusion: These results suggest that 6 CY pulses are less effective than 12 CY pulses to treat severe PAN and MPA, particularly with respect to the risk of relapses.

113-116

CUTANEOUS PANARTERITIS NODOSA IN CHILDREN

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Cutaneous panarteritis nodosa (PAN) is rare vasculitic disorder in children. The main histologic feature is the necrotizing vasculitis small- and medium-sized arteries of the skin without visceral involvement. Infectious triggers of disease onset, ie, Strep and HBV, have been discussed. Ocular involvement has been noted in adults.

Objective: Define the clinical and laboratory features of the disease onset, the treatment and the outcome of cutaneous PAN in children.

Methods: A retrospective chart review of all patients seen over an 11-year period (1991–2001) at HSC was performed. Twelve patients were identified to have cutaneous PAN. The clinical presentation and laboratory data at onset, treatment and outcome were reviewed.

Results: Twelve patients (8m/4f) with a mean age at diagnosis of 7.5 yr (range: 1.2-13.4 yr) were included. The clinical presentation was skin lesions in terms of painful nodules and local swelling (12/12), fever (11/12), local pain (12/12), lym-

phadenopathy (9/9), arthritis (10/12), and splenomegaly (8/10). The skin lesions were noted on the legs (10/12), feet (8/12), hand/wrists (2/12), thorax/neck (2/12). 2 patients developed uveitis. 2 patients had severe vessel involvement leading to gangrene. 6/12 patients were pre-treated with antibiotics for a q. Strep infection. Lab features at onset included raised ESR (mean: 102, range: 52-132), high WBC (m: 20.5, r: 9.8-40.0), elevated Polys (m: 10.5, r: 8.26-33.6), anemia (10/12), thrombocytosis (9/12), low albumin (8/11) and high C4 complement levels (6/9 patients). 4/12 patients had significant ANA titers of >80, all showed speckled patterns, no other specific auto-antibodies were found, when tested. 7/12 patients had positive ASOT (3/12 high titers >160, 4/12 low titers, 5/12 neg). No other positive serology (HBV, EBV) was detected. The treatment was prednisone in 10/12 patients, plus penicillin 3/10, penicillin alone 2/12, or additional cyclophosphamide (2/12 for initial non-responsiveness, 1/12 relapse treatment). The mean duration of prednisone treatment was 35 months (range 4-120 months). 2/12 children relapsed after remission (1 skin manifestation, 1 uveitis relapse).

Conclusion: Cutaneous PAN in children is a rare but well-characterized disease entity, which has the typical clinical onset feature of painful skin lesions plus fever, lymphadenopathy, arthritis, and splenomegaly. Lab tests reveal severe inflammation including raised ESR, WBC, and Poly count, anemia, thrombocytosis and elevated C4 complement levels. More than 50% of the vasculitides are associated with Strep. Treatment mainly consists of prednisone as immunosuppression for a mean duration of 35 months. Cyclophosphamide is effective either in the case of non-responsiveness or as relapse treatment. 10/12 patients had a monocyclic course of the disease. Ocular involvement was seen in 2/12 patients, both with significant ANA titers.

114-117

BROAD SPECTRUM OF VASCULITIS AFFECTING THE AORTA

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Background: The spectrum of vasculitis affecting the aorta requiring surgical repairs is not well documented.

Methods: Data and routine histological examination with H&E and elastin stains were collected prospectively on patients undergoing 403 ascending aorta aortic arch operations; 87 of the patients were found to have inflammatory vasculitis of the aorta. Forty were male and 47 were female. Coronary artery disease was present in 34, aortic dissection in 33, peripheral vascular disease in 22, and 16 had a history of stroke. Twenty had emergency surgery and 26 urgent surgery with rupture being present in 14. Macroscopic atheroma in the aorta was present in 57.

Results: In addition to the aortic repair, 54 patients had aortic valve replacements and 13 had the entire or nearly entire aorta replaced. Specimens were available in 75 of the 87 patients. The associated findings were giant-cell arteritis in 13 (4 with temporal arteritis, 3 with polymyalgia rheumatica, and one of each of the following: TB or myasthenia gravis or cocaine abuse). Takayasu was present in 6, severe rheumatoid arthritis in 5, history of chest radiotherapy for carcinoma in 3, severe osteoarthritis in 3 (one with ankylosing spondylitis), Buerger's in 2, one of each with Behcet's, relapsing polychondritis, lupus, Cogan's, Hashimoto's, Erdheim-Chester, and 1 chronic myeloid leukemia. Past history of chronic, non-active infections included 7 bacterial endocarditis, 3 likely syphilis, 3 TB, 2 rheumatic fever, 1 chronic bronchiectasis and 1 chronic dental carries with aphthous ulcers. Two patients had systemic malaise and fatigue of unknown etiology treated by steroids. In 12 patients with aortitis, no other associated findings were present. Three had Marfan syndrome. For the 403 patients, the 30-day survival rate was 98% and for the 87 aortitis patients 95% (84/87). Three suffered a stroke (3.5%).

Conclusion: The incidence of inflammatory vasculitis associated with diverse etiologies is not as uncommon as thought when the aortic histology is examined and the patients are carefully questioned for associated pathologies.

115-124

MYCOPHENOLATE MOFETIL (MMF) THERAPY FOR ANCA-ASSOCIATED VASCULITIS (AAV)

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Rationale/Methods: Induction and maintenance of remission of AAV can be limited by drug toxicity or inefficacy. MMF has recently been introduced as an alternative agent for the treatment of AAV. To determine the efficacy and side effect profile in AAV patients, we performed a retrospective analysis of the 14 AAV patients treated with this drug at our institution.

Results: In three patients, MMF was part of a post-renal transplant regimen. In three patients, MMF was used for remission induction because other agents were contraindicated or had failed. In the remaining eight, MMF was used for remission maintenance, also primarily because of initial agent failure or contraindication. Twelve patients had Wegener's granulomatosis; two were diagnosed with microscopic polyangiitis. Seven (88%) of the maintenance patients remained in remission for an average of 17 months (range 11-26 mo), four (57%) without concomitant prednisone therapy. One relapsed after 24 months, but was found to have subtherapeutic drug levels. All of the maintenance patients in remission had therapeutic drug levels. One transplant patient suffered a meningeal flare, prompting cyclophosphamide therapy. Of the three patients for whom induction of remission was intended, relapse occurred within an average of 4 months, one episode occurring during taper of steroids. Lymphocyte counts did not seem to predict disease course. MMF was generally well tolerated, with only mild possible drug-related adverse effects including leukopenia, thrombocytopenia and gastrointestinal upset.

Conclusion: MMF appears to be an effective agent with few side effects when used for remission maintenance in patients with AAV. However, it may not be effective for induction of remission in AAV. Based on this encouraging preliminary data, further prospective controlled trials to define the exact role of MMF in the therapy of AAV seem warranted.

116-126

EFFICACY AND SAFETY OF LF 15-0195 (ANISPERIMUS) IN PERSISTENT OR RELAPSING PRIMARY SYSTEMIC VASCULITIS

The Laboratoires Fournier LF 15-0195 Vasculitis Study Group.

An open-label, non-controlled, multicenter phase II study evaluating the efficacy and safety of repeated administration of LF 15-0195 (anisperimus) was performed in 18 patients with persistent or relapsing primary systemic vasculitis. Immunosuppressives were withdrawn at entry and prednisolone dose adjusted according to clinical status. LF 15-0195, 0.025 mg/kg/day, was administered by subcutaneous injection on five consecutive days at four-week intervals for four cycles. Those in remission after two cycles reduced to 0.0125 mg/kg/day. From four months to the study end at 12 months, patients continued on prednisolone alone. Immunosuppressives were allowed to be restarted in case of relapse.

Of 9 patients completing the study to date, mean age at entry was 51 years and disease duration 6 years. Diagnoses were: Wegener's granulomatosis 7, microscopic polyangiitis 1, and polyarteritis nodosa 1. Seven were receiving immunosuppressives up to the time of entry (cyclophosphamide 3, azathioprine 3, methotrexate 1). By the end of the treatment period 4 patients achieved full remission (BVAS = 0), 3 partial remission (BVAS <50% baseline) and 2 had no response. At 12 months, of the 7 responders, 5 had had a minor and 2 a major relapse. Mean prednisolone doses fell from 34 mg/day at entry to 10 mg/day after three months. No severe adverse reactions associated with the trial medication were reported. Minor infections occurred in 3 and neutropenia in 1. Immunosuppressives were restarted during the follow-up phase in 5 (cyclophosphamide 2, methotrexate 1, leflunomide 1, infliximab 1).

These preliminary results in refractory vasculitis indicate potential efficacy of LF 15-0195 without major toxicity. However, remission was not sustained after the treatment period by prednisolone alone. Further results will be available in 2002.

Treating the Permanent Sequelae of Vasculitis

117-001

NASAL CAVITY SQUAMOUS CELL CARCINOMA IN WEGENER'S GRANULOMATOSIS

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Wegener's granulomatosis (WG) is well known for its chronic, debilitating nature, multiple organ system involvement, variable course, and myriad of complications causing morbidity and mortality. Although the occurrence of a variety of malignancies has been recognized in patients with long-standing WG undergoing cyclophosphamide therapy, only one case of squamous cell carcinoma (SCC) of the upper aerodigestive tract has been recorded. We report two cases of nasal cavity/sinus cavity SCC diagnosed in patients with quiescent WG. Both patients presented with progressive facial discomfort which did not respond to therapies directed to treat possible WG recurrence or infection. Tissue diagnosis was eventually positive for SCC. Because the nasal and sinus findings in WG may conceal the overt appearance of malignancy, diagnosis is very difficult and may be delayed, resulting in a suboptimal clinical outcome. Physicians who manage patients with WG should recognize that worsening nasal and sinus symptoms might not only be due to disease exacerbations or secondary infection but may less commonly signal the development of carcinoma.

118-048

BRONCHOSCOPIC FINDINGS IN WEGENER'S GRANULOMATOSIS (WG)

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Background: Airway involvement leading to chronic, irreversible lesions occurs in up to 20% of WG patients.

Objective: To describe findings and propose early bronchoscopy in patients with WG and airway involvement.

Patients and Methods: 7 of 32 WG patients (21.8%) underwent 15 bronchoscopic procedures (range 1-5).

Results: They relate to the number of procedures. Airway symptoms (prior at least one month) were found in all except 3 occasions (80%): dyspnea, 60%; stridor and dysphonia, 53%; and cough, 20%. Mean BVAS score was 4.6 ± 3.3 (0-12). Prior nasofibrolaryngoscopy was performed in 8/15 showing stenosis ≥50% in 5. Fibrobronchoscopy was performed in 13/15 procedures, rigid bronchoscopy in 2/15 and both in 2/15. In all but 4, subglottic and/or tracheal stenosis ≥50% was found. An active lesion in the left bronchial carina was seen in one procedure. On follow-up evaluation of this lesion, a 70% chronic stenosis was seen which after mechanical dilatation was reduced to 30%. Mechanical dilatation was done in 11/15 procedures (including both tracheal), being progressive in 7, and not possible or not done in 2 each. Biopsies were performed in 7/15 (5 tracheal, 5 subglottic). Chronic inflammation and/or fibrosis was seen in 3 tracheal and 4 subglottic biopsies, acute inflammation and necrosis in 3 tracheal. All patients with acute inflammation received systemic treatment (cyclophosphamide in 2, methotrexate in one). Dilatation and intralesional steroid injection, including one on the main left bronchus, was done in 3/15 according to the technique by Langford et al, with successful results in all three. One of the patients subject to this technique was lost to follow-up at the time a second procedure was planned and had subglottic restenosis when reevaluated. Complications were observed in 2/15 procedures. On the first patient (with 95% subglottic stenosis), tracheoplasty was performed at the same time of first bronchoscopic evaluation; on the second, after successful pro-

gressive dilatation, the patient developed sudden laryngeal edema which required tracheostomy. Laryngotracheomalacia was observed in two patients. Mean follow-up is 18.8 ± 20.2 months (3-61).

Conclusions: We found both chronic and new lesions coexisiting in 3 patients, requiring both local and systemic therapy. The procedure proposed by Langford et al seems effective in both settings. In general, BVAS was not useful to predict the nature (acute or chronic) of the lesions observed. We therefore propose fibrobronchoscopy for evaluation of all the airway (as we also found significant tracheal lesions) to be performed in patients with both new and past airway involvement to precise nature of the lesions and decide on early treatment.

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TREATMENT OF ENDOBRONCHIAL WEGENER'S GRANULOMATOSIS WITH LOCAL APPLICATION OF HUMANIZED ANTI-LYMPHOCYTE ANTIBODY

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Wegener's granulomatosis is a form of necrotizing small-vessel, systemic vasculitis which commonly involves the upper airways, lungs, and kidneys.[1] A minority of patients with Wegener's granulomatosis develop tracheal and bronchial stenoses, which may occur in the absence of systemic disease.[2] These pose a significant therapeutic challenge in that they are often unresponsive to standard immunosuppressive agents. We would like to report a series of cases treated with mechanical dilatation and intra-mucosal injection of anti-CD52 monoclonal antibody. Systemic anti-CD52 antibody has previously been found to be effective in the treatment of refractory systemic Wegener's granulomatosis.[3]

Five patients with Wegener's granulomatosis with tracheal and/or bronchial stenoses were treated between 1997 and 1999. Patient details: 4 female, 1 male. Mean age: 50 years (34-63 years). Endobronchial involvement: subglottic stenosis + unilateral bronchial stenosis (n=1), subglottic stenosis + bilateral bronchial disease (n=2), single bronchial stenosis (n=2). Patients were treated with 20 mg anti-CD52 monoclonal antibody injected submucosally and concurrent mechanical dilatation. Patients received between 1 and 9 treatments. Following treatment, stenoses were noted to be more amenable to dilatation. All patients had improvement in their pulmonary function tests, and in one patient there was resolution of the bronchial stenosis.

Complications of treatment included lower respiratory tract infection (n=4) and goiter with associated thyrotoxicosis (n =1).

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120-123

MANAGEMENT OF ACUTE AND CHRONIC MANIFESTATIONS OF TRACHEOBRONCHIAL WEGENER'S GRANULOMATOSIS

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Purpose: To evaluate the clinical spectrum and course of endobronchial lesions in Wegener's granulomatosis (WG), and to assess the efficacy of bronchoscopic intervention in providing long-term airway patency.

Methods: Review of medical records of patients with WG who underwent initial bronchoscopy between January 1990 and November 2001. Specific data assessed included symptoms, frequency of bronchoscopy, pulmonary function testing, and treatment.

Results: 90 patients with a diagnosis of WG underwent bronchoscopy. Acute manifestations of tracheobronchial WG included ulcerating tracheobronchitis, inflammatory pseudotumor, and mucosal cobblestoning. Systemic disease activity did not correlate with endobronchial disease activity. Chronic manifestations included luminal narrowing or occlusion from endobronchial scarring. Eight patients underwent dilatation for symptomatic airway narrowing, and four patients had airway Silastic stents placed due to refractory symptoms related to the severity of their stenosis. Mean follow-up for patients receiving stents was 6.2 years. Patients with stents underwent bronchoscopy approximately every 8 to 12 months for inspection of stent function, replacement and evaluation of disease progression. Stent migration or mucoid impaction were the most common reasons for stent replacement. One patient died two months following stent placement, of an unrelated cause. One patient had two stents placed: a tracheal stent that was removed after 2 years, and a right mainstem bronchus stent that was removed after 4 years. After 6 years of follow-up, there have been no recurrent symptoms or stenosis requiring further intervention. One patient had a left mainstem bronchus stent removed after 4 years. Recurrent symptomatic stenosis necessitated replacement of the stent 2 years later. One patient had a left mainstem bronchus stent placed due to severe stenosis. Progressive stenosis of the right mainstem bronchus necessitated placement of a Y-stent 6 months later. There were no reported complications due to the stents in any of the patients.

Conclusions: Tracheobronchial manifestations of WG can result in significant airway stenosis necessitating bronchoscopic intervention. Bronchoscopic dilatation and stent placement can provide long-term symptomatic and functional improvement in patients with WG and endobronchial disease.

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