# Granuloma formation, implications for the pathogenesis of vasculitis

MICHAEL C. SNELLER, MD

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.

From the Immunologic Diseases Section, Laboratory of Immunoregulation, National Institute of Allergy, Immunology, and Infectious Diseases, National Institutes of Health, Bethesda, MD. Address correspondence to M.C.S., Chief, Immunologic Diseases Section, LIR/NIAID/NIH, Bldg. 10, Room 11B13, 9000 Rockville Pike, Bethesda, MD 20892. E-mail: sneller@nih.gov

## 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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