The Search for Pathogenic T Cells and the Genetic Basis of Psoriasis Using a Severe Combined Immunodeficient Mouse Model

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The immunologic and genetic bases of psoriasis are under active investigation throughout the world. Rather than pursue the genetic linkage to psoriasis to discover the gene(s) responsible for causing the disease, we have focused on the cellular immunology and basic biology using a novel animal model. We reasoned by identifying specific cellular and molecular abnormalities involved in the biologic responses that initiate lesion formation, that the genes involved in such a pathologic process would lead us to the correct causative DNA-based abnormality that determines disease susceptibility and inheritance. To pursue this line of inquiry, we utilized an animal model in which severe combined immunodeficient (SCID) mice were engrafted with symptomless skin (PN skin), and bona fide psoriatic plaques (PP skin) were created using specific pathogenic T cell subsets. This model can be used experimentally not only to study the mechanism by which PP skin is converted to PN skin, but also to create PP skin from PN skin. The clinical, histologic, immunologic, and pharmacologic validation of this SCID mouse model will be presented. This summary will also highlight the value of such a model, which has recently led to the discovery of previously overlooked types of immunocytes that are associated with induction of psoriatic lesions. Finally, a novel hypothesis linking the immunology and the genetics of psoriasis, based on findings using this animal model, will conclude this presentation.

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Physicians for the past 2000 years have recognized the clinical hallmarks of psoriasis including cutaneous symmetrical, well-circumscribed erythematous plaques (PP skin).[1,2] Although it is a fairly easy diagnosis to make with such classic findings, the cause of the disease and effective therapy that sustains remission without side effects present greater problems to the practicing dermatologist.[3] Treatment approaches and our basic understanding of the disease, as regards both the patterns of inheritance and the pathophysiology of psoriasis, have been greatly impeded by the apparent absence of psoriasis among various other species and lack of a suitable animal model.[4] Until the last three years, investigative skin biologists have had to rely primarily on tissue culture-based results, clinical pharmacologic trials, and random whole-genome scans to try to determine the etiology, pathophysiology, genetics, and treatment of psoriasis.[5,6] Although it is somewhat reassuring that even though we do not currently know the genetic basis, etiology, or many details as regards the pathophysiology of psoriasis, over 90% of patients can be induced into clinical remission (of various duration) with available treatment options.[7] Unfortunately, since psoriasis often begins in the first two decades of life and remains a lifelong disease process, the unwanted side effects of all available effective therapies in producing remissions cannot be continuously used to sustain the disease-free interval or necessarily prevent the onset of new lesions.[3] In an attempt to decipher the genetic code that causes psoriasis and to better understand the cellular and molecular basis for psoriasis, we set out to create an animal model that would permit us to both produce and eliminate skin lesions under tightly-controlled experimental conditions.

Animal Model

Since psoriasis is apparently confined to humans, we reasoned that the best approach to creating an animal

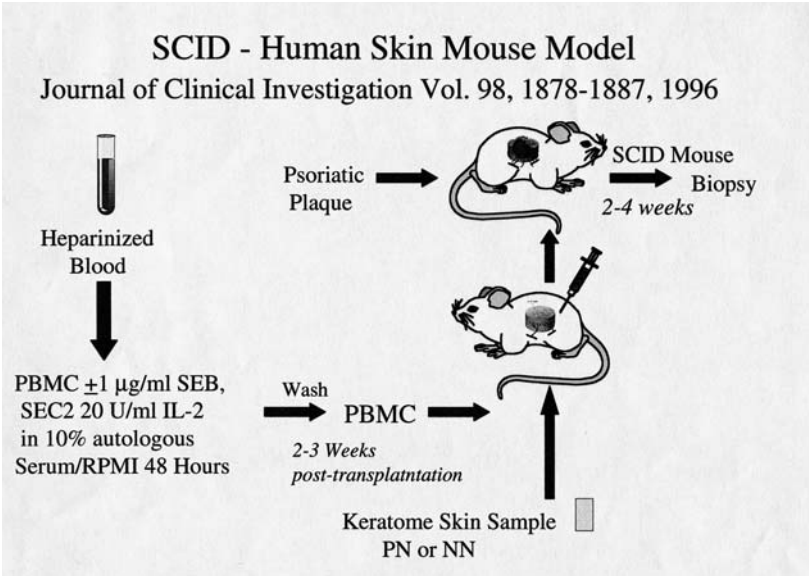

FIGURE 1. Overview of SCID mouse-human skin xenograft model for psoriasis. This summary depicts both the use of engrafted pre-existing psoriatic plaques, as well as the method by which either PN or NN skin can be converted into PP skin using blood-derived, activated immunocytes.

model would be to use entirely human cellular constituents. For decades, debate was constantly centered around the role of the skin-derived keratinocyte versus the bone marrow-derived T cell as the most likely candidate cell type for harboring the genotype responsible for the characteristic clinical phenotype.[8,9] Thus, our experimental approach (Figure 1) was to transplant relatively large keratome samples of human skin (about the size and thickness of a postage stamp with a peel-off backing) onto severe combined immunodeficient (SCID) mice.[10] Since SCID mice lack both T cells and B cells, they do not reject the human skin, which can remain engrafted for months, and even up to one year. The graft includes all the human epidermal cell types (i.e., keratinocytes, Langerhans cells, melanocytes) as well as a confederacy of dermal cell types (i.e., fibroblasts, T cells, macrophages, dendritic cells, mast cells), including human post-capillary venules lined by human endothelial cells that anastamose with murine vessels in the deep dermal and subcutaneous tissue during the wound-healing response after surgical transplantation. Two of the important aspects of this approach are: 1) that all of the human cellular constituents retain their native anatomical configuration; and 2) that SCID mice have thick, well-vascularized skin. Once the human skin is engrafted, all of these dermal and epidermal cell types can be sustained in a viable state by such a well-perfused graft.

Skin Graft Success

Having overcome potential problems with immunologic graft rejection and inadequate blood supply, the first question that we addressed was whether such large grafts of either normal skin obtained from donors without psoriasis (NN skin), or, more importantly, PN and PP skin, would retain their phenotypes after transplantation.[10] Indeed, NN skin post-engraftment clinically and histologically was indistinguishable when comparing pre- to post-engraftment biopsies (Figure 2), and, most importantly, both PN and PP skin also had stable phenotypes (Figure 3). The PP skin grafts provided an opportunity to explore therapeutic options in which PP skin was reversed back to PN skin. To date, we have determined that this PP model system can be converted back to PN skin using cyclosporin A corticosteroids, and 1,25-dihydroxyvitamin D3, in a similar manner as occurs during direct patient clinical trials.[11] Thus, this model system can be used in preclinical studies to screen a drug or other compound, or treatment protocol, without directly subjecting patients to any adverse effects of the prospective new therapeutic agent. We have also used both PP and NN skin to examine the tissue response to various important viruses in an entirely human system such as human immunodeficiency virus-1 and human herpesvirus-8.[12] Obviously, for ethical reasons, studies including direct injection of these viruses into human skin could not otherwise be performed, but the use of SCID-mouse human skin xenografts have permitted us to explore treatment responses and to determine the role of human immunodeficiency virus-1 in AIDS-related psoriasis, as well as to try to fulfill Koch's postulates relative to human herpesvirus-8 and cutaneous Kaposi's sarcoma.

Creating Psoriatic Lesions

Perhaps the most relevant experiments for this review article pertain to our attempts to actually create psoriatic lesions from either PN or NN skin.[13] Since neither PN or NN skin spontaneously develops psoriatic lesions

in a reproducible fashion after engraftment, it was an ideal setting to address the long-standing question as to whether the key cell type was keratinocytes or an immunocompetent, bone marrow-derived cell (i.e., immunocyte).[9,10] When engrafted PN skin was injected with various cytokines or growth factors, no psoriasis was produced. However, when autologous blood-derived immunocytes were pre-activated and then injected intradermally, skin lesions developed in the PN skin that resembled PP skin within 2 to 3 weeks. The clinical lesions included prominent skin thickening, scale production, erythema and neovascularization in the dermis. Histologically, and by immunophenotyping, all of the characteristic changes of a psoriatic lesion were created using this approach. While many other transgenic mice and other types of rodent models have been labeled as "psoriasiform" or "psoriasis-like," none of these animal models has all of the characteristic clinical, histologic or immunologic features identical to PP skin.[14,15]

Having established that PN skin can be converted to bona fide PP skin, the next question was whether NN skin could be converted to PP skin. This was an important issue to at least try to examine, because it would be one of the most definitive methods to formally prove that psoriasis was caused by pathogenic immunocytes, and that the genotype of keratinocytes was not of primary importance in the appearance of skin lesions. In other words, individuals with psoriasis have exactly the same type of keratinocyte in their skin as non-psoriatics, but what distinguishes the psoriatic population is that they have circulating immunocytes that can, upon entry into skin, rapidly and profoundly trigger substantial keratinocyte and endothelial cell hyperplasia.[16] Even though one would expect the injection of activated blood-derived immunocytes from a psoriatic patient to cause graft-versus-host disease when injected into a different person's (i.e., NN) skin, we have observed in about half of the experiments what appears to be creation of psoriatic lesions.[7] Although some grafts did show evidence of graft-versus-host disease, at least three different combinations revealed that NN skin could be converted to PP skin (B.J. Nickoloff, unpublished observation). Clearly, much more work remains to be explored in this regard, but it was these unexpected results that led us to consider a previously unimagined scenario for psoriasis that involved a novel type of immunocyte.

Immunologic and Genetic Investigation

To try to understand how a pathogenic T cell from one patient could interact with epidermal cells from a different individual and not produce cytopathic graft-versus-host disease, the possible presence of inhibitory receptors was explored.[17] Such receptors have begun to be well-characterized as natural killer (NK) cells to explain the molecular basis by which NK cells attack virally infected or tumor cells, but leave normal cells unscathed.[18] Natural killer cells have both killer inhibitory receptors and killer activating receptors.[19] These NK receptors (NKRs) are capable of recognizing various major histocompatibility complex (MHC) class I alleles such as HLA-B or HLA-C.[20,21] Furthermore, a small subset of T cells also expresses such receptors, and these are referred to as NK-T cells.[22,23]

To determine whether such receptors may be present in the allogeneic T cell reactions involving the SCID mouse model, immunostaining was performed and lymphocytes bearing NKRs were found to be present. Specifically, immunocytes in the epidermis of acutely generated psoriatic lesions (i.e., PN to PP skin) were found to be positive for CD94, CD158a, and CD158b. Another ligand/receptor pair involving NK-T cells was also observed, including CD161 on the immunocytes and CD1d on the keratinocytes in an allogenetic combination of transplanted NN skin and psoriatic-blood-derived immunocytes.[24] A previously overlooked population of immunocytes bearing NKRs was also documented in chronic plaques taken directly from patients and has led to a novel hypothesis linking the genetics and immunologic basis for psoriasis.[25]

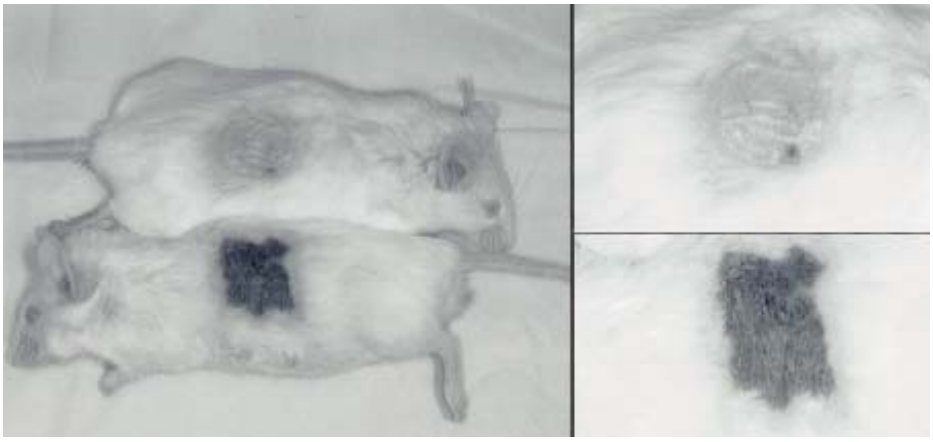

FIGURE 2. Clinical appearance of two different mice with engrafted normal (NN) human skin grafts. Note the retention of the normal appearance of human skin, which becomes well-vascularized with no evidence of graft rejection using the SCID mice as recipients.

Conclusions

The basis for this hypothesis is twofold. First, it has been increasingly evident that psoriasis is linked to the MHC region on chromosome 6, and certain class I MHC alleles such as HLA-Cw6 have been suggested as important proteins for the genetic basis of the disease. Second, certain NKRs recognize and interact with some of these same MHC class I alleles.[20] It is possible that, if a certain type of NK-T cell is not appropriately deleted via negative selection, it could produce an autoreactive immune reaction if it were triggered by a specific class I MHC allele. Thus, an autoreactive NK-T cell could produce psoriasis in any epidermal environment if its receptor were triggered by the appropriate class I MHC allele to produce cytokines that set into motion a cascade of cellular and molecular events to produce a psoriatic lesion.[25] Although the exact nature of additional antigens (either peptides or nonpeptides—i.e., lipids) necessary to interact with the T cell receptor remain to be defined, NK-T cells represent exciting new candidates for future studies into the genetics, etiology, and pathophysiology of psoriasis.[26]

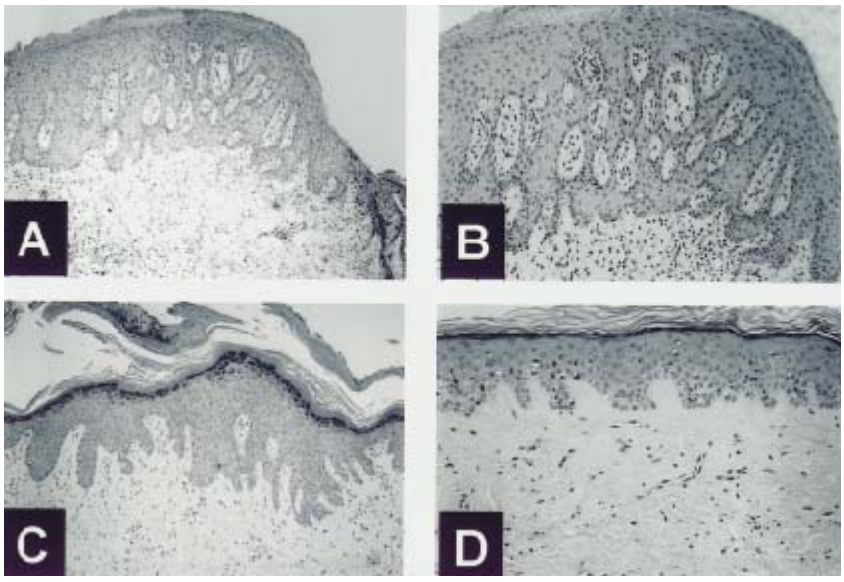

FIGURE 3. Histologic appearance of engrafted human skin onto a SCID mouse. PP skin after transplantation retains its highly characteristic clinical appearance with confluent parakeratotic scale, epidermal hyperplasia with elongation of rete pegs, loss of granular cell layer, and lymphocytic infiltration (A and B). Engrafted PN skin has focal parakeratotic scale, and slightly increased epidermal thickness, but there is no loss of the granular cell layer or elongation of the rete pegs (C). Transplanted NN skin has a basket-weave stratum corneum and an unremarkable dermis (D).

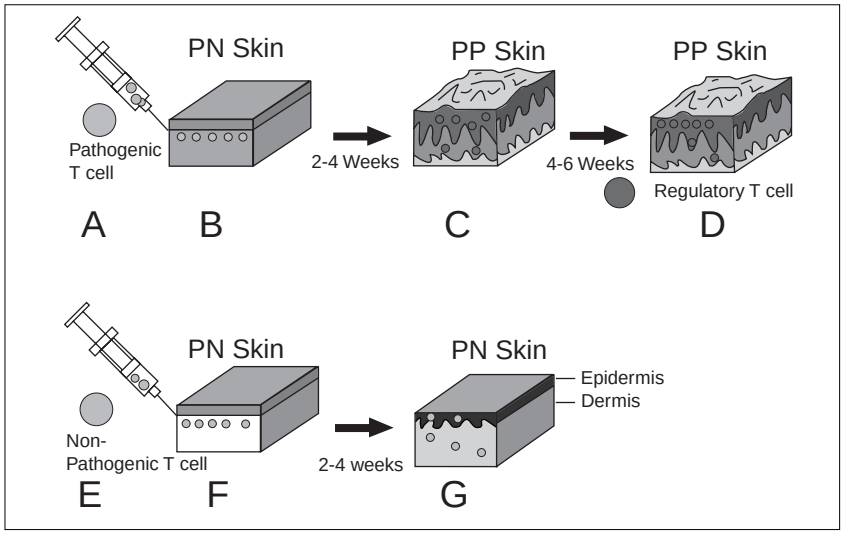

FIGURE 4. SCID mouse-based animal model can be used to determine whether a clone of T cells is either pathogenic or non-pathogenic on the basis of its ability to convert PN skin to PP skin.

Figure 4 depicts the value of this SCID mouse-based model in which pathogenic T cells can be distinguished from non-pathogenic T cells on the basis of their ability to convert PN skin to PP skin. In addition, this model may be used to isolate immunoregulatory T cells that may be dormant in PN skin, but become activated upon injection of pathogenic T cells. Being able to distinguish pathogenic versus non-pathogenic T cells provides investigators with an opportunity to determine the phenotype of a given clone of T cells, and ultimately may assist in furthering our understanding as to how a pathogenic T cell triggers psoriasis.

Finally, the potential importance of NK-T cells suggests a link between the innate immune system (i.e., NK cells) and the adaptive (i.e., acquired) immune system (i.e., T cells). The innate immune aspects may be important in combating bacterial, viral, and fungal infections and also provide a hint as to why psoriasis is so prevalent worldwide, and why it may have persisted for so many generations.[27] It has been observed that patients with psoriasis are less likely to suffer from infec-

tious assaults, and have lower incidence of skin cancer (even though many patients are exposed to carcinogens, mutagens, tumor promoters, and immunosuppressives).[6] Perhaps having enhanced numbers and function of NK-T cells, while predisposing to psoriasis, may at the same time be protective against deadly infections and neoplastic assaults. This is not to minimize the terrible pain and suffering that accompanies the lifelong presence of psoriatic plaques, but to provide a plausible (and, more importantly, testable) hypothesis that suggests that there may be a beneficial component to this complex, chronic, and enigmatic disorder of the immune system that manifests itself in the skin. In conclusion, much progress has been made in the past 15 years and it has become clear using the SCID mouse animal model that psoriasis is indeed a disease of the immune system.[28]

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