

The Role of Superantigens in Skin Disease

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***Staphylococcus aureus* and streptococci secrete a large family of exotoxins involved in the pathogenesis of toxic-shock-like syndromes and have been implicated in several autoimmune disorders. These toxins act as prototypic superantigens capable of binding to major histocompatibility complex proteins on antigen-presenting cells outside the antigen peptide-binding groove and can thereby stimulate cytokine release from macrophages. The superantigen-major histocompatibility complex unit is recognized primarily by the variable region of the T-cell receptor**

beta chain, and by engaging this region, can activate a large portion of the T-cell repertoire. It is thought that the capacity of these toxins to cause the massive stimulation of T cells and accessory cells such as macrophages, Langerhans cells, and activated keratinocytes accounts for most of their pathologic effects. The current review examines the evidence that implicates a role for these superantigens in the pathogenesis of certain skin diseases. *J Invest Dermatol* 105:37S-42S, 1995

BACKGROUND

The term "superantigen" was first coined by White *et al* [1] in 1989 and refers to a group of microbial antigens that differ in several respects from conventional peptide antigens (reviewed in [2,3]). Similar to peptide antigens, superantigens are presented by class II major histocompatibility complex (MHC) molecules. However, they do not engage the MHC peptide-antigen binding groove. Instead, the intact (unprocessed) superantigen binds to conserved amino acid residues that are on the outer walls of the peptide-antigen binding groove. Thus, whereas recognition of peptide antigens by the T-cell receptor (TCR) is restricted by MHC alleles, recognition of superantigens is generally not MHC restricted. More important, the recognition of superantigens by TCRs depends predominantly on the variable domain of the TCR beta chain (V β). Conventional peptide antigens require recognition by all five variable elements (i.e., V β , D β , J β , V α , J α) of the TCR. Therefore, the responding frequency to a conventional antigen is usually much less than one in 1000. In contrast, because the relative number of V β genes is restricted, the frequency of T cells responding to these molecules exceeds that of conventional peptide antigens by several orders of magnitude, hence their name "superantigens."

During the past 5 years, there has been an explosion of information regarding the biochemical and immunologic properties of superantigens. Furthermore, an increasing number of bacterial-, mycoplasmal-, and viral-derived proteins have been identified as superantigens [2,3]. The potential role of superantigens in human disease, particularly with regard to the skin, is of considerable interest. However, because of the complexities of studying cause-and-effect relations in the clinical setting, information on the role of

superantigens in mediating human disease remains limited. The current review examines the immunologic effects of superantigens, particularly as they may relate to the skin.

IMMUNOBIOLOGY OF SUPERANTIGENS IN THE SKIN

The unique capacity of superantigens to bind directly to MHC class II molecules on the one hand, and to stimulate a large fraction of T cells expressing relevant TCR V β chains on the other hand, provides a number of immune mechanisms by which these potent molecules can participate. As shown in Table I, staphylococcal enterotoxins (SE) and streptococcal exotoxins have been demonstrated to act as prototypic superantigens [4]. These superantigens have been demonstrated to act as potent inducers of interleukin (IL)-1 and tumor necrosis factor (TNF)- α from monocytes [5]. SE-mediated stimulation of monocytes is a consequence of binding and transducing a positive signal through MHC class II molecules on monocytes [6,7]. Although SEs bind to class II molecules in an unrestricted manner, human leukocyte antigen (HLA)-DR alleles differ in their ability to present SEs to T cells [8].

Recently, several laboratories have examined the ability of epidermal Langerhans cells and/or keratinocytes to act as accessory cells in the T-cell response to staphylococcal superantigens. Murine splenic T cells were stimulated with staphylococcal enterotoxin B (SEB) and exfoliative toxin in the presence of Langerhans cells or epidermal cells isolated from mice pretreated with recombinant interferon (IFN)- γ , a procedure that induces the expression of MHC class II molecules on keratinocytes [9]. Similarly, cultured human keratinocytes that have been induced *in vitro* to express MHC class II molecules on their cell surface by IFN- γ acquire the ability to activate T cells in the presence of superantigens [10,11]. This activation was demonstrated to require direct contact between keratinocytes and T cells, because monoclonal antibodies to human HLA-DR, HLA-DQ, intercellular adhesion molecule-1 (ICAM-1), and lymphocyte function-associated antigen-1 (LFA-1) blocked SEB-induced T-cell stimulation. Of interest, unlike Langerhans cells, keratinocytes bearing MHC class II are unable to present conventional peptide antigens to resting T cells. These findings,

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Abbreviations: APC, antigen-presenting cell(s); SE, staphylococcal enterotoxins; SPE, streptococcal exotoxins; TCR, T-cell receptor; V β , variable region of the TCR β chain.

Table I. Human TCR V β Specificities of Bacterial Superantigens^a

Bacterial Exotoxin	TCR V β Specificity
SEA	1.1,5,6,7,3-7.4,9.1
SEB	3,12,14,15,17,20
SEC1	3,6,4,6,9,12,15
SED	5,12
SEE	5.1,6,8,12
TSST-1	2
Exfoliative toxin	2
SPEA	8,12,14,15
SPEB	2,8
SPEC	1,2,5,1,10
Mycoplasma arthritis mitogen	3,17

^a Reviewed in [3].

however, suggest that under certain pathologic conditions, e.g., during *S. aureus* or streptococcal skin infections, class II (+) keratinocytes can serve as accessory cells.

These recent studies have also demonstrated that murine and human keratinocytes can release cytokines with T-cell-activating activities after exposure to staphylococcal toxins. In particular, the production of TNF- α , but not IL-1 α , was enhanced by SEB [9]. The capacity of keratinocytes to release cytokines in response to superantigenic stimulation is of interest because it is well established that T-cell differentiation is influenced by the cytokines released during antigen-driven stimulation. In this regard, Goodman *et al* [12] compared the profile of cytokines produced by T cells stimulated by SEB in the presence of MHC class II (+) keratinocytes with the response seen using dermal dendritic cells. T cells stimulated in the presence of dermal dendritic cells produced the Th1 cytokines IL-2 and IFN- γ , whereas T cells stimulated in the presence of activated keratinocytes produced only the Th2 cytokine IL-4. This dichotomy in T-cell responses appears to relate to the inability of keratinocytes to produce IL-12 after SEB stimulation. More important, these observations suggest that the nature of the primary T-cell response can be determined by the accessory cell used for antigen presentation.

Studies in experimental animals have demonstrated that superantigens have three principal *in vivo* effects on T cells bearing the relevant V β elements [2,3]. First, exposure of developing thymocytes to superantigens causes intrathymic deletion of T cells of appropriate V β specificity, with resultant skewing of the T-cell repertoire. Second, exposure of peripheral T cells to superantigens results in profound activation and lymphocytosis of T cells of appropriate V β specificity, and under certain conditions, this may be followed in time by anergy and/or deletion of the stimulated T-cell clones. Finally, exposure of peripheral T cells to superantigens can have profound physiologic consequences, related largely to massive cytokine release by T cells and antigen-presenting cells (APC). The particular response of T cells to superantigens depends on a variety of factors, including their developmental and activation state as well as the accessory signals provided by the superantigen-presenting cell. The extent to which these observations, mainly achieved in experimental animal models, relate to actual human diseases remains to be clarified. Nevertheless, the marked immunomodulatory effects of superantigens on T cells has led to widespread speculation that these molecules may provide the basis by which microbes may mediate a variety of diseases associated with immunodeficiency, autoimmunity, and/or inflammation (reviewed in [3]).

IDENTIFICATION OF SUPERANTIGENS IN HUMAN DISEASE

The role of superantigens in immune-mediated human disease is an area of intense investigation. Superantigens are thought to play a role in the pathogenesis of a number of systemic disease conditions

in which the development of skin inflammation is a prominent feature. These include staphylococcal toxic shock syndrome (TSS) and Kawasaki syndrome (KS). Before considering superantigens in primary skin diseases, it is instructive to review the data that have been used to support a role for superantigens in staphylococcal TSS and KS, as they provide potential criteria that may be useful to establish a cause-and-effect relation between superantigens and T-cell-mediated diseases.

Staphylococcal TSS TSS is an acute disease characterized by fever, rash, shock, and multiorgan involvement [13]. More than 90% of *S. aureus* strains isolated from menstruation-related cases produced TSS toxin-1 (TSST-1). The absence of specific antibody to TSST-1 appears to be a major risk factor for acquiring the disease. In nonmenstrual cases, TSST-1 is implicated in less than 50% of cases, and SEB and SEC production plays a more important role. Chio *et al* [14] demonstrated that the majority of T cells stimulated by TSST-1 in culture specifically expressed V β 2. Based on these *in vitro* findings, studies were carried out to determine the T-cell repertoire in patients with TSS. The majority of patients with TSS were found to have a marked and selective expansion of V β 2+ T cells. Serial evaluation of patients during the convalescent phase of their illness showed a gradual return of V β 2 levels toward normal, suggesting that the increase in V β 2+ T cells occurs after the onset of TSS.

The mechanisms by which TSST-1 produces the manifestations of TSS are unknown. Cytokines such as TNF- α have been implicated as a cause of shock. This cytokine is released in cultures of monocytes after the addition of TSST-1, and elevated levels of TNF- α have been documented during acute disease [5]. However, studies in animals also support an important role for T cells in superantigen-mediated lethal shock. Pretreatment of mice with D-galactosamine leads to the development of lethal shock when the mice are challenged with low doses of either lipopolysaccharide or SEB. Shock in both cases can be shown to be mediated by TNF. It is interesting that the lethal shock triggered by SEB was shown to be mediated by T cells [15]. In contrast, endotoxin (lipopolysaccharide)-induced shock appeared to be independent of T cells.

Consistent with a role for T cells in the pathogenesis of shock, shock-like syndromes have been reported in cancer patients being given large amounts of T-cell-derived IL-2 [16] and in allograft recipients being treated with anti-CD3 monoclonal antibodies [17]. Taken together, the observations of T-cell-dependent shock in animals injected with superantigen, the massive stimulation of V β 2+ T cells during TSS, and the isolation of TSST-1-, SEB-, or SEC-secreting *S. aureus* from patients with TSS support the concept that superantigens play an important role in the pathogenesis of this disease.

Kawasaki Syndrome KS is an acute multisystem vasculitis that primarily affects infants and young children (reviewed in [18]). The primary clinical features of acute KS include high fever, nonexudative conjunctival injection, inflammation of the oropharynx, erythema and edema of the hands and feet, polymorphous rash, and cervical lymphadenopathy. Although acute KS is generally self limited, coronary artery abnormalities due to vasculitis can develop in up to 25% of untreated patients. In Japan and the United States, KS has become one of the most common causes of acquired heart disease in children. Treatment of acute KS with high doses of intravenous gamma globulin significantly decreases the occurrence of coronary artery abnormalities.

The etiology of KS remains unknown. However, it is widely believed that KS is caused by an infectious agent because of the acute self-limited nature of this disease, its seasonal incidence, the geographic clustering of outbreaks, the clinical symptoms of fever and exanthem mimicking bacterial toxin-mediated infectious diseases, and the unique susceptibility of infants and young children. The observation that children younger than 6 months or older than 8 years rarely develop acute KS suggests that the lack of a protective antibody against the putative KS agent may be an important risk factor for development of this disease.

A number of studies have demonstrated that the acute, but not the convalescent, phase of KS is associated with marked activation of T cells and monocytes/macrophages (reviewed in [19]). Furthermore, successful treatment of KS with intravenous gamma globulin is associated with a significant reduction in immune activation [20]. Based on these immunologic features, which are characteristic of diseases caused by bacterial toxins acting as superantigens, studies were conducted on the T lymphocytes of patients with acute KS for evidence of selective TCR V β gene usage. In a study from our laboratory, peripheral blood mononuclear cells from 19 patients with acute KS were analyzed for TCR V β gene expression. Patients with KS demonstrated elevated levels of V β 2+, and to a lesser extent V β 8.1+ T cells compared with cells from normal donors and from controls with other febrile illnesses [21,22]. During convalescence, the proportions of V β 2+ and V β 8.1+ T cells returned to normal levels. None of the other TCR V β populations were found to be elevated. These results are consistent with the hypothesis that the immune stimulation of KS is triggered by a bacterial toxin with superantigenic activity.

As noted above, superantigens stimulate T cells primarily through the V β portion of the TCR and therefore induce an expansion of T cells independent of the TCR β -chain junctional or CDR3 (diversity) regions. To determine whether V β 2+ and V β 8+ T cells were expanded in a superantigen-like (polyclonal) as opposed to an oligoclonal manner in acute KS, sequences of random cDNA clones containing V β 2 and V β 8 gene segments were analyzed [22]. In patients with a two- to threefold increase in V β 2 gene expression, one might expect that up to 60% of the sequences would reflect the abnormally expanded T cells. However, none of the V β 2 sequences were found to be identical to another. These data indicate that in the expansion of V β 2+ T cells in acute KS, V β itself plays the dominant role in recognition. Together with the high frequency of responding V β 2+ T cells in the circulation, which is disproportionate to that expected for a response to a conventional antigen, the results appear to be most consistent with the hypothesis that T-cell activation during acute KS is mediated by a superantigen.

To address the hypothesis that a bacterial toxin might be the cause of the superantigenic immune activation, Leung *et al* [23] carried out the following study. Skin (groin, axilla) and mucous membrane (rectum, throat) cultures were obtained from 16 patients with acute KS and 15 control patients with fever and/or rash. All group A β -hemolytic streptococci and all coagulase-positive *S. aureus* isolates were screened for toxin production in a blinded fashion. Bacteria-producing superantigens were found in 13 of 16 KS patients, but in only one of 15 control patients ($p < 0.0001$). Eleven of 13 toxin-positive cultures from patients with KS were due to TSST-elaborating *S. aureus*, and two of 13 were due to streptococci-producing pyogenic exotoxins B and C. Each of these toxins is known to possess V β 2 stimulatory activity.

Of interest, the toxin-producing *S. aureus* from the 11 KS patients were unusual because they produced less lipase, hemolysin, and protease than conventional *S. aureus* and because the bacteria were white rather than the customary gold appearance. These findings suggest that the immune activation associated with the acute phase of KS may indeed be caused by a novel strain of *S. aureus* or to a lesser extent by streptococci that colonize mucous membranes and elaborate toxins with superantigenic activity. Furthermore, the observation that the different bacterial toxins associated with KS are potent activators of V β 2+ T cells suggests an important role for this T-cell subset in the pathogenesis of this disease.

The capacity of bacterial exotoxins (superantigens) to bind to MHC class II molecules on monocytes/macrophages or to stimulate T cells bearing particular TCR V β s provides several mechanisms by which a putative superantigen could cause acute KS. First, exotoxins secreted at the inflamed mucosal surface could be absorbed and engage HLA-DR on local or circulating macrophages to stimulate the production of IL-1 β and TNF- α , which both have potent proinflammatory properties, and thus contribute to vascular inflammation. Second, as in TSS, T-cell stimulation may be critical

for cytokine release, with the subsequent induction of adhesion molecules that localize inflammatory cells to the vascular wall, and may thus play an important role in the pathogenesis of vasculitis in acute KS. Third, superantigens could induce autoreactive T cells that recognize vascular antigens and are involved in the more persistent manifestations of KS.

SUPERANTIGENS AND SKIN DISEASES

Several primary skin disorders are associated with the presence of bacteria, in particular *S. aureus* and streptococci, that produce superantigenic toxins. These include staphylococcal scalded-skin syndrome, in which exfoliation of the skin occurs. Exfoliation is primarily due to the separation of granular-layer keratinocytes, possibly by binding of exfoliative toxin to desmoglein I [24]. In addition, it is well known that bacterial infection can exacerbate a variety of chronic inflammatory skin diseases such as atopic dermatitis and certain forms of psoriasis. Such associations of skin disorders and superantigens have led a number of investigators to examine the immunomodulatory consequences of bacterial toxins in these patients.

Atopic Dermatitis Atopic dermatitis is a chronic pruritic inflammatory skin disorder characterized by the local infiltration of T cells and monocytes/macrophages (reviewed in [25]). Several observations suggest that the expansion of T-helper type 2 (Th2) cells and allergen sensitization play a role in the pathogenesis of this disease. First, the majority of patients with atopic dermatitis have a personal or family history of atopic disease, e.g., asthma or allergic rhinitis. Second, serum IgE levels are elevated in about 80% of patients with atopic dermatitis, and their serum IgE levels often correlate with the extent of skin disease. Third, most patients with atopic dermatitis produce IgE to various food and inhalant allergens, and double-blind, placebo-controlled food challenge studies have demonstrated that food allergies can trigger cutaneous reactions in a subset of children with atopic dermatitis. Fourth, mite-specific Th2-type lymphocytes have been cloned from the peripheral blood and skin lesions of atopic dermatitis patients with IgE-specific antibody to dust mites [26,27]. More recently, *in situ* hybridization studies have demonstrated that acute atopic dermatitis skin lesions express increased levels of IL-4 and IL-5, but not IFN- γ mRNA [28].

Although food- and inhalant-induced eczema has been reported in this disease, it has been argued that such reactions may involve only a minority of patients with atopic dermatitis. Numerous reports have shown that *S. aureus* can exacerbate this skin disease. Leyden *et al* [29] first demonstrated that *S. aureus* could be isolated from the affected skin of more than 90% of patients with atopic dermatitis. By quantitative methods, the density of *S. aureus* on inflamed atopic dermatitis lesions can reach up to 10^7 colony-forming units/cm² [30]. Clinically, it is also well known that patients with chronic atopic dermatitis show a better clinical response to combined treatment with antistaphylococcal antibiotics and topical corticosteroids than to corticosteroids alone [31].

To study potential mechanisms by which *S. aureus* can exacerbate atopic dermatitis, Leung *et al* [32] characterized *S. aureus* from the skin of patients with atopic dermatitis for their capacity to secrete toxins known to have superantigenic properties. More than half of atopic dermatitis patients secreted identifiable toxins, primarily SEA, SEB, and TSST-1. The capacity of SEs to bind to MHC class II molecules and to stimulate T cells bearing particular TCR V β s suggests several mechanisms by which *S. aureus* could exacerbate atopic dermatitis. First, SEs secreted at the skin surface could penetrate inflamed skin and engage HLA-DR on epidermal macrophages or Langerhans cells to stimulate the production of IL-1 and TNF- α . IL-1 and TNF- α have potent proinflammatory properties, which contribute to skin inflammation. Second, SEs could stimulate a high proportion of T cells via their TCR V β to proliferate and secrete cytokines, which modulate tissue inflammation. Indeed, Th2 cells expanded *in vitro* with superantigens have been demonstrated to induce inflammation when injected into the

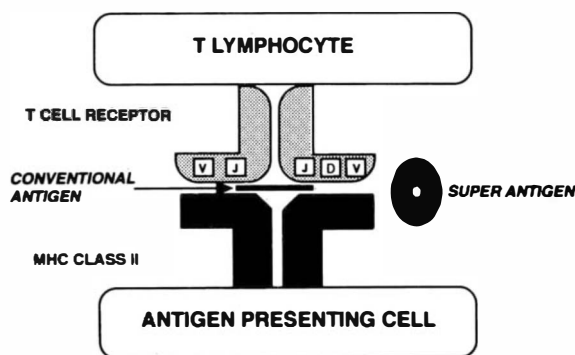


Figure 1. Antigen presentation to T lymphocytes.

skin of naive animals [33]. Furthermore, preliminary studies from our laboratory indicate that acute atopic dermatitis lesions can be associated with the selective expansion of T cells expressing specific TCR V β gene segments.

Because SEs are globular proteins of 24–30 kD, the possibility that they could act as allergens was also investigated [32]. In this regard, it has been found that nearly half of patients with atopic dermatitis produce IgE directed to staphylococcal toxins, particularly SEA, SEB, and TSST-1. To study the potential functional role of IgE antistaphylococcal toxins, we investigated the capacity of SEs to induce histamine release from freshly isolated basophils of normal individuals and patients with AD. Basophils from normal controls and atopic dermatitis patients who lacked IgE antitoxin failed to release any histamine on exposure to the various staphylococcal exotoxins. In contrast, basophils from atopic dermatitis patients who produced IgE antitoxin released histamine on exposure to the relevant exotoxin, but not in response to exotoxins in which there is no IgE response [32]. These findings suggest the possibility that local production of staphylococcal exotoxins at the skin surface could cause IgE-mediated histamine release and thereby trigger the itch-scratch cycle that can exacerbate atopic dermatitis.

Psoriasis Immune activation, particularly of T cells, is thought to play a critical role in the pathogenesis of psoriasis. This concept is supported by numerous observations. First, histologic examination of early skin lesions has demonstrated that the epidermal hyperplasia that is a key histologic feature of psoriasis is preceded by the infiltration of T lymphocytes and macrophages into the skin [34]. In support of a key role for T cells in the pathogenesis of psoriasis, T-lymphocyte clones grown from psoriatic lesions have been reported to release growth factors that induce keratinocyte proliferation [35]. The cytokine release into lesional and lesion-free psoriatic skin has been found to be a T-helper type 1 (Th1) cell-mediated response, i.e., increased expression of IL-2, IFN- γ , and TNF- α [36]. Second, certain subtypes of psoriasis exhibit a strong association with particular HLA types [37]. Third, immunosuppressive drugs that inhibit T-cell activation and cytokine secretion such as anti-CD3, corticosteroids, and cyclosporin A are effective in treating psoriasis [38].

The release of cytokines as the result of ongoing cellular immune responses is thought to contribute to the formation and persistence of psoriatic plaques. Localized collections of HLA-DR+ keratinocytes are seen in psoriatic plaques [39] and likely reflect a response to the increased production of IFN- γ by Th1 cells. Other cytokines that mediate skin inflammation and vascular endothelial adhesion molecule expression, such as IL-1 β , IL-6, IL-8, and TGF α , have also been reported to be increased in psoriatic plaques [40].

The mechanisms by which keratinocytes, T cells, and monocytes/macrophages achieve and maintain their activated state in psoriatic skin lesions are poorly understood. Although this process is multifactorial, there are a number of reports suggesting that bacterial infection can trigger psoriasis [41–43]. In this regard, 50%

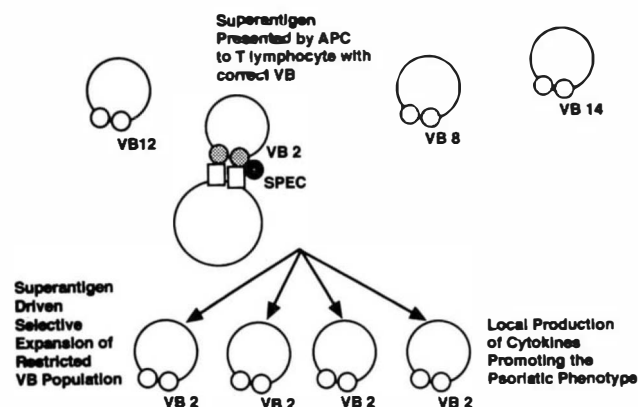


Figure 2. Proposed mechanism for local activation by superantigens of restricted population(s) of T lymphocytes in psoriasis.

of patients with psoriasis harbor *S. aureus* on their skin [42]. Furthermore, in patients with guttate psoriasis, it has been reported that acute exacerbation is preceded by throat infection with *Streptococcus pyogenes* and by increases in serum antistreptococcal titers [43]. Patients with this form of psoriasis frequently improve with systemic antibiotic therapy.

In support of these clinical observations, Colè and Wuepper [44] first reported that alcohol precipitates of cell-free culture filtrates from *Streptococcus pyogenes* (strain NY-5) induced increased keratinocyte proliferation after intradermal injection into rabbit skin. These culture filtrates also had marked mitogenic activity upon human cultured lymphoid cells and are now known to have contained high levels of the streptococcal exotoxins SPEA, SPEB, and SPEC. Subsequently, it was reported that psoriatic lesions could be induced by intradermal injection of small amounts of killed streptococcal material [45]. Furthermore, *in vitro*, both guttate and chronic plaque psoriasis have been shown to have enhanced T-lymphocyte responses to group A streptococcal antigens [46].

These observations suggested the hypothesis that bacterial toxins derived from *S. aureus* and streptococci, acting as superantigens, could mediate the activation of epidermal HLA-DR+ keratinocytes as well as infiltrating T cells and monocytes in at least a subset of patients with psoriasis. As illustrated in Figs 1 and 2, this selective expansion of T-cell populations locally could lead to the release of cytokines, which promote keratinocyte proliferation, endothelial cell activation, and other features of psoriasis. This superantigen-driven T-cell activation might be involved in both the induction and persistence of psoriatic lesions (Fig 3).

With respect to the induction of psoriatic lesions, superantigen(s) from epicutaneous sources or from a circulating source could bind to MHC class II bearing APC in the dermis or epidermis (Langer-

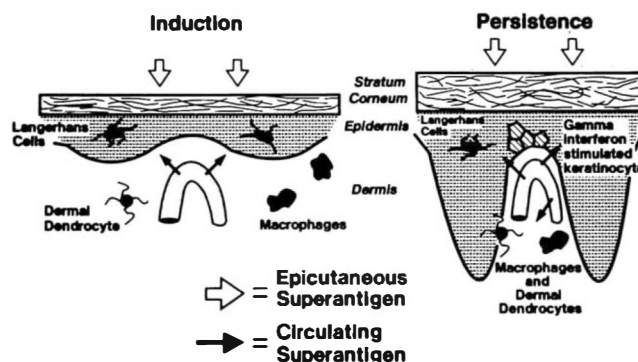


Figure 3. Potential populations of MHC class II-positive APC in either the induction or persistence of psoriasis.

hans cell, dermal dendrocyte, dermal macrophage). If the appropriate V β -bearing T lymphocyte were present, APC/lymphocyte interaction could lead to T-cell activation by superantigen. T-cell proliferation, cytokine release, and the characteristic psoriatic phenotype would then ensue (Fig 2). On the other hand, in the persistence of psoriatic lesions, MHC class II-positive keratinocytes might also be added to the list of potential APC that could facilitate T-cell activation by superantigen (Fig 3). It is unlikely, however, that in a complex immune reaction such as psoriasis, keratinocytes would represent the only APC involved in T-cell activation. T lymphocytes recruited into an ongoing psoriatic lesion would then be subject to activation if a superantigen capable of binding to their V β chain were presented by appropriate MHC class II-bearing APC.

As a proof of this concept, it is important to determine whether certain subtypes of psoriasis are associated with the selective expansion of T cells bearing particular V β gene segments characteristic of superantigenic stimulation. Furthermore, bacteria grown from these patients, especially during a flare of their disease, should secrete toxins known to act as superantigens. The demonstration that selective expansion of TCR V β s in the skin infiltrate reflects the superantigen secreted at the skin surface or in the patient's pharynx would offer strong evidence that the bacteria are truly pathogenic and the immune activation is superantigen driven.

It should be noted that a previous study of the TCR repertoire in skin lesions of patients with chronic nonpustular psoriasis did not demonstrate any specific selection of a single V region [47]. However, this may have reflected the use of a limited number of monoclonal anti-V β s or the recruitment of bystander T cells not involved in the initial recognition of the inciting antigen. Because the acute exacerbation of guttate psoriasis has clearly been demonstrated to be triggered by streptococcal infections, it is of considerable interest to determine the potential role of streptococcal superantigens in the pathogenesis of this form of psoriasis.

In a recent report, Lewis *et al* [48] enumerated T lymphocytes in peripheral blood and skin biopsy specimens from patients with guttate or chronic plaque psoriasis for the expression of nine TCR V β gene families, using a range of monoclonal antibodies. A selective expansion of V β 2+ T lymphocytes was observed in the skin lesions of patients from both psoriatic groups as compared with T lymphocytes in their peripheral blood. A less marked dermal increase in V β 5.1+ T cells was also observed in these patients. Recently, we have also analyzed skin specimens from patients with guttate psoriasis and found a marked increase in the percentage of V β 2+ T cells in the skin of these individuals (Leung and Kotb, unpublished observations). Furthermore, analysis of streptococcal isolates from the pharynx of five patients with guttate psoriasis revealed that all secreted SPEC, a toxin (superantigen) known to stimulate the selective expansion of V β 2+ T lymphocytes (Leung and Schlievert, unpublished observations). These data strongly support the concept that guttate psoriasis is triggered by a superantigen, most likely SPEC.

Of interest, other V β families have also been reported to be elevated in the T-cell skin infiltrate of selected patients with psoriasis. For example, in the study by Lewis *et al* [48], two of the 10 patients with guttate psoriasis showed an increase in V β 12 in lesional skin compared with that in peripheral blood T cells. Conversely, in three of eight patients with chronic plaque psoriasis, an increase in the number of V β 8+ T cells was observed. This latter increase was not observed in guttate psoriasis. The observation that patients with psoriasis can demonstrate divergent T-cell responses suggests that different antigens can trigger this disease.

Indeed, in an earlier study by Leung *et al* [49] of two patients with plaque psoriasis triggered by infection, each demonstrated a different pattern of V β expansion as a response to the particular microbial antigens expressed during their infection. Cultures from the psoriatic skin lesion of patient 1, who had increased V β 5.1 and V β 12 expression in her skin infiltrate, grew *S. aureus* secreting the superantigen SEB. In contrast, the skin lesion from patient 2, with increased expression of V β 5.1 and V β 8, grew *Candida albicans*. SEB

is known to stimulate T cells *in vitro* to express the V β 12 but not the V β 5.1, 5.2, 6.7, or 8.1 gene segments [11]. In contrast, *C. albicans* stimulates V β 5.1 and V β 8.1 in delayed-type hypersensitivity skin reactions, but not V β 5.2, 6.7, or 12. Thus, the TCR V β skewing observed in the local infiltrate of these patients showed some correlation with the superantigen being secreted at their skin surface. The observation that the V β skewing did not correspond exactly with what is seen when purified superantigen is introduced into the skin or peripheral blood was not surprising, because T-cell activation in the local psoriatic lesion may be influenced by a variety of exogenous and endogenous antigens.

Taken together, superantigen-driven T-cell activation might contribute to the heterogeneity in psoriasis on two different levels. First, reactions to different superantigens may produce lesions with different V β patterns. Second, reactions to particular superantigens may be relatively genetically restricted. Although most MHC class II haplotypes are able to present superantigens, different alleles vary in the ability to present particular superantigens [8]. Hence, the development of particular types of psoriasis may be favored by MHC class II haplotypes associated with preferential presentation of particular superantigens.

These data suggest that superantigen-driven psoriasis is one of many potential mechanisms of T-cell activation in psoriasis. The pattern of activation would be expected to be different among different patients. Further studies will be needed to determine whether TCR skewing is seen in the acute lesion of all forms of psoriasis or whether it is restricted to the subset of psoriasis associated with infection. Nevertheless, the current observations provide a new direction for investigating the pathogenesis of this fascinating skin disorder.

CONCLUSIONS

Cutaneous effects of bacterial toxins may be categorized into at least three types. The first type is the superantigen reaction, in which skin-infiltrating T cells bearing particular V β elements respond to the toxins presented by macrophages, Langerhans cells, and/or class II+ keratinocytes. A second type is the capacity of toxins to affect directly the production by skin cells of cytokines and/or mediators of inflammation. Among such cells, keratinocytes are quantitatively the major population, and keratinocytes produce cytokines with T-cell-proliferative activity in response to bacterial toxins. The third type of toxin-mediated intracutaneous activity involves nonimmune actions, e.g., the capacity of exotoxins such as exfoliative toxin to induce intercellular separation of granular-layer keratinocytes. Other examples of toxin effects that are nonsuperantigenic include the production of vomiting and diarrhea by SEs in humans and primates. In these experiments, the enterotoxigenic effects of SEB could be separated from its T-cell-stimulatory properties by carboxymethylation of the SEB [50].

Because of its critical location, the skin is constantly exposed to a wide variety of potentially harmful environmental agents, including bacteria. Several skin disorders are associated with the presence of bacteria, in particular *S. aureus* and streptococci, that produce superantigenic toxins. It is likely that many of the pathologic effects of these toxins are due to their ability to activate T cells, macrophages, Langerhans cells, and/or keratinocytes. Identification of the antigens or superantigens that trigger particular skin diseases, such as psoriasis or atopic dermatitis, may allow researchers to identify earlier events in the pathogenesis of these skin conditions and to develop new strategies to cope with the aberrant responses to these triggers.

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REFERENCES

- White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marrack P: The V β -specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 56:27-35, 1989
- Möller G: Superantigens. *Immunol Rev* 131:1-200, 1993
- Kotzin BL, Leung DYM, Kappler J, Marrack P: Superantigens and human disease. *Adv Immunol* 54:99-166, 1993
- Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. *Science* 248:705-711, 1990
- Fast DJ, Schlievert PM, Nelson RD: Toxic shock syndrome-associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. *Infect Immunol* 57:291-296, 1989
- Grossman D, Cook RG, Sparrow JT, Mollick JA, Rich RR: Disassociation of the stimulatory activities of staphylococcal enterotoxins for T cells and monocytes. *J Exp Med* 172:1831-1841, 1990
- Sperduti F, Spits H, Geha RS: Staphylococcal toxins deliver activation signals to human T-cells via major histocompatibility complex class II molecules. *Proc Natl Acad Sci USA* 88:7533-7537, 1991
- Herman A, Croteau G, Sekaly R-P, Kappler J, Marrack P: HLA-DR alleles differ in their ability to present staphylococcal enterotoxins to T cells. *J Exp Med* 172:709-717, 1990
- Tokura Y, Yagi J, O'Malley M, Lewis JM, Takigawa M, Edelson RL, Tigelaar RE: Superantigenic staphylococcal exotoxins induce T-cell proliferation in the presence of Langerhans cells or class II-bearing keratinocytes and stimulate keratinocytes to produce T-cell-activating cytokines. *J Invest Dermatol* 102:31-38, 1994
- Strange P, Skov L, Baadsgaard O: Interferon gamma-treated keratinocytes activate T cells in the presence of superantigens: involvement of major histocompatibility complex class II molecules. *J Invest Dermatol* 102:150-154, 1994
- Nickoloff BJ, Mitra RS, Green J, Zheng X-G, Shimizu Y, Thompson C, Turka LA: Accessory cell function of keratinocytes for superantigens: dependence on lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. *J Immunol* 150:2148-2159, 1993
- Goodman RE, Nestle F, Naidu YM, Green JM, Thompson CB, Nickoloff BJ, Turka LA: Keratinocyte-derived T cell costimulation induces preferential production of IL-2 and IL-4 but not IFN- γ . *J Immunol* 152:5189-5198, 1994
- Bohach GA, Fast DJ, Nelson RD, Schlievert PM: Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* 17:251-272, 1990
- Choi Y, Lafferty JA, Clements JR, Todd JK, Gelfand EW, Kappler J, Marrack P, Kotzin BL: Selective expansion of T cells expressing V β 2 in toxic shock syndrome. *J Exp Med* 172:981-984, 1990
- Miethke T, Wahl C, Heeg K, Echtenacher B, Krammer PH, Wagner H: T cell-mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. *J Exp Med* 175:91-98, 1992
- Beldegrun A, Webb DE, Austin HA, Steinberg SM, White DE, Linehan WM, Rosenberg SA: Effects of interleukin-2 on renal function in patients receiving immunotherapy for advanced cancer. *Ann Intern Med* 106:817-822, 1987
- Chatenoud L, Ferran C, Reuter A, Legendre C, Gevaert Y, Kreis H, Franchimont P, Bach JF: Systemic reaction to the anti-T-cell monoclonal antibody OKT3 in relation to serum levels of tumor necrosis factor and interferon-gamma. *N Engl J Med* 320:1420-1421, 1989
- Leung DYM: Kawasaki disease. *Curr Opin Rheumatol* 5:41-50, 1993
- Leung DYM: Immunologic aspects of Kawasaki syndrome. *J Rheumatol Suppl* 24:15-18, 1990
- Leung DYM, Burns J, Newburger J, Geha RS: Reversal of immunoregulatory abnormalities in Kawasaki syndrome by intravenous gamma globulin. *J Clin Invest* 79:468-472, 1987
- Abe J, Kotzin BL, Jujo K, Melish ME, Glode MP, Kohsaka T, Leung DYM: Selective expansion of T cells expressing T cell receptor variable regions V β 2 and V β 8 in Kawasaki disease. *Proc Natl Acad Sci USA* 89:4066-4070, 1992
- Abe J, Kotzin BL, Meissner C, Melish ME, Masato T, Fulton D, Romagne F, Malissen B, Leung DYM: Characterization of T cell repertoire changes in acute Kawasaki disease. *J Exp Med* 177:791-796, 1993
- Leung DYM, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM: Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* 342:1385-1388, 1993
- Resnick SD, Elias PM, Fritsch PO: The staphylococcal scalded skin and toxic shock syndromes. In: *Physiology, Biochemistry, and Molecular Biology of the Skin*. Oxford University Press, New York, 1991, pp 1287-1312
- Leung DYM: Immunology of atopic dermatitis. *Clin Rev Allergy* 11:447-470, 1993
- Van der Heijden F, Wierenga EA, Bos JD, Kapsenberg JL: High frequency of IL-4 producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 97:389-394, 1991
- Wierenga EA, Snoek M, de Groot C, Chrétien I, Bos JD, Jansen HM, Kapsenberg ML: Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. *J Immunol* 144:4651-4656, 1990
- Hamid Q, Boguniewicz M, Leung DYM: Differential cytokine gene expression in acute vs chronic atopic dermatitis. *J Clin Invest* 94:870-876, 1994
- Leyden JE, Marples RR, Kligman AM: *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 90:525-530, 1974
- Hauser C, Wuethrich B, Matter L, Wilhelm JA, Sonnabend W, Schopfer K: *Staphylococcus aureus* skin colonization in atopic dermatitis patients. *Dermatologica* 170:35-39, 1985
- Lever R, Hadley K, Downey D, Mackie R: Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. *Br J Dermatol* 119:189-198, 1988
- Leung DYM, Harbeck R, Bina P, Hanifin JM, Reiser RF, Sampson HA: Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis: evidence for a new group of allergens. *J Clin Invest* 92:1374-1380, 1993
- Müller KM, Jaunin F, Masouye I, Saurat JH, Hauser C: Th2 cells mediate IL-4-dependent local tissue inflammation. *J Immunol* 150:3254-3262, 1993
- Baadsgaard O, Fisher G, Voorhees JJ, Cooper KD: The role of the immune system in the pathogenesis of psoriasis. *J Invest Dermatol* 95:32S-34S, 1990
- Strange P, Cooper KD, Hansen ER, Fisher G, Larsen JK, Fox D, Krag C, Voorhees JJ, Baadsgaard O: T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. *J Invest Dermatol* 101:695-700, 1993
- Uyemura K, Yamamura M, Fivenson DP, Modlin RL, Nickoloff BJ: The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. *J Invest Dermatol* 101:701-705, 1993
- Baker BS, Swain AF, Fry L, Valdimarsson H: Epidermal T lymphocytes and HLA-DR expression in psoriasis. *Br J Dermatol* 110:555-564, 1984
- Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, Cooper KD, Baadsgaard O, Duell EA, Annesley TM: Cyclosporine improves psoriasis in a double-blind study. *JAMA* 256:3110-3116, 1986
- Kaplan G, Witmer MD, Nath I, Steinman RM, Laal S, Prasad HK, Sarno EN, Elvers U, Cohn ZA: Influence of delayed immune reactions on human epidermal keratinocytes. *Proc Natl Acad Sci USA* 83:3469-3473, 1986
- Krueger JG, Krane JF, Carter DM, Gottlieb AB: Role of growth factors, cytokines, and their receptors in the pathogenesis of psoriasis. *J Invest Dermatol* 94:135S-140S, 1990
- Henderson CA, Highet AS: Acute psoriasis associated with Lancefield group C and group G cutaneous streptococcal infections. *Br J Dermatol* 118:559-562, 1988
- Marples RR, Heaton CL, Kligman AM: *Staphylococcus aureus* in psoriasis. *Arch Dermatol* 107:568-570, 1973
- Whyte JH, Baughman RD: Acute guttate psoriasis and streptococcal infection. *Arch Dermatol* 89:350-356, 1964
- Cole GW, Wuepper KD: Isolation and partial characterization of a keratinocyte proliferative factor produced by *Streptococcus pyogenes* (strain NY-5). *J Invest Dermatol* 71:219-223, 1978
- Rosenberg EW, Noel PW: The Koebner phenomenon and the microbial basis of psoriasis. *J Am Acad Dermatol* 18:151-158, 1988
- Baker BS, Bokth S, Powles A, Garioch JJ, Lewis H, Valdimarsson H, Fry L: Group A streptococcal antigen-specific T lymphocytes in guttate psoriatic lesions. *Br J Dermatol* 128:439-499, 1993
- Schmitt-Egenolf M, Boehncke W-H, Christophers E, Stander M, Sterry W: Type I and type II psoriasis show a similar usage of T-cell receptor variable regions. *J Invest Dermatol* 97:1053-1056, 1991
- Lewis HM, Baker BS, Bokth S, Powles AV, Garioch JJ, Valdimarsson H, Fry L: Restricted T-cell receptor V β gene usage in the skin of patients with guttate and chronic plaque psoriasis. *Br J Dermatol* 129:514-520, 1993
- Leung DYM, Walsh P, Giorno R, Norris DA: A potential role for superantigens in the pathogenesis of psoriasis. *J Invest Dermatol* 100:225-228, 1993
- Alber G, Hanauer DK, Fleischer B: Relationship between enterotoxin- and T lymphocyte-stimulating activity of staphylococcal enterotoxin. *J Immunol* 144:4501-4506, 1990