

Cellular and immunologic mechanisms in atopic dermatitis

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Atopic dermatitis is a chronic inflammatory skin disease that is frequently associated with respiratory allergies. Atopic dermatitis develops as a result of a complex interrelationship of environmental, immunologic, genetic, and pharmacologic factors. Efforts to understand the relative contributions of these factors have led to research seeking to identify the relevant effector cells and mediators involved in the pathogenesis of atopic dermatitis. These factors include the pattern of local cytokine release, the differentiation of helper T cells, multiple roles of IgE, skin-directed cell responses, infectious agents, and superantigens. This article reviews these cellular and immunologic mechanisms underlying atopic dermatitis and discusses how an understanding of their role in the inflammatory process may lead to improved treatments for atopic dermatitis. (J Am Acad Dermatol 2001;44:S1-12.)

Atopic dermatitis (AD) is a highly pruritic, recurring inflammatory skin disease. It usually develops in early childhood and is frequently seen in children with a personal history of respiratory allergy and/or a family history of atopic disease.^{1,2} The prevalence of AD has increased in recent years, and it is now estimated to affect up to 20% of the general population.^{3,4} The term *atopic dermatitis* suggests a close association between AD and respiratory allergy, although there has been considerable debate over whether AD is primarily an allergen-induced disease or simply an inflammatory skin disorder found in association with respiratory allergy. However, recent data strongly suggest that AD is indeed an allergic disorder with important immunologic parallels to asthma.

Overall, atopy can be defined as a familial hypersensitivity of skin and mucous membranes against

Abbreviations used:

AD:	atopic dermatitis
cAMP-PDE:	cyclic adenosine monophosphate-phosphodiesterase
CD:	cluster differentiation
CLA:	cutaneous lymphocyte-associated antigen
GM-CSF:	granulocyte-macrophage colony-stimulating factor
IFN:	interferon
IgE:	immunoglobulin E
IL:	interleukin
LPR:	late phase reaction
PBMC:	peripheral blood mononuclear cell
PDE:	phosphodiesterase
PGE2:	prostaglandin E ₂
TNF:	tumor necrosis factor

environmental substances, which is associated with increased IgE production and/or altered nonspecific reactivity in the skin of patients with AD and in the lungs of patients with asthma. Allergic reactions appear to play a role in some, but not all patients. A small subgroup (<30%) of patients with AD has a negative history of allergic diseases, normal levels of IgE, an absence of specific IgE antibodies, and no immediate-type hypersensitivity skin reactions.⁵ In many patients factors such as a disturbance of skin function, infection, and stress appear to be more important than allergic reactions. Immunologic disturbances are reflected in the increased IgE production and T-cell dysregulation observed in AD. Altered nonspecific reactivity is reflected in increased secre-

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tions from mediator cells and bronchial, nasal, and skin hyperreactivity.

An understanding of the immunologic basis of AD may have important implications for clinical management of the disease. The skin is readily accessible for tissue analysis, and so information obtained regarding the immunopathogenesis of AD may provide important insights into potential mechanisms that contribute to chronic tissue inflammation in other allergic diseases such as asthma. This article reviews the immunopathogenesis of AD, as well as factors contributing to T-cell differentiation and the role of IgE and infectious agents in the development of AD.

SKIN REACTION PATTERNS IN AD

The most notable features of AD are cutaneous reactivity and intense pruritus leading to scratching. Scratching may be present throughout the day, but is usually worse in the early evening and at night, and often leads to disruption of normal sleep patterns in patients with chronic AD. The pathogenesis of cutaneous pruritus is not well understood. Pruritus can be induced experimentally by intradermal injection of various products of inflammatory effector cells such as histamine, neuropeptides, leukotrienes, and proteolytic enzymes. Pruritus and scratching can be exacerbated by allergens, reduced humidity, excessive sweating, and irritants such as wool, acrylic, soaps, and detergents. Patients with active AD have significantly greater irritant skin responses to graded dilutions of sodium lauryl sulfate than patients with inactive AD and respiratory allergy, who in turn have increased irritancy compared with normal control subjects.⁶ Thus "atopic skin" is usually associated with a lowered threshold of responsiveness to irritants.⁷

The various stages of AD are characterized by different types of skin lesions. Acute skin lesions are characterized by intensely pruritic, erythematous papules over erythematous skin. These are associated with extensive excoriations, erosions, and serous exudate. Subacute dermatitis is associated with erythematous, excoriated, scaling papules. Chronic dermatitis is characterized by thickened skin, accentuated skin markings (lichenification), and fibrotic papules. In chronic AD, all 3 types of skin reactions frequently coexist in the same individual. The skin distribution pattern changes with age. During infancy and early childhood AD is usually more acute and mostly involves the face, scalp, and extensor surface of the extremities. In adolescents and adults the skin becomes lichenified and lesions occur mainly in the flexural folds of the extremities.

IMMUNOPATHOGENESIS OF AD

Support for the immunologic basis of AD comes from observations that patients with primary T-cell

immunodeficiency disorders frequently have elevated serum IgE levels, eosinophilia, and eczematous skin lesions that are indistinguishable from AD. AD is likely mediated by bone marrow-derived cells, as suggested by the observation that in patients with Wiskott-Aldrich syndrome, resolution of their skin lesions occur after successful bone marrow transplantation to correct the immunologic defect.⁸ In addition, nonatopic recipients of bone marrow transplants from atopic donors have been reported to develop positive immediate skin tests and atopic symptoms after successful engraftment.⁹

Peripheral blood abnormalities

Several peripheral blood abnormalities have been noted in patients with AD, which reflect some of the cellular and biochemical changes observed in atopic skin. These abnormalities include increased IgE levels, eosinophilia, chronic macrophage activation (with increased secretion of granulocyte-macrophage colony-stimulating factor [GM-CSF], prostaglandin E₂ [PGE₂], and interleukin [IL] 10, increases in IL-4- and IL-5-secreting T_H2-type cells, decreases in interferon gamma (IFN- γ)-secreting T_H1 cells, and increased spontaneous histamine release from basophils.

The serum levels of IgE are elevated with eosinophilia. The increased serum levels of eosinophil cationic protein and soluble E-selectin (sE-selectin) correlate with disease activity, and sE-selectin levels also correlate with eosinophilia.¹⁰ The increased IgE and eosinophilia are thought to reflect the altered expression of T_H2 cytokines, that is, increased IL-4, IL-5, and IL-13, and decreased IFN- γ expression, observed in patients with AD.¹¹⁻¹⁴ A significant inverse correlation between IFN- γ generation in vitro and IgE serum concentrations in vivo in AD has been reported.¹³ Several studies have shown increased frequency of allergen-specific T cells producing increased IL-4 and IL-5, but little IFN- γ in the peripheral blood and skin lesions of patients with AD.^{15,16}

Immunohistology of AD

The histologic features of AD depend on the acuity and therefore the duration of skin lesions.^{17,18} Even clinically normal-appearing (uninvolved) skin of patients with AD is histologically abnormal. Uninvolved skin demonstrates traces of hyperkeratosis and a sparse perivascular cellular infiltrate consisting primarily of T lymphocytes. Acute skin lesions are characterized by extensive degrees of intercellular edema (spongiosis) of the epidermis and intracellular edema (ie, ballooning of the keratinocytes). A sparse epidermal infiltrate consisting mainly of lymphocytes is also frequently observed. In the der-

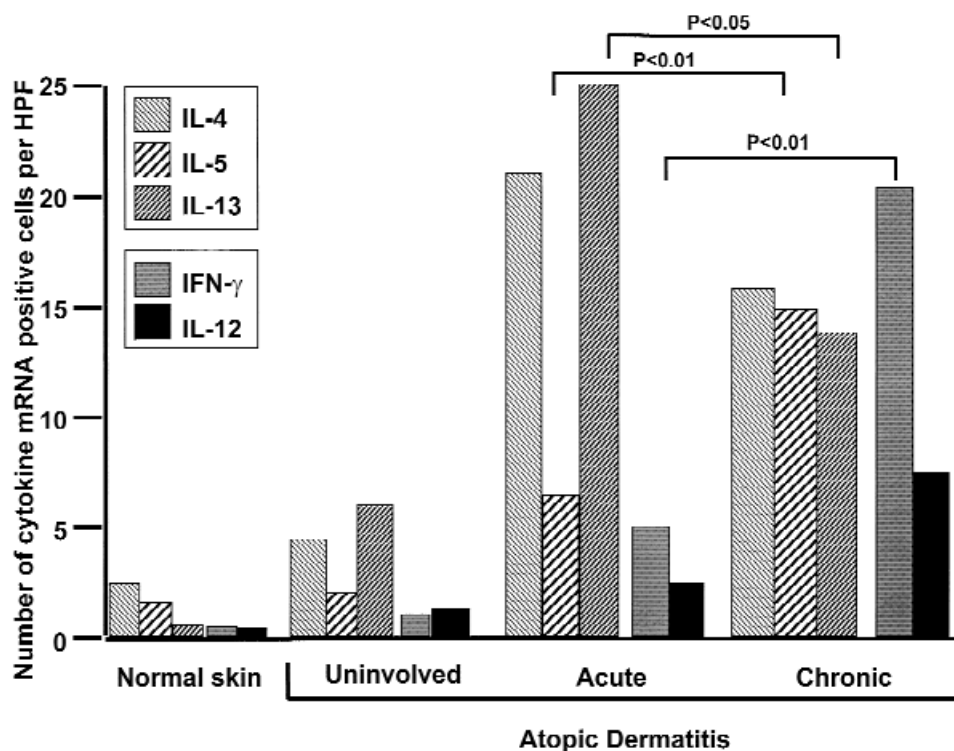


Fig 1. Biphasic cytokine expression in atopic dermatitis. Acute skin lesions are rich in T_H2 (IL-4 and IL-13) cytokine expression. Chronic skin lesions show increased expression of IL-5 and IFN- γ and IL-12, with significant decrease in IL-4 and IL-13 as compared with acute lesions.

mis, there is a marked perivenular inflammatory cell infiltrate consisting predominantly of T lymphocytes, and occasional monocyte-macrophages. Mast cells, in various stages of degranulation, are present in normal numbers, but eosinophils, basophils, and neutrophils are rarely present in acute lesions.

In chronic lichenified lesions, the epidermis is hyperplastic with elongation of the rete ridges, prominent hyperkeratosis, and minimal spongiosis. There is an increased number of Langerhans cells in the epidermis, and macrophages dominate the dermal mononuclear cell infiltrate. Mast cells are increased in number and are usually fully granulated. Increased numbers of eosinophils are also observed in chronic AD skin lesions. The eosinophils show various stages of degeneration, with abnormal granules, and loss of cytoplasm and/or nuclear membranes.¹⁹ An eosinophil-derived extracellular major basic protein has been detected in a fibrillar pattern associated with the distribution of elastic fibers through the upper dermis, supporting a functional role of eosinophils in chronic AD lesions.²⁰ Extracellular major basic protein deposition was much more extensive in the involved area than in the uninvolved areas of skin. Eotaxin is a CC chemokine that acts via the receptor CCR3. Immunoreactivity and transcripts of eotaxin and

CCR3 are increased in lesional skin of chronic AD.²¹ In addition, both the content in the skin lesions and plasma levels of RANTES (regulated upon activation, normal T cell expressed and secreted), a CC chemokine that is chemotactic for eosinophils, were increased in patients with severe AD.²² Eosinophils are thought to contribute to tissue injury in AD through the production of reactive oxygen intermediates and the release of cytotoxic granules. Eosinophil cationic protein is elevated in AD sera and correlates with disease severity.²³

In both acute and chronic lesions, the venular endothelial cells are enlarged and contain enlarged nuclei with clumped chromatin and prominent nucleoli. Vascular basement membrane alterations include reduplication and thickening.¹⁷ The endothelial cells exhibit E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1.²⁴

Biphasic pattern of cytokine expression in atopic skin lesions

The pattern of local cytokine expression plays an important role in modulating tissue inflammation, and in AD this pattern depends on the acuity or duration of the skin lesion. The expression of IL-4, IL-5, IL-13, and IFN- γ messenger (m)RNA in skin

biopsy specimens from clinically normal (uninvolved), acute (erythematous AD lesions of <3 days' duration), and chronic (>2 weeks' duration) skin lesions of patients with AD has been investigated by means of in situ hybridization.^{25,26} Uninvolved skin of patients with AD had significantly more cells expressing IL-4 and IL-13 mRNA than normal skin, but no increased expression of IL-5 or IFN- γ mRNA. Acute skin lesions had significantly more cells positive for IL-4, IL-5, and IL-13 mRNA than normal or uninvolved skin, but no significant expression of IFN- γ mRNA. Acute skin lesions also have an increased expression of IL-16.²⁷ In chronic AD skin lesions, expression of IL-4, IL-5, IL-13, and IFN- γ mRNA was significantly increased compared with normal and uninvolved skin.^{25,26} However, compared with acute skin lesions, chronic skin lesions had significantly fewer IL-4 and IL-13 mRNA-expressing cells and increased numbers of IL-5 and IFN- γ mRNA-expressing cells (Fig 1). Other studies have demonstrated overexpression of IL-12 and GM-CSF in chronic AD lesions.^{26,28}

These data indicate a biphasic pattern of cytokine expression in AD, with acute skin inflammation associated with a predominance of IL-4 and IL-13 expression, and chronic inflammation associated with increased IL-5 and IFN- γ expression. Support for this biphasic role of cytokines in the pathogenesis of AD skin inflammation is provided by recent studies of in vivo expression of cytokine receptor mRNA.²⁹ These studies demonstrated that acute AD is associated with a high expression of IL-4 receptor, whereas chronic AD is associated with increased IL-5 receptor and GM-CSF receptor mRNA. IL-12 plays a key role in T_H1-cell development, and its expression in eosinophils and/or macrophages is thought to initiate the switch to T_H1-cell development in chronic skin lesions.³⁰ In addition, IL-16 may promote the infiltration of cluster differentiation (CD)4⁺ T cells into acute lesions and GM-CSF is likely to enhance cell survival of eosinophils and macrophages in chronic skin lesions.²⁷

The biphasic pattern of T-cell activation has also been well demonstrated in studies on skin biopsy specimens of atopic patch-test reaction sites.³⁰ Increased expression of IL-4 mRNA and protein was observed at 24 hours after allergen application, after which IL-4 expression declined to baseline levels. In contrast, IFN- γ mRNA expression was not detected in 24-hour patch-test lesions, but was strongly overexpressed at the 48- to 72-hour time points. These results were consistent with studies demonstrating that allergen-specific T_H2-cell clones were associated with early time points of patch-test reactions, whereas a predominantly T_H1- or T_H0-type cytokine profile

was associated with later timepoints (>48 hours). The increased expression of IFN- γ mRNA in atopic patch-test lesions is preceded by a peak of IL-12 expression, suggesting that IL-12 has a role in the development of the T_H1-type response. This increased IL-12 expression coincides with the infiltration of macrophages and eosinophils, which are known to express IL-12. These observations have led to the proposal that initiation of AD is driven by allergen-induced activation of T_H2-type cells, whereas the chronic inflammatory response is dominated by a T_H1-type response driven by the infiltration of IL-12-expressing eosinophils and macrophages, which accompanies the initial T_H2 response.

FACTORS AFFECTING THE DIFFERENTIATION OF HELPER T CELLS

T_H0 cells can develop into either the T_H1 or T_H2 phenotype, and the pathway followed depends upon several factors, including the cytokine environment in which T-cell development takes place, the genetic background of the host, pharmacologic factors, and the costimulatory signals involved in T-cell activation.

Cytokine environment

The cytokine environment at the time of antigen exposure is one of the major determinants directing helper T cells toward the T_H1 or T_H2 phenotype. T_H1 cells are induced by IL-12, which is produced by macrophages and dendritic cells. The binding and signal transducing subunit of the IL-12 receptor is present on T_H1, but not T_H2, cells. Expression of this receptor is induced by IL-12 and IFN- γ , but inhibited by IL-4. IL-4 also inhibits IFN- γ production and down-regulates the differentiation of T_H1 cells.³¹ In contrast, IL-4 promotes the development of T_H2 cells. T cells in unaffected skin and acute skin lesions, as well as circulating T cells, have been shown to express increased amounts of IL-4, IL-5, and IL-13, but decreased amounts of IFN- γ . This cytokine environment would tend to promote the development of T_H2 cells and decrease the production of T_H1 cells.

Genetic factors

Although more than 20 genes are likely to be involved in the development of allergic diseases, the presence of a clustered family of cytokine genes (IL-3, IL-4, IL-5, IL-13, and GM-CSF) on chromosome 5q31-33 has led to particular interest. Several studies have reported linkage between total IgE, bronchial hyperreactivity, and asthma with markers around this gene cluster.^{32,33} Additional data support the concept that IL-4 gene expression plays a critical role in the expression of atopy. One study reported an asso-

ciation in families with asthma between a polymorphism in the IL-4 promoter and elevated total serum IgE levels.³⁴ Chan et al³⁵ found that abnormal IL-4 gene expression may be linked to alterations in nuclear protein interactions with IL-4 promoter elements in AD. A recent study in Japan, which examined linkage between markers at and near the IL-4 gene and AD, showed a significant preferential transmission to AD offspring of the T allele of the -590C/T polymorphism of the IL-4 gene.³⁶ The data suggested that genetic differences in transcriptional activity of the IL-4 gene influences AD predisposition. Another study in a small group of patients with AD reported an association of atopy with a gain-of-function mutation in the α subunit of the IL-4 receptor.³⁷ The authors speculated that the R576 allele may predispose persons to allergic diseases by altering the signaling function of the receptor.

Pharmacologic factors

Leukocytes from patients with AD have increased cyclic adenosine monophosphate (cAMP)-phosphodiesterase (PDE) enzyme activity.³⁸ Since IgE and IL-4 production can be decreased in vitro by a PDE inhibitor, this cellular abnormality appears to contribute to the increased IgE synthesis by B cells and IL-4 production by T cells in AD.³⁹ AD monocytes have a unique, highly active PDE isoenzyme,⁴⁰ and the elevated cAMP-PDE in atopic monocytes contributes to the secretion of increased levels of IL-10 and PGE₂.^{41,42} This in turn inhibits IFN- γ production by T cells and may contribute to the decreased IFN- γ production by cultured AD peripheral blood mononuclear cells (PBMCs).

Costimulatory signals

Activation of resting T cells requires costimulatory signals independent of the engagement of T-cell receptors with the major histocompatibility complex on antigen-presenting cells. Studies in mice have shown that the generation of T_H2 cells is mainly dependent on the interaction of cluster differentiation (CD) 28 with B7.2.⁴³ Various data demonstrate the predominant B-cell expression of B7.2 in AD, but not in psoriasis, and suggest a novel role for this molecule in IgE synthesis. The expression of B7.2 on B cells of patients with AD is significantly higher than in normal subjects and patients with psoriasis, but there is no significant difference in B7.1 expression among these subjects.⁴⁴ Total serum IgE from patients with AD and normal subjects correlated significantly with B7.2 expression on B cells suggesting a role for B7.2⁺ B cells in IgE synthesis, and purified B7.2⁺ B cells produced significantly more IgE than B7.2⁻ B cells in vitro. Monoclonal antibody to human B7.2, but not to

B7.1, significantly decreased IgE production by PBMCs stimulated with IL-4, and anti-CD40 monoclonal antibody B7.2 expression on B cells is also induced by IL-4 and IL-13, thereby providing an amplification loop for IgE synthesis in AD.⁴⁵

In AD, presentation of aeroallergens by Langerhans cells may also be important in sustaining T_H2 and IgE responses because Langerhans cells predominantly express B7.2 rather than B7.1.⁴⁶

MULTIFUNCTIONAL ROLE FOR IGE IN ATOPIC SKIN INFLAMMATION

IgE contributes to the inflammatory cell infiltrate in AD by several mechanisms, including a biphasic immediate/late phase reaction, allergen presentation by IgE-bearing Langerhans cells, allergen-induced activation of IgE-bearing macrophages, and IgE autoreactivity to human proteins.

Biphasic immediate/late phase reaction

Clinically significant allergen-induced reactions are associated with an IgE-dependent biphasic response. Mast cells bearing IgE directed against the relevant allergen release various mediators, cytokines, and leukocyte chemotactic factors into local tissue within 15 to 60 minutes of allergen exposure. This immediate reaction likely contributes to the acute pruritus and erythema observed after allergen exposure in patients with AD.

Three to 4 hours after the immediate reaction starts to subside, there is the onset of an IgE-dependent late phase reaction (LPR). This reaction is characterized initially by expression of leukocyte adhesion molecules on postcapillary venular endothelium, followed by the infiltration of eosinophils, neutrophils, and mononuclear cells. The maximum cell accumulation of granulocytes is reached at 6 to 8 hours, and the cellular infiltrate consists predominantly of mononuclear cells by 24 to 48 hours after onset of the LPR. The cellular infiltrate in allergen-induced LPRs has been shown to express increased mRNA for IL-3, IL-4, IL-5, and GM-CSF, but no mRNA for IFN- γ .⁴⁷ These results suggest that the T cells infiltrating into the allergen-induced LPR are T_H2-like cells.

LPRs are also associated with the release of cytokines such as IL-1 and tumor necrosis factor (TNF),⁴⁸ which, together with IL-4, can induce leukocyte adhesion molecules.⁴⁹ AD skin lesions and cutaneous LPR are associated with the expression of leukocyte adhesion molecules such as E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1.²⁴ The induction of adhesion molecules like E-selectin in allergen-stimulated atopic skin explants can be blocked by neutralizing antibodies to IL-1 and TNF.⁵⁰ Thus the release of these cytokines by

resident skin cells likely represents an important initiating event in the local accumulation of inflammatory cells at the site of allergic reactions.

Langerhans cell- and macrophage-associated IgE

Langerhans cells and macrophages infiltrating into AD skin lesions bear IgE on their cell surface.^{51,52} High-affinity IgE receptors on Langerhans cells and low-affinity IgE receptors on macrophages have been demonstrated.^{53,54} Allergens have been shown to activate IgE-bearing macrophages in an IgE-dependent manner, with the formation of leukotrienes, platelet-activating factor, IL-1, and TNF.⁵⁵ Patients with AD have circulating autoantibodies to IgE, which can also activate IgE-bearing macrophages.⁵⁶ The activation of IgE-bearing Langerhans cells and macrophages by allergens and autoantibodies to IgE could contribute to atopic skin inflammation. IgE-bearing Langerhans cells from AD skin lesions are capable of presenting house dust mite allergen to T cells.⁵⁷ Thus IgE bound to Langerhans cells could facilitate the binding of cutaneous allergens to Langerhans cells before their presentation to T_H2 cells.

IgE autoreactivity

Recent studies have suggested that IgE autoreactivity may be a pathogenic factor in AD. The majority of patients with AD have circulating IgE antibodies directed against human proteins,⁵⁸ which are not detected in patients with chronic urticaria, systemic lupus erythematosus, or graft-versus-host disease, or in healthy control subjects. This suggests that while IgE immune responses are initiated by environmental allergens, allergic inflammation is maintained by endogenous human antigens.

Although acute pruritus in AD is triggered by the release of a variety of mediators into the skin after allergen exposure, the actual development of eczematous skin lesions is dependent on the skin trauma inflicted by scratching.⁵⁹ Once the itch-scratch cycle commences, scratching likely promotes inflammation of the skin by inducing keratinocytes to release a variety of proinflammatory cytokines.^{60,61} This includes the release of IL-1, TNF- α , IL-4, and CC chemokines, which are critical cytokines in the induction of adhesion molecules, as well as directing lymphocytes, macrophages, and eosinophils into cutaneous sites of inflammation. At this stage, a wide variety of resident and infiltrating cells are then capable of secreting cytokines and mediators that sustain the inflammation. AD therefore results from a combination of different specific and nonspecific cellular mechanisms which serve to trigger and maintain skin inflammation.

SKIN-DIRECTED T_H2-LIKE CELL RESPONSES IN AD

Allergic rhinitis or asthma eventually develops in nearly 80% of children with AD, and many patients outgrow their AD as they are developing respiratory allergy. This observation is consistent with the concept that the clinical expression of allergic disease is determined in part from local tissue allergen sensitization and compartmentalization of the immune response in the skin versus the respiratory mucosa. Because allergic diseases involve organ-specific allergic inflammatory responses, the mechanisms that control recruitment of T_H2 lymphocytes to different tissue sites are of considerable interest. Studies in animal models have shown heterogeneity in the ability of memory T cells to migrate to different tissues.⁶² This tissue-selective homing is regulated primarily by interaction of differentially expressed T-cell homing receptors with vascular endothelial cell surface antigens. In humans, the most extensively studied lymphocyte/endothelial cell adhesion molecule pair that participates in T-cell homing in skin is the cutaneous lymphocyte-associated antigen (CLA) and its counter-receptor, E-selectin.

T cells migrating into the skin of allergen-induced reactions express significantly higher levels of CLA than T cells isolated from the airways of patients with asthma.⁶³ Thus the propensity of patients to develop AD instead of asthma likely depends on differences in T cell skin-homing versus lung-homing characteristics. Patients with milk-induced eczema were found to have significantly higher levels of CLA in casein-reactive T cells than in *Candida albicans*-reactive T cells as well as significantly higher levels of CLA than casein- or *C albicans*-reactive T cells from normal control subjects or noneczematous atopic patients.⁶⁴

The relationship between CLA and cutaneous T-cell responses has also been studied by analysis of the expression of CLA on circulating T cells in patients with AD and those with asthma sensitized with house dust mites.⁶⁵ The mite-specific T-cell proliferation response in patients with AD was localized to CLA⁺ T cells. In contrast, mite-sensitive asthmatic patients had a mite-dependent proliferation response in their CLA⁻ T cells, consistent with the observation that airway T cells have a low-level CLA expression. In addition, freshly isolated CLA⁺ T cells from patients with AD, but not normal subjects, expressed the HLA-DR activation antigen and spontaneous production of IL-4, but not IFN- γ . Recent studies have also demonstrated that CLA⁺ T cells in AD spontaneously secrete IL-5 and IL-13, functionally prolong eosinophil survival, and induce IgE synthesis.¹²

The T-cell expression of CLA is regulated by various cytokines.⁶² Transforming growth factor β , IL-12,

and IL-6 increase CLA expression, but IL-1, IL-2, IL-3, IL-4, IL-5, IL-7, and IFN- γ do not. Furthermore, when bacterial toxins with superantigenic activity are used to stimulate T cells in the presence of accessory cells, CLA is up-regulated in an IL-12-dependent manner.⁶⁶ Thus the nature of the antigen, the cytokine environment in which the immune responses occur, and the location of the response all contribute to the regulation of CLA.

THE ROLE OF INFECTIOUS AGENTS AND SUPERANTIGENS IN AD

In addition to food and inhalant allergens, AD can be exacerbated by fungal, bacterial, and viral skin infections (including herpes simplex, vaccinia, warts, molluscum contagiosum, and papillomavirus). Superficial fungal infections, such as *Trichophyton rubrum*,⁶⁷ also appear to occur more frequently in atopic individuals. Recurrence of dermatophyte infections has also been documented to coincide with flaring of AD. *Malassezia furfur* is a lipophilic yeast commonly present in the seborrheic areas of the skin, and IgE antibodies against *M furfur* are commonly found in patients with AD, most frequently in those with head and neck dermatitis.⁶⁸ Positive allergen patch-test reactions to this yeast have been demonstrated.⁶⁹ IgE reactivity directed against *M furfur* is associated with AD. Some allergens from *M furfur* have been cloned, and all of these recombinant polypeptides were capable of binding serum IgE from patients with AD.⁷⁰ The potential importance of *M furfur* and other dermatophyte infections is supported by the reduction in severity of AD after treatment with antifungal agents, such as ketoconazole, in patients with these infections.^{71,72}

Staphylococcus aureus is found in more than 90% of AD skin lesions,⁷³ but in only 5% of normal subjects, and thus many studies have examined the contribution of *S aureus* colonization and infection to the severity of AD. The density of *S aureus* on inflamed AD lesions without clinical superinfection can reach up to 10⁷ colony-forming units per square centimeter.⁷⁴ Patients with impetiginized AD and AD patients without superinfection show clinical response to combined treatment with antistaphylococcal antibiotics and topical corticosteroids.⁷⁵

A variety of data suggest that one method by which *S aureus* exacerbates or maintains skin inflammation in AD is by secreting a group of toxins known to act as superantigens, which stimulate marked activation of T cells and macrophages.⁷⁶⁻⁷⁸ First, more than 50% of patients with AD have *S aureus* cultured from their skin that secretes superantigens such as enterotoxins A and B, and toxic shock syndrome toxin-1. Second, most patients with AD make specif-

ic IgE antibodies directed against the staphylococcal toxins found on their skin.⁷⁶ Third, there is a correlation between the severity of AD and the presence of IgE antisuperantigens.^{77,78} With the use of a humanized murine model of skin inflammation, *S aureus* toxin plus allergen was shown to have an additive effect in inducing cutaneous inflammation.⁷⁹ Superantigens have also been shown to induce corticosteroid resistance, suggesting that several mechanisms exist by which superantigens could aggravate the severity of AD.⁸⁰ Fourth, staphylococcal enterotoxin B applied to the skin can induce erythema and induration.⁸¹ In a prospective study of patients recovering from toxic shock syndrome, it was found that 14 of 68 patients developed chronic eczematous dermatitis, whereas no patients recovering from gram-negative sepsis developed eczema.⁸² Superantigens have recently been demonstrated to induce T-cell expression of the skin homing receptor via stimulation of IL-12 production.⁶⁶

Taken together, these data suggest that in AD, staphylococcal superantigens secreted at the skin surface could penetrate inflamed skin and stimulate epidermal macrophages or Langerhans cells to produce IL-1, TNF, and IL-12. Local production of IL-1 and TNF induces the expression of E-selectin on vascular endothelium, allowing an initial influx of CLA⁺ memory/effector cells. Local secretion of IL-12 could increase CLA expression on those T cells activated by allergen or superantigen, and thereby increase the efficiency of T-cell recirculation to the skin. IL-12 secreted by toxin-stimulated Langerhans cells (which migrate to skin-associated lymph nodes and serve as antigen-presenting cells) could up-regulate the expression of CLA and influence the functional profile of virgin T cells activated by the toxins, thereby creating additional skin-homing memory-effector T cells. Superantigen obtained from the skin of patients with AD showed appropriate VB gene segments within the CLA⁺ subset of CD4 and CD8 T cells in peripheral blood. Thus superantigens can increase the frequency of memory T cells in AD lesions.⁸³ Together, these mechanisms would tend to markedly amplify the initial cutaneous inflammation in AD and possibly create conditions favoring staphylococcal skin colonization.

Finally, PBMCs from children with AD have been reported to have significantly higher proliferative responses to *S aureus* and staphylococcal enterotoxin B,⁸⁴ as well as diminished production of IFN- γ and increased production of IL-4. Impaired IFN- γ production in response to *S aureus* *in vivo* may result in failure to eradicate this organism from the skin. Persistence on the skin could also contribute to inflammation by causing continued T-cell activation,

release of proinflammatory mediators, and corticosteroid resistance. Outside the pollen season, toxic shock syndrome toxin-1 increased total IgE production by PBMCs only in the presence of IL-4, whereas during pollen season IgE production was enhanced without exogenous IL-4 and was associated with lower IFN- γ . Superantigens can augment allergen-specific IgE synthesis and B7.2 expression.⁸⁵ Furthermore, by eliciting an IgE response, staphylococcal toxins could exacerbate AD by activating mast cells and basophils. It should be noted, however, that other staphylococcal proteins/toxins such as protein A and alpha toxin could also participate in the induction of skin inflammation by releasing TNF- α from epidermal keratinocytes or by direct cytotoxic effects on skin keratinocytes.⁸⁶

ROLE OF THE EPIDERMIS IN BARRIER FUNCTION

The stratum corneum constitutes the main barrier for the diffusion of substances through the skin. It consists of corneocytes and intracellular lipids, especially ceramides, sterols, and free fatty acids. Ceramides are involved in the water-holding properties and stratum corneum barrier function. Dry skin is a feature of AD with increased transepidermal water loss, which is a reflection of impaired barrier function of the stratum corneum. There is a reduction in ceramides in the epidermis of patients with AD, with an abnormal expression of sphingomyelin deacylase, which hydrolyzes sphingomyelin to yield sphingosulphosphorylcholine rather than ceramide.⁸⁷⁻⁸⁹ This leads to a reduction in ceramide and deleterious effects on the function of the stratum corneum.⁹⁰ Moreover, the bacterial skin flora obtained from both lesional and nonlesional skin of patients with AD secrete ceramidase, which metabolizes ceramide to sphingosine and fatty acids, and further adds to the deficiency of ceramide in the stratum corneum.⁹¹ The reduction of ceramides and their ratios affects the function of the stratum corneum as a permeability barrier, with water loss and consequent dry skin. The dry skin may provide a portal of entry for allergens, irritants, and skin pathogens.

IMPLICATIONS FOR THE MANAGEMENT OF AD

Current therapy for AD is directed at hydrating the skin, reducing inflammation, and relieving symptoms such as pruritus and sleep disturbance. Depending on its severity, AD is usually managed with the liberal use of emollients, topical corticosteroids, antihistamines, and antibiotics. For patients who have severe or long-standing disease, extensive body surface area involvement, or facial lesions, the

therapeutic options are more limited. Mid- to high-potency topical steroids are not recommended for long-term use or use on the face, neck, and intertriginous areas because of the potential for atrophy and other side effects. The long-term use of oral corticosteroids may also lead to adverse effects.

Therapeutic trials using several experimental immunomodulators have been reported. In particular, IFN- γ , a cytokine which downregulates T_H2 cell function, has been found to reduce the clinical severity of AD and decrease total circulating eosinophil counts.^{92,93} In addition, the new high-potency phosphodiesterase inhibitors may be useful in targeting the increased PDE activity in atopic monocytes and have demonstrated promising preliminary clinical results.³⁹ However, the response to many of these immunomodulators has been modest or they appear to act in only a subset of patients with AD, presumably because patients are in different stages of immune activation. Oral cyclosporine, a potent immunosuppressive drug which down-regulates cytokine production, has also been reported to be effective for AD, but concerns over systemic toxicity have limited its use.⁹⁴ Topical therapy with cyclosporine has been attempted, but patients failed to show significant improvement, probably because of insufficient penetration into the skin.

Tacrolimus (FK506), a macrolide lactone isolated from *Streptomyces tsukubaensis*, is a potent immunosuppressive agent with a spectrum of activity similar to cyclosporine. Its smaller molecular size and higher potency compared with cyclosporine suggested it could be effective as a topical agent. Experimental evidence suggests that tacrolimus diffuses across cell membranes and binds to a class of ubiquitous peptidyl-prolyl *cis-trans* isomerases (PPIase), designated FK506-binding proteins. This binding inhibits the ability of calcineurin to dephosphorylate the transcription factor (nuclear factor of activated T cells) that activates IL-2 gene transcription.^{95,96} Consequently, synthesis and release of IL-2 are inhibited.⁹⁷ Tacrolimus also inhibits the transcription and release of other cytokines (eg, IL-3, IL-4, IL-5, IFN- γ , TNF- α , GM-CSF), which can contribute to allergic inflammation.⁹⁸ Indeed, recent clinical trials have shown that topical tacrolimus is highly effective in reducing the symptoms of AD.⁹⁹

Skin biopsy specimens obtained after 3 and 7 days of treatment with topical tacrolimus revealed markedly diminished T-cell and eosinophilic infiltrates.¹⁰⁰ Cell types important in allergic skin inflammation including mast cells, basophils, eosinophils, keratinocytes and Langerhans cells have FK506-binding proteins and down-regulate their mediator or cytokine expression after treatment with tacrolimus.

Because recent studies suggest that the T-cell activation in AD is biphasic with activation of the T_H2 -like cytokines during the acute phase and increased expression of the T_H1 -cytokines in chronic lesions, the capacity of tacrolimus to inhibit the activation of multiple cell types and different cytokines may account for its ability to effectively reduce skin inflammation in AD.

CONCLUSIONS

AD develops as a result of a complex interrelationship of genetic, environmental, immunologic, and pharmacologic factors. Allergic reactions appear to play a role in some patients, but in other patients, factors such as a disturbance of skin function, infection, and stress may be more important. Factors identified as being involved in the pathogenesis of AD include the pattern of local cytokine expression, the differentiation of helper T cells, multiple roles for IgE, skin-directed cell responses, infectious agents, and superantigens.

The pattern of local cytokine expression plays an important role in modulating tissue inflammation and depends on the acuity or duration of the skin lesion. There appears to be a biphasic pattern of cytokine expression in AD, with acute skin inflammation associated with a predominance of IL-4 and IL-13 expression and chronic inflammation associated with increased IL-5 and IFN- γ expression.

The differentiation of T_H0 cells into T_H1 or T_H2 cells also appears to play an important role in AD. The pathway followed depends on several factors including the cytokine environment, the genetic background of the host, pharmacologic factors, and various costimulatory signals. The initiation of AD appears to be driven by allergen-induced activation of T_H2 -type cells, whereas the chronic inflammatory response is dominated by a T_H1 -type response driven by the infiltration of IL-12 expressing eosinophils and macrophages.

IgE contributes to the inflammatory cell infiltrate in AD by several mechanisms, including a biphasic immediate/late phase reaction, allergen presentation by IgE-bearing Langerhans cells, allergen-induced activation of IgE-bearing macrophages, and IgE autoreactivity to human proteins. It is unlikely that AD skin lesions can be classified discretely into an IgE-mediated LPR or a T-cell-mediated immune reaction. The mononuclear cell infiltrate in the AD skin lesion probably reflects a combination of both IgE-dependent mast cell/basophil degranulation and T_H2 -cell-mediated responses elicited during acute exposures to allergens and other antigens.

The clinical expression of allergic disease is determined in part from local tissue allergen sensitization,

compartmentalization of the immune response in the skin versus the respiratory mucosa, and by the ability of memory T cells to migrate to different tissues. This tissue-selective homing is regulated primarily by interaction of differentially expressed T-cell homing receptors with vascular endothelial cell surface antigens, such as CLA.

AD can be exacerbated by various skin infections. In particular, *S aureus* may exacerbate or maintain skin inflammation in AD by secreting a group of toxins known to act as superantigens. These toxins stimulate marked activation of T cells and macrophages and amplify the initial cutaneous inflammation in AD by a variety of mechanisms.

An understanding of the role of some of the factors involved in AD has already led to the development of potentially useful new treatments.

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