

Immune mechanisms leading to atopic dermatitis

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The incidence of atopic dermatitis is increasing, and this poses a major burden on health care costs. A precise understanding of the genetic and immunologic mechanisms is crucial for development of effective treatment strategies for atopic dermatitis. Various studies indicate that it has a multifactorial cause, with activation of complex immunologic and inflammatory pathways. The current review will examine recent advances that have been made in our understanding of the genetic and pathophysiologic prerequisites that form the basis of this common recalcitrant skin disorder. (*J Allergy Clin Immunol* 2003;112:S128-39.)

Key words: Atopic dermatitis, dendritic cells, IgE, inflammation, skin

Atopic dermatitis (AD) presents as a chronic relapsing skin disease with a characteristic phenotype and typically distributed skin lesions, which often make the diagnosis of AD very simple and clear-cut (see review by Spergel and Paller¹). In contrast, the underlying pathophysiologic and genetic mechanisms leading to the manifestation of AD are often not obvious. Challenged by this puzzle, scientific approaches during the last few years have made considerable progress in gaining insights into the complexity of the mechanisms that cause AD.²

In the past few years, there has been increasing interest in this disease triggered by its increasing prevalence in western societies and its contribution to increasing health care costs.³ AD offers a wide clinical spectrum ranging from minor forms presented by a few dry eczematous patches to major forms with large areas of erythematous rash.⁴ The cardinal features of AD are erythematous eczematous skin lesions, flexural lichenifications, or papules accompanied by intense pruritus and cutaneous hyperreactivity^{3,5-7} in combination with one or more typical atopic signs, such as palmar hyperlinearity, the Dennie Morgan infraorbital fold, the Hertoghe sign of the lateral eyebrows, xerosis, and lichenification of the skin. Immunohistologically, AD is hard to differentiate from other inflammatory eczematous skin diseases. Although

Abbreviations used

AD: Atopic dermatitis
APC: Antigen-presenting cell
APT: Atopy patch test
CLA: Cutaneous lymphocyte antigen
IDEC: Inflammatory dendritic epidermal cell
LC: Langerhans' cell
PG: Prostaglandin
SNP: Single nucleotide polymorphism
SP: Substance P

nonlesional skin of patients with AD represents a mild hyperkeratosis and a superficial perivascular cellular infiltrate, the histologic features of acute AD lesions are represented by spongiosis, epidermal hyperplasia, thickening of the papillary dermis, parakeratosis, and a perivascular infiltrate, which consists mainly of T cells, monocytes, macrophages, and antigen-presenting cells (APCs).^{2,8}

Over the last 100 years, various names have been created for AD and mirror the wide clinical, genetic, and immunologic spectrum of this entity and the rapid progress in our understanding of the disease (Table I). Nevertheless, many questions in regard to the exact pathophysiologic network in AD remain unanswered.⁹⁻¹²

EXTRINSIC AND INTRINSIC AD

Two types of AD have been identified (Fig 1): the allergic, or extrinsic, form, which affects about 70% to 80% of the adult patients with AD and occurs in the context of sensitization toward environmental allergens and increased serum IgE levels, and the intrinsic, nonallergic form, which affects 20% to 30% of adult patients with AD accompanied by low IgE serum levels and the absence of any detectable allergen sensitization.¹¹ Recently, the concept of a pure type of AD without any previous or actual associated respiratory diseases has been distinguished from the mixed counterpart associated with sensitizations against aeroallergens or food allergens, implying that increased IgE serum levels can be associated with AD but are not an obligate parameter for defining this disease.

THE GENETIC BACKGROUND OF AD

It is a challenge to finely characterize the genetic background underlying AD because this complex skin disease has a high level of genetic heterogeneity and genetic actions on different phenotypic and immunologic levels. AD has a high familial occurrence, with a 2-fold

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increased risk for a child to develop AD when one of the parents is affected and a 3-fold increase in cases in which both of the parents are affected. These observations and the early age of onset, in combination with the high concordance rate of 77% in monozygotic twins and 15% in dizygotic twins, suggest that AD represents a genetically complex disease.¹³

To date, 2 different approaches have been used to identify candidate genes: linkage gene analysis studies and the studies of candidate genes. The first strategy is aimed at detecting an association of the AD phenotype with any unknown chromosomal region. In contrast, candidate gene studies are restricted to a single, already known gene locus and investigate the association of polymorphisms of a specific gene with the phenotype of AD.

Genome-wide linkage studies done to characterize susceptibility loci for AD demonstrated that this disease is linked to chromosome 3q21, a region that encodes the costimulatory molecules CD80 (B7-1) and CD86 (B7-2).^{14,15}

Evidence has been obtained that a defect in the *SPINK5* gene mapping to chromosome 5q31, which encodes the serine protease inhibitor LEKTI, is operative in patients with Netherton syndrome,¹⁶ and precedence for an association of single nucleotide polymorphisms (SNPs) in this *SPINK5* gene not only in patients with Netherton syndrome but also in patients with AD in general comes from a recent Japanese study.¹⁷ In another approach, the arachidonate metabolite thromboxane A2 and thromboxane A2 receptor polymorphisms have been considered to be related to chronic inflammatory diseases, such as AD, and might provide a major determinant for high serum IgE levels in AD.¹⁸

Furthermore, a pivotal role of the *IL13* gene in AD, which is located at chromosome 5q31-33, has been estimated in linkage and association analysis studies. This is in agreement with recent results of a Japanese study, which suggest that an SNP in the *G4257A* allele of the *IL13* gene is of functional relevance in AD.¹⁷ Most interestingly, a variant of the *RANTES* gene, which results in a new consensus binding site for the GATA transcription factor family, has been identified as a functional mutation in the proximal promoter of the *RANTES* gene on chromosome 17q11.2.¹⁹ This mutation might be related to the upregulation of C-C chemokines detectable in a high number of patients with AD.

Common candidate genes of AD with Crohn's disease and psoriasis

Of special note are recent observations that indicate that AD and psoriasis share loci on chromosomes 1q21 and 17q25, implicating that common candidate genes are involved in the control of general mechanisms of skin inflammation, which might form the basis of both of these chronic inflammatory skin diseases.²⁰ In addition, there is a high probability of a shared genetic background of AD and Crohn's disease, a chronic inflammatory bowel disease with an impaired recognition of microbial components, which might be the reason for a higher fre-

TABLE I. Different names that have been used for AD

Prurigo diathésique	Besnier (1892)
Névrodermite diffuse	Brocq (1902)
Prurigo Besnier	Rasch (1903)
Eczéma constitutionnel	Brocq (1927)
Early and late exsudative eczematoid	Rost (1928)
Eczema infantum	
Eczema flexurarum	
Neurodermatitis disseminata and pruriginosa	
Hay fever eczema-asthmatic eczema	
Neurodermitits	Rost and Marchionini (1932)
Atopic dermatitis	Wise and Sulzberger (1935)
Endogenous eczema	Korting (1954)
Neurodermitis constitution- alis sive atopice	Schnyder and Borelli (1967)
Neurodermitis atopica sive constitutionalis	Wuthrich (1983)
Pure-mixed atopic eczema	Wuthrich (1989)
Atopiforme dermatitis	Bos (1998) (2002)
Extrinsic-intrinsic atopic eczema	Wuthrich (1989)
IgE-mediated-non-IgE-mediated atopic eczema	
Allergic-nonallergic atopi eczema dermatitis syndrome	Johansson (2001)

quency of SNPs in the caspase recruitment domain observable in both AD and Crohn's disease.²¹

Candidate genes related to the high-affinity receptor for IgE

The gene locus 11q13, a region encoding for the β -chain of the high-affinity receptor for IgE has also been linked to the AD phenotype. Variants of this gene seem to exert a regulatory effect on IgE production by patients with AD. Additionally, evidence has been obtained for a maternal imprint effect, as shown by a significant sharing of maternally inherited alleles in region 11q13 in sib pairs with atopic IgE responsiveness.²² In this context, recent observations support the hypothesis that as a consequence of alternative splicing, the *Fc ϵ RI β* gene encodes 2 proteins with opposing and competing functions. According to this model, one isoform represents the classical Fc ϵ RI β chain, whereas the other truncated variant (β T) prevents Fc ϵ RI surface expression by inhibiting Fc ϵ RI α chain maturation.^{23,24}

The gene encoding the human γ chain of the IgE receptor (Fc ϵ RI γ) has been characterized on chromosome 1q23 and seems to have evolved from a common ancestor by duplication of the γ chain of the T-cell receptor.²⁵ Both genes show an analogous organization of their exons, and γ and δ chains are essential for surface expression of their respective receptors. Together, these mechanisms might be involved in the control of the surface expression of the high-affinity receptor for IgE and thereby the outcome of allergic disorders, such as AD (for summary, see Table II).

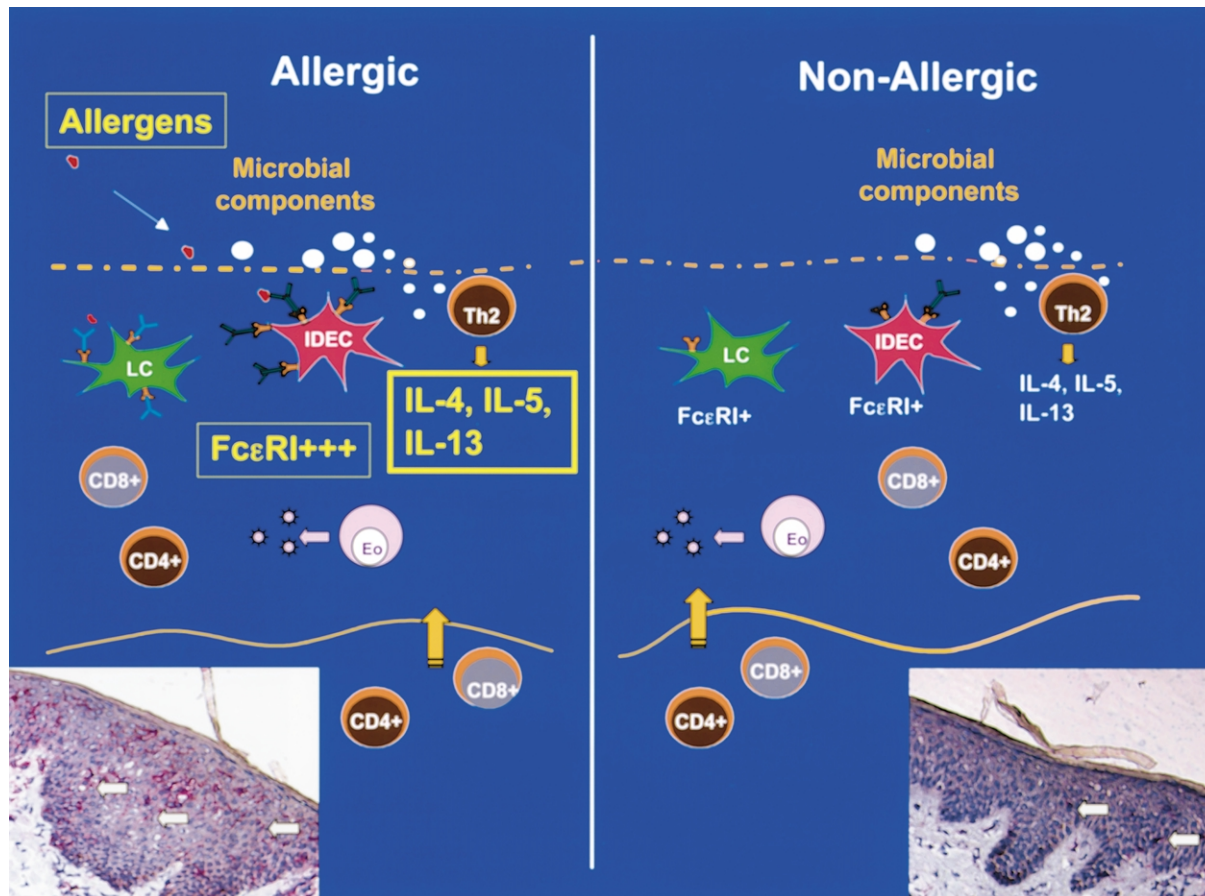


FIG 1. The pathophysiologic puzzle of AD. The differences between the allergic subtype of AD (*left*) and the nonallergic subtype of AD (*right*) are indicated in yellow. In addition, immunohistochemical stainings of FcεRI on CD1a⁺ LCs and IDECs (indicated by *arrows*) are shown on the bottom of each figure. *eo*, Eosinophils.

EPIDEMIOLOGY

The hygiene hypothesis

Although it is clear that the general propensity of AD is genetically determined, the genetic background alone cannot explain the rapid increase of atopic disorders in recent times,²⁶ and despite the expenditure of considerable energy and resources over the last decades, fundamental questions about the epidemiology of AD remain unsolved. Therefore several hypotheses have been put forward to explain the tendency to undergo an atopic career. One favored hypothesis is that in genetically susceptible individuals influences from the environment in utero and the early years of life can lead to the manifestation of AD. The generally held belief is that human fetal lymphocytes are skewed toward a T_H2 profile as a consequence of intrauterine priming by placental cytokines and hormones and possibly by transplacental allergen exposure.^{27,28} The downregulation of the T_H2-type immunity and the T_H2 skewing at the maternofetal interface during gestation is regarded as beneficial for the maintenance of pregnancy. Furthermore, it is hypothe-

sized that T_H1 cytokines, such as IFN- γ , can damage the placenta by the activation of cytotoxic cells.

In general, T-cell responses to allergens seem to occur prenatally, and the cutoff level for a possible T-cell priming with inhalant and nutritive allergens has been proposed at greater than 22 weeks. The human fetus contains B cells that are primed to undergo IgE class switching from the earliest stages of ontogeny and can produce endogenous IgE after 20 weeks of gestation, although IgE-producing cells are rare until 9 months after birth. During the postnatal period, in nonatopic individuals the T_H2 profile switches to a T_H1 profile, probably as a consequence of immune system stimulation with different components of infectious agents, including endotoxin.

Infections induce IFN- γ -dominated T_H1 immunologic immune responses, which suppress the T_H2 immune responses in vivo and in vitro. It is important to note that the relationship of endotoxin exposure and disease development is influenced by the time point of the endotoxin exposure; the nature, dose, and frequency of the stimulus; and the genetic variations in the interindividual capability to respond to endotoxins, for example through respective

TABLE II. Candidate genes for atopy and AD

Region	Candidate gene	Phenotype
1p32-31	Unknown	Positive prick test response
1q21; 17q25		Common loci of psoriasis and AD
2q21-q23	Unknown	Specific IgE
3q21	Costimulatory molecules CD80 and CD86	AD
3p24.2-22	Unknown	Atopy-allergic asthma
3p24	Chemokine <i>RANTES</i>	AD
4q35.2	Unknown	Atopy-allergic asthma
5q31.1	<i>IL4</i>	Total IgE
5q32-q33	Glucocorticosteroid receptor β -Adrenergic receptor	Total IgE Bronchial hyperreactivity/asthma Common loci of Crohn's disease and AD
5q31		AD
5q31-33	<i>IL13</i>	
5q31	<i>SPINK5</i>	Association wit Netherton syndrome
6p21.3	MHC class II	Specific IgE
6p21.3	<i>TNFA</i>	Total IgE
8p23-p21	Unknown	Specific IgE
11q13	<i>FCER1B</i>	Atopy, asthma, total IgE, specific IgE
12q15-q24.1	<i>IGF1, SCF</i>	Atopy, total IgE
13q12-14	Unknown	AD
14q11.2	T-cell receptor	Specific IgE
	Mast cell chymase	AD
14q32	IgG heavy chain	Atopy
16p11.2-12.1	<i>IL4RA</i>	Total IgE, intrinsic-extrinsic AD
16q24.1	Unknown	Atopy, allergic asthma
17q11.2	<i>RANTES</i>	AD
19q13	Unknown	Allergic asthma, total IgE
19p13.3	<i>TXA2</i>	Total IgE, AD
21q21	Unknown	Atopy, allergic asthma

surface structures, such as toll-like receptors. In this regard it has also been suggested that atopic individuals display a deficient capability to respond to endotoxins caused by some functional variants in their toll-like receptors, which might be the result of, for example, SNPs in the respective gene regions. Factors of the modern lifestyle, such as the use of antibiotics, the reduction of family size, and the increase of hygienic strategies leading to decreased bacterial stimulation, support the development of a T_H2 predominance in the immune system.²⁸⁻³¹

Other risk factors for atopic diseases include low birth weight, maternal smoking, early infection with respiratory syncytial virus, and vaccination against *Bordetella pertussis*.³² Furthermore, alterations in the intestinal microbial microflora in infants in western societies underlie a deficit of specific microbial strains, which drive a protective effect on the development of allergic diseases by the induction of contraregulatory cytokines, such as IL-10 and TGF- β .¹⁹ Supporting this view, the perinatal and postnatal systemic administration of the probiotic *Lactobacillus rhamnosus* has been shown to lead to a 50% reduction in the development of AD in children at risk in a Finnish study.³³

PROVOCATION FACTORS

Food allergens

Food allergies have a pathogenetic role in a subset of patients with AD, particularly in infants, and contribute to the severity of the disease through the triggering of

pruritus and the induction of skin lesions. In most of the children with AD (>90%), these food allergens are derived from egg, wheat, milk, soy, or peanut. Food allergen-specific T cells can be cloned from the lesional and nonlesional skin of patients with food allergy.³⁴ Furthermore, antigen-specific T cells in the peripheral blood of these patients are highly activated and are supposed to induce a strong food-specific T-cell response after food-allergen challenge.³⁵

Typically, children with food allergies generally have positive immediate skin test responses or serum IgE levels directed against these allergens. In view of a high frequency of false-positive results with these tests, the relevance of each positive test result should be evaluated in light of the respective clinical history. Furthermore, double-blind food challenges or exclusion diets can be performed to confirm or to exclude the food hypersensitivity. Although most of the children outgrow their allergies on milk, egg, wheat, and soy, clinical reactivity to peanuts, tree nuts, and fish often becomes manifest.³⁴ Therefore rechallenges in the follow-up period are necessary to test the putative development of tolerance to food allergens. The atopy patch test (APT) has been proposed within the last years as an adjunctive test for detection of food allergy in patients with AD.^{36,37} For suspected cow's milk allergy, the APT has been reported to have a positive predictive value of about 95%.³⁶ In the subgroup of patients with AD in which a food allergy can be proved by using 2 or more of the test methods mentioned above, dietary treatment for a limited time period is of value.

The association between breast-feeding and AD has been controversial. In most studies a protective effect of breast-feeding on the development of atopic diseases in children at risk has been observed.³⁸ On the other hand, it has been suggested that an immunologic imprinting and transfer of immunoglobulins and other factors in the breast milk of the mother might influence the higher risk for the development of sensitization in these children.^{39,40} In general, a delayed exposure to solid foods in combination with prolonged breast-feeding and, in some cases, even a restricted diet for the mother during lactation has been recommended.

Aeroallergens

Several studies have demonstrated the relevance of aeroallergens that elicit or aggravate eczematous skin lesions and contribute to the flare-ups of AD in a subgroup of patients with AD. It has been repeatedly demonstrated that intranasal or bronchial inhalation challenge with aeroallergens, such as house dust mite or animal dander, can lead to the worsening of AD skin lesions. The degree of IgE sensitization to aeroallergens is directly associated with the severity of the disease, although the reduction of exposures to some common allergens, such as house dust mite, is associated with a significant improvement of AD, at least in some cases.^{41,42}

Recently, a paradoxical relationship between the exposure to animal dander and the development of sensitization in atopic children has been discussed. Research during the past few years has implicated different windows of opportunity for environmental exposures that might be the reason for the absence of manifestation of sensitization despite exposure to aeroallergens in a subgroup of these atopic children. Because of this model, early exposure of children to animal dander in combination with the protective effect of the endotoxin comprising dander might lead to increased stimulation of T_H1 immune responses, favoring a decrease of the T_H2 predominance. In contrast, the exposure to animal dander at later time points might not derive any protective effect on the immune system and indeed might lead to the development of sensitizations.⁴³⁻⁴⁶ In a subgroup of children with AD at risk for asthma who display sensitization against aeroallergens, a preventive effect of orally administered antihistamines has been proved in the ETAC study,⁴⁷ and this presents another mode of preventive action on the development of AD.

APT

Application of aeroallergens, such as cat dander or house dust mite, in the APT shows that it is possible to elicit eczematous skin lesions solely by means of external application of aeroallergens to the skin.^{37,48,49} Positive APT reactions were significantly more frequent in patients with a typical air-exposed predilection for the skin lesions.⁵⁰ In recent studies, even in some patients with intrinsic AD, a reactivity to aeroallergens or food allergens has been shown with the APT,⁵¹ but the mechanisms by which this occurs remain unclear. In these

APTs a rapid influx of inflammatory dendritic epidermal cells (IDECs) is observed within only 72 hours in the freshly elicited skin lesions in the initial phase. This initial event is followed by an alteration of the phenotype of Langerhans' cells (LCs) and IDECs within these skin lesions.⁵² In contrast, a specific upregulation of FcεRI on epidermal dendritic cells (DCs) in APT lesions has been observed to be limited to the extrinsic form of AD (Fig 1). The data appraised in these APTs suggest that an intermittent or continuous flow of aeroallergens and autoantigens in the process of facilitated allergen presentation might define the pathophysiology of the recurrent, self-perpetuating course of AD.⁵³⁻⁵⁷

SKIN BARRIER

Impairment of the epidermal skin barrier

The skin encounters a high number of antigens at the interface between the body and the surrounding environment.^{58,59} Intense pruritus and scratching, in combination with a cutaneous hyperreactivity and reduced threshold for pruritus, underlie the vicious circle of continuous mechanical stimulation and a dysregulated cytokine release by keratinocytes as a consequence of scratching in patients with AD.⁶⁰

The altered lipid composition of the stratum corneum is a basic defect of AD, which is responsible for the xerotic aspect of the skin and results in a higher permeability to allergens and irritants, which can be typically found in patients with AD.^{60,61} Ceramides serve as the major water-retaining molecules in the extracellular space of the cornified envelope, and the barrier function of these complex structures is provided by a matrix of structural proteins that are bound to ceramides.⁶²⁻⁶⁵ Even the noninvolved skin of patients with AD is characterized by severe dryness and an impairment of the barrier function of the stratum corneum, as reflected by increased transepidermal water loss. A reduced content of ceramides has been reported in the cornified envelope of both lesional and nonlesional skin in patients with AD. This abnormal expression of ceramides leads to an abnormal expression of sphingomyelin deacylase-like enzymes. As an additional factor, ceramidase, which breaks down ceramide into sphingosine and fatty acid, is secreted from the bacterial flora obtained from the lesional and nonlesional skin of patients with AD. Another contributing factor to the active phase of the disease is the shift in pH values toward alkalinity at eczematous and uninvolved skin sites.⁶⁴ Altogether, the susceptibility to irritants in AD can be described as a primary defect of epidermal differentiation in the presence of inflammation-induced skin damage in combination with a further impairment of the skin barrier inflicted by the active inflammatory phase of AD.

The intrinsic defect of keratinocytes

Keratinocytes of patients with AD manifest an intrinsically abnormal chemokine-cytokine production pattern that contributes to the recruitment of distinct leukocyte

subsets to the inflammatory site and maintains the inflammatory immune response of AD skin.⁶⁶ At the level of signal transduction, an altered transcriptional control and enhanced expression of activator protein 1, in combination with an overexpression of the transcription factors c-Jun, Jun-B, and c-Fos induced by functional abnormalities in the protein kinase C translocation, might lead to the enhanced GM-CSF release of AD keratinocytes.⁶⁷

As a consequence of the altered cytokine synthesis in AD, keratinocytes also release high amounts of the pro-inflammatory cytokines TNF- α and IL-1 β . Furthermore, in response to TNF- α and IFN- γ , epidermal keratinocytes of patients with AD overexpress soluble epidermal growth factors, which induce the release of monocyte chemoattractant protein 1, RANTES, IP-10, and IL-8.^{68,69} On the basis of recent evidence, the cytokine milieu in the skin environment is thought to be crucial to the process of DC activation and maturation. Thymic stromal lymphopoietin, which is an IL-7-like cytokine, is another soluble factor that is produced in high amounts by keratinocytes of patients with AD and contributes to the initiation of the allergic cascade by means of induction of the migration of LCs into the lymph nodes.^{70,71} Thymic stromal lymphopoietin-stimulated DCs prime naive T cells to produce soluble factors, such as IL-5, IL-13, and TNF- α , and initiate the production of chemokines by DCs, such as macrophage-derived chemokine or thymus and activation-regulated chemokine, which attract T cells of the T_H2 type and can be found in high amounts in patients with AD.⁷²

We contend that the ability of epicutaneous application of allergens to cause allergic airway inflammation is contingent on the presence of an immunologic milieu in the skin microenvironment that enhances T_H2 responses.¹ Recently, a role of T cell-mediated keratinocyte apoptosis through FAS ligand, which leads to the rapid downregulation of the adhesion molecule E-cadherin, was reported.^{73,74} In contrast, desmosomal cadherins are resistant to this process. Together, this leads to the formation of spongiosis, which is a typical histologic feature of eczematous AD lesions.^{75,76} Furthermore, keratinocytes, which undergo apoptosis, release chemotactic factors and recruit more T cells into the epidermis, which themselves enhance the amount of IFN- γ and Fas, as well as the apoptosis of keratinocytes.^{77,78} Taken together, a dynamic interaction between the inflammatory and remodeling components in the skin serves to enhance and prolong the inflammatory immune responses in AD.

ω -6 Fatty acids

Immunologic and biochemical alterations in AD have been attributed to a deficient conversion of ω -6 fatty acids (ie, linoleic acid, γ -linoleic acid, and dihomo- γ -linoleic acid) to prostaglandin E₁ (PGE₁). There is evidence for a deficiency of essential long-chain ω -6 fatty acids and E-type prostaglandins, which are important for thymic T-cell maturation and thymus hormone action. This proposed concept links the alterations in cell-mediated and humoral immunity in atopy to impaired PGE-

mediated thymic maturation of T-suppressor lymphocytes and diminished activation of T-suppressor lymphocytes of the peripheral atopic immune system. The decreased sensitivity of atopic T lymphocytes to PGE, explained by a reduction of PGE₂ receptors on atopic lymphocytes, is regarded as a common underlying defect in atopy. A second defect, the δ -6-desaturase deficiency affects the supply of PGE precursors. Deficiencies of 6-desaturated ω -6 fatty acids have been observed in plasma phospholipids, epidermal red cell phospholipids of patients with AD, umbilical cord plasma lecithin of newborn infants with increased cord blood IgE levels, cord blood T cells of "atopy-at-risk" newborn infants, atopic monocytes, adipose tissue lipids of patients with AD, and breast milk lipids of mothers with a history of AD. Furthermore, a reduced release of arachidonic acid has been measured in atopic monocytes and platelets. Diminished formation of PGE₂, which suppresses the IL-4-induced IgE synthesis, has been observed in atopic monocytes and in the epidermis of patients with AD.

MICROBIAL COMPONENTS AND DEFENSINS Microbes

Pityrosporum ovale is a lipophilic yeast commonly found to colonize AD skin and is thought to elicit immediate and late-phase reactions in these patients. In more than 60% of patients with AD, IgE to *P ovale* can be detected in the peripheral blood, supporting the hypothesis of the importance of this organism as an allergic trigger factor in AD.⁷⁹ Many reports clearly indicate the role of *P ovale*, especially in AD located in the head and neck area. Furthermore, skin prick test responses to *P ovale* are positive in a subgroup of patients with AD. In vitro assays it has been shown that immature DCs can take up *P ovale* through the mannose receptor and might thereby induce sensitization against *P ovale*, even in the absence of IgE.

Staphylococcus aureus is found in more than 90% of patients with chronic AD skin lesions.⁸⁰ Acute exudative skin lesions can contain over 10 million of these organisms per square centimeter, and increased numbers have been found even in normal skin and the nasal vestibula or intertriginous areas of patients with AD. Scratching is an important factor, enhancing the binding of the bacteria by disturbing the skin barrier and exposing extracellular matrix molecules known to act as adhesins to *S aureus* (eg, fibronectin, collagens, fibrinogen, elastin, or laminin).⁸¹ In addition, bacterial binding seems to be higher at skin sites with T_H2-mediated inflammation and the respective cytokine milieu because of the induction of an enhanced production of these adhesions and downregulation of antimicrobial peptides needed to control the replication of *S aureus*.^{82,83} The important role of T_H2-mediated inflammation in contributing to *S aureus* colonization is supported by clinical data that *S aureus* counts are reduced in AD skin after treatment with effective anti-inflammatory agents.⁸⁴ *S aureus* secretes toxins, such as enterotoxin A and B or toxic shock syndrome toxin 1, at the skin surface, which serve as so-called

superantigens in AD.⁸⁰ This mechanism of superantigenic stimulation contributes to the amplification of the allergic inflammatory skin immune response that characterizes AD.⁸⁵

In vitro stimulation of PBMCs with staphylococcal superantigens leads to insensitivity to glucocorticoids by means of the upregulation of glucocorticoid receptor β , a protein isoform of the classical glucocorticoid receptor α , which acts as a potent inhibitor of the glucocorticoid action.⁸⁶ This observation raises the possibility that the bacterial colonization and the release of enterotoxins by these bacteria might lead to the development of an insensitivity of some patients with AD to glucocorticoids and contribute to difficult-to-manage AD. A fundamental step in the proposed sequence of events has been the finding that AD skin is deficient in antimicrobial peptides, which are a component of the innate immune system needed for efficient and rapid host defense against bacteria, fungi, and viruses in the skin.^{82,83} Together, the higher colonization with microbial hosts in combination with an inadequate host defense might perpetuate the course of AD.

Autoallergens

IgE autoreactivity has been implicated as an immunopathogenetic factor in AD. In addition, molecular analysis of allergens has revealed striking similarities between environmental allergens and human proteins, leading to the hypothesis that autoimmune reactions also might play a role.^{87,88} Recently, IgE-reactive autoantigens directed against human proteins have been cloned from human epithelial copy DNA (cDNA) expression libraries and have been found to primarily represent intracellular proteins.⁸⁹ Autoantigens characterized for AD include Hom s 1-5 and DSF70.^{90,91} These autoantigens seem to act as adjuvants to the immunologic mechanisms, especially in patients with AD who have increased IgE levels because they have been detected in IgE-immune complexes of AD sera. The release of autoallergens from the damaged tissue, putatively triggered by mechanical factors, such as scratching, could trigger responses mediated by mast cells, basophils, T cells, and IgE.⁹² This notion is supported by the observation that IgE autoallergen levels decrease as a consequence of successful treatment.⁹³ In view of these findings, a picture emerges that immune responses that are primarily initiated by environmental allergens might be maintained by human endogenous antigens.

Neuroimmunologic factors

One important provocation factor of AD that can lead to the exacerbation of this disease is stress. It is well documented that stress can alter levels of circulating lymphocyte subsets and eosinophils in patients with AD.⁹⁴ Even though the exact mechanisms of the interaction of the skin immune system and the nervous system have not yet been identified, it is believed that this phenomenon might be mediated by neuroimmunologic factors, such as neuropeptides, which can be found in the blood and within the epidermal nerve fibers in close association with epi-

dermal LCs.⁹⁵⁻⁹⁸ In AD these neuropeptides might dysregulate the production of cytokines and other factors, which results in a reduced host defense. Increased levels of nerve growth factor and substance P (SP) can be found in the plasma of patients with AD and correlate positively with the disease activity.⁹⁹ Some of these neuroimmunologic mediators, such as calcitonin gene-related peptide, are able to exert an inhibitory effect on the antigen-presenting capacity of LCs and might be defective or only present in low amounts in patients with AD.¹⁰⁰ Increased SP and calcitonin gene-related peptide levels in the lesional skin of patients with AD, which have been shown recently, might stimulate keratinocytes to release inflammatory mediators.¹⁰¹ Furthermore, the intradermal injection of SP leads to wheal-and-flare reactions and the release of histamine. Therefore it is possible that these mechanisms might contribute to the intense itching seen in AD. In addition, activated keratinocytes are able to produce the neuropeptide proopiomelanocortin-derived hormones, which in turn seems to promote IL-10 secretion to induce negative feedback signals for the downregulation of the inflammatory reactions in the skin.¹⁰² This neuroimmunologic network in the blood and the skin of patients with AD might provide new behavioral therapeutic intervention strategies for the future, including stress management and intervention strategies to interfere with the stress-induced immune imbalances.

The role of monocytes in AD

APCs, like monocytes, LCs, and DCs from atopic donors, express 3 different IgE-binding structures on their cell surface: the high-affinity receptor for IgE (Fc ϵ RI), the low-affinity receptor for IgE (Fc ϵ RII, CD23), and the IgE-binding lectin galectin-3.¹⁰³⁻¹⁰⁵ In human subjects the classical tetrameric Fc ϵ RI ($\alpha\beta\gamma_2$) is constitutively expressed on effector cells of patients experiencing anaphylaxis (ie, mast cells and basophils), whereas a trimeric form of Fc ϵ RI ($\alpha\gamma_2$), which lacks the β chain, is variably expressed on APCs. The expression of Fc ϵ RI and Fc ϵ RII on monocytes in the peripheral blood is increased in atopic subjects and is significantly higher in patients with extrinsic AD than in patients with intrinsic AD. Recent concepts support the hypothesis that Fc ϵ RI-bearing monocytes in the peripheral blood might be the source of subtypes of IgE-bearing DCs in the epidermal lesions of patients with AD, which are recruited in the acute phase or during exacerbation of the disease into the inflammatory skin by chemokines, cytokines, and other mediators. In addition to its role in antigen uptake and presentation, Fc ϵ RI on APCs seems to play a pivotal role in modulating the differentiation. This hypothesis is supported by the recent observation that cross-linking of Fc ϵ RI on APCs leads to profound alterations in the phenotypic and functional characteristics of the developing DCs. This effect is mediated mainly by a significantly increased production of the tolerogenic cytokine IL-10, which is released by the activated cells themselves.¹⁰⁶ In addition, Fc ϵ RI ligation on monocytes of atopic donors induces indoleamine-dioxygenase,

which is similar to IL-10 in that it is involved in the control of T-cell responses and the induction of tolerance in the immune system.^{106,107}

Some studies have shown that leukocytes from patients with AD have increased levels of cAMP-phosphodiesterase activity, an enzyme abnormality that results in reduced intracellular cAMP, creating a permissive effect on cell function.^{108,109} This increased activity accounts for subnormal cAMP responses and correlates with increased PGE₂ production of monocytes, which inhibits T_H1 responses and accentuates IL-4 secretion by T_H2 cells. It appears that in addition to PGE₂, IL-10 acts to regulate the balance between T_H1 and T_H2 functional responses, accounting for many atopic features, including increased IgE synthesis, decreased IFN- γ production, impaired cell-mediated immune responses, and IL-4, IL-5, and IL-6 production by T cells. All of these abnormalities can be related to increased phosphodiesterase activity in atopic monocytes. On the basis of this evidence, therapeutic approaches in clinical studies using topical type 4 phosphodiesterase inhibitors have shown significant but not dramatic improvement of the skin lesions in patients with AD.¹¹⁰

Characterization of DCs in patients with AD

A hyperstimulatory capacity of epidermal DCs isolated from AD lesions was shown as early as 1992.^{111,112} The outcome of antigen presentation to T cells is determined by the convergence of signals delivered to them by APCs. However, it was the combination of immunomorphologic and ultrastructural characterization that led to the delineation of 2 different epidermal DC populations in AD: LCs and IDECs.^{113,114} Classical LCs are characterized ultrastructurally by a clear cytoplasm, a lobulated nucleus, and the lack of desmosomes, melanosomes, or Merkel cell granules and, most importantly, by the presence of highly specific tennis racket-shaped, cytoplasmic Birbeck granules. LCs show a relatively invariable CD1a⁺⁺⁺, Fc ϵ R2⁺⁺, HLA-DR⁺⁺⁺, CD1b⁻ immunophenotype.^{112,114,115} However, the Fc ϵ R1 levels on LCs in the lesional skin of patients with AD are significantly higher than in uninvolved areas, although LCs at nonlesional sites still bear higher receptor numbers than in nondiseased skin.

The ultrastructure of IDECs resembles that of LCs because of their clear cytoplasm, lobulated nucleus, and lack of desmosomes, melanosomes, and Merkel cell granules, but IDECs do not contain any Birbeck granules. Although a number of authors have claimed that they have identified LCs as the IgE-binding and Fc ϵ R1-expressing epidermal cell population in AD lesions, it is now clear that IDECs and LCs are the relevant IgE-binding and Fc ϵ R1-expressing cells. In comparison with LCs, IDECs are additionally expressing CD1b, CD11a, CD11b, and CD11c; the thrombospondin receptor CD36; and the mannose receptor (CD206), which is known to be involved in the uptake of bacterial components.¹¹⁶ Both cell types (ie, LCs and IDECs) express the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) and MHC I and II molecules on their cell surface. There is

some evidence that the quality and quantity of the signal transduction events triggered in LCs from normal individuals and individuals with AD differ profoundly. First, it has been shown that cross-linking of Fc ϵ R1 on LCs leads to rapid tyrosine phosphorylation of several proteins, including p72, p77, and p95. However, significant calcium mobilization could only be detected in LCs from the lesional skin of patients with AD. In contrast, LCs from healthy donors, even in the presence of significant Fc ϵ R1 expression, show no calcium mobilization. Calcium mobilization is dependent on tyrosine phosphorylation and the activation of PLC- γ , which does not occur in LCs from healthy donors but, in contrast, does occur in LCs from atopic donors. These data imply that some steps of the Fc ϵ R1-mediated signaling cascade might be upregulated in patients with AD.

The high expression of Fc ϵ R1 on LCs and IDECs in patients with AD could be detected with a high sensitivity and specificity from other inflammatory skin diseases and provides the diagnostic application of a standardized phenotyping procedure, in which the expression of the Fc ϵ R1/Fc γ R2 ratio is used to distinguish between extrinsic and intrinsic AD or other inflammatory skin diseases, which do not differ histologically.^{51,117}

Antigen presentation in AD

AD is a biphasic inflammatory skin disease characterized by an initial phase predominated by T_H2 cytokines that switches to a second and more chronic T_H1-dominated eczematous phase. From the pathophysiologic point of view, Fc ϵ R1-expressing LCs in the epidermis have been suspected of playing a crucial role in the initial events of AD because they might represent the missing link between aeroallergens penetrating the epidermis and antigen-specific cells infiltrating the skin lesions. It is assumed that allergens that could penetrate into the epidermis because of the reduced skin barrier function in patients with AD are taken up by the Fc ϵ R1-bound IgE molecules of epidermal LCs. In this way APCs could extend their ability to react toward allergens by binding large amounts of IgE molecules with various specificities, which are efficiently processed and targeted to MHC class II-rich compartments, ultimately leading to a higher density of specific peptides in the grooves of surface MHC class II molecules. Because the outcome of antigen presentation to T cells is determined by the convergence of signals delivered to them by APCs, this in turn leads to an optimal antigen presentation to T cells as a first-line mechanism for antigen recognition.^{118,119}

Thereby the initial contact of DCs with antigens in AD launches a series of events that equip DCs with the necessary apparatus to migrate to the lymph nodes and activate T cells. Furthermore, Fc ϵ R1 activation of LCs leads to the release of chemokines, such as monocyte chemoattractant protein 1, IL-16, thymus and activation-regulated chemokine, and macrophage-derived chemokine, which might be responsible for the recruitment of other pro-inflammatory cells into the skin. Because the polarization of naive T cells is a unique feature of APCs and

this polarization process might play a pivotal role in the outcome and course of AD, it has been shown recently that LCs activated by FcεRI drive naive T cells into IL-4-producing T cells of the T_H2 type.

Recent concepts support the hypothesis that IDECs, like DCs generated in vitro, which are assumed to be of monocytic origin, are recruited in the acute phase of AD into the epidermis by signals mediated from cells of the inflammatory micromilieu, such as LCs. Therefore the number of FcεRI-bearing monocyte precursors of IDECs decrease in the peripheral blood in the exacerbation phase of AD and increase again after clinical improvement of the skin lesions. As an adjuvant, IDECs, like DCs generated in vitro expressing high FcεRI receptor densities after their migration into the skin lesions, display a full activation on FcεRI ligation and induce the synthesis and release of mediators that contribute to an enhancement of subsequent antigen presentation. Furthermore, FcεRI-activated IDECs, like DCs, prime naive T cells into IFN-γ-producing T cells of the T_H1 type and release IL-12 and IL-18 in vitro, which together might lead to the switch of the initial T_H2 immune response to a T_H1 immune response in vivo (Novak et al, unpublished data). As another indicator for a pivotal role of IDECs as amplifiers of the allergic-inflammatory immune response in the skin, after successful topical treatment of AD lesions, the number of IDECs in the epidermis decreases to less than the detection level.^{120,121}

T-cell homing

Skin-selective homing of memory and effector T cells represents an important immunologic event in the pathogenesis of AD. Cutaneous lymphocyte antigen (CLA) is a skin-homing receptor that defines a subset of circulating memory T cells. CLA interacts with the vascular receptor E-selectin on inflamed dermal postcapillary venules, which forms the basis of the selective and immediate recruitment of CLA⁺ T cells into the skin on invasion of foreign antigens and allergens.^{76,122,123} CLA⁺CD45RO⁺ T cells of patients with AD are activated, whereas CLA resting memory T cells seem to exert immunoprotective properties toward allergens through the release of IFN-γ and the induction of IgG4.¹²⁴ Furthermore, it has been shown recently that the interaction of the skin-associated chemokine CCL27 and its receptor CCR10 mediate the chemotactic response of skin-homing T cells in vitro and in vivo.¹²⁵ Other important cofactors in this homing process are α-6 integrin, vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and IL-8, which can be found in higher levels in the peripheral blood of patients with AD.^{5,126} After antigen presentation by DCs, T_H0 precursors are triggered to differentiate into T_H1 or T_H2 cells. Although the T_H1 response is associated with delayed-type hypersensitivity reactions and the predominance of IFN-γ and IL-2 secretion of T cells, the T_H2 pattern is associated with an increased secretion of IgE and IgE-mediated reactions as even as the predominance of IL-4, IL-5, and IL-13.^{56,127} In this regard AD is unique because it is clinically a mixture of cell-mediated type I and type IV hypersensitivity reactions.

Transcriptional regulation of T_H1 and T_H2 cells

It is hypothesized that the differentiation and polarization of T cells is regulated at the transcriptional level. Recently, it has been demonstrated that *T-bet*, which belongs to the T-box family of transcription factors, is induced in the initial phase of the development of T_H1 T cells and is absent in T_H2 T cells.¹²⁸ *T-bet* seems to promote the polarization of CD4 T cells into T_H1 cells and CD8 T cells into T_C1 cells. It inhibits the polarization of T_H2 and T_C2 cells through the activation of T_H1 genetic programs and the repression of T_H2 programs.¹²⁸⁻¹³² A lower *T-bet* expression of CD4⁺ T cells in the airways of asthmatic patients might be the reason for the T_H2 predominance in these tissues. In vitro, the T_H2-associated GATA-3 transcription factor is rapidly upregulated after allergen-specific reactivation of CD4⁺CD45RA⁺ T cells of atopic subjects, whereas GATA-3 is downregulated in nonatopic subjects.¹³³ Furthermore, GATA-3 intrinsically suppresses *T-bet*. During the development to T_H2 T cells, these T cells enhance their GATA-3 expression, downregulate their IL-12Rβ chain, and thereby lose their capacity to respond to IL-12.¹²⁸ These mechanisms involving a transcriptional regulation of the development of T_H1 and T_H2 cells might also play a role in the pathogenesis of AD and should be evaluated in more detail in future studies.

Eosinophils

Increased levels of circulating eosinophils and eosinophil granule proteins in the sera and the urine of patients are a characteristic feature of AD.¹³⁴ They correlate with disease activity and decrease in response to therapy for AD. Furthermore, activated and histologically hypodense eosinophils display an enhanced survival, which is achieved by means of delayed programmed cell death.¹³⁵ During flares of AD, the amount of IL-5 and eosinophil chemotaxins, such as eotaxin, increases in the peripheral blood,¹³⁶ and this might lead to eosinophil extravasation through selectins and recruitment of eosinophils to the skin.¹³⁷ In the skin lesions of patients with AD and in the lesions induced by APT reactions, eosinophil degranulation and eosinophil cytolytic degeneration is indicated by high amounts of eosinophil cationic protein, eosinophil-derived neurotoxin, and major basic protein deposits, whereas the amount of intact eosinophils is low.^{138,139} An additional trigger factor, staphylococcal enterotoxins, the levels of which are high in the skin of patients with AD, have been shown to modulate the effector function of eosinophils and the course of AD by inhibiting eosinophil apoptosis, increasing surface activation antigen expression, and enhancing oxidative burst of eosinophils in vitro.¹³⁵

CONCLUSIONS

AD is a common skin disease that has been known from antiquity, and its increasing incidence has attracted public interest in this disease during the last few years. Despite intensive research and significant progress in the fields of immunodermatology, epidemiology, and genet-

ics, a unifying pathogenetic concept of AD has not been established. However, the pathophysiologic complexity of this disease is being elucidated. The complex interactions between genetic and environmental mechanisms, together with an intensive network of cytokines and chemokines orchestrating the development of disease-related mechanisms, are actively being investigated. APCs, like LCs and epidermal DCs bearing IgE-binding structures on their cell surface, have been the subject of research in recent years and shed light on the underlying mechanisms of allergic skin reactions. Taken together, future approaches in clinically oriented research will be needed to complete our understanding of this skin disease. It is hoped that this will result in the development of effective therapeutic strategies and prevention mechanisms for this ever-challenging disease.¹⁴⁰

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