



Review

Pathogenetic Mechanisms of Atopic Dermatitis

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Abstract. Atopic dermatitis (AD) is a chronic inflammatory disease which results from complex interactions between genetic and environmental mechanisms. An altered lipid composition of the stratum corneum is responsible for the xerotic aspect of the skin and determines a higher permeability to allergens and irritants. Keratinocytes of AD patients exhibit a propensity to an exaggerated production of cytokines and chemokines, a phenomenon that can have a major role in promoting and maintaining inflammation. Specific immune responses against a variety of environmental allergens are also implicated in AD pathogenesis, with a bias towards Th2 immune responses. In particular, dendritic cells expressing membrane IgE receptors play a critical role in the amplification of allergen-specific T cell responses. Cross-linkage of specific IgE receptors on dermal mast cells provokes the release and synthesis of a vast series of mediators. Following their recruitment and activation into the skin, eosinophils are also thought to contribute relevantly to tissue damage. Thus, a complex network of cytokines and chemokines contributes to establishing a local milieu that favors the permanence of inflammation in AD skin.

Key words: skin; atopy; keratinocytes; dendritic cells; T lymphocytes.

Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease with an early onset and characterized by typically distributed skin lesions in different age groups. Acute lesions present with erythematous macules and papules, associated with excoriations and erosions. Chronic AD is characterized by thickened skin with accentuated skin markings and excoriated papules^{43, 49}. AD is a major health problem world-wide, affecting 5 to 20% of children⁵², and in the last decades AD as well as other atopic disorders have become steadily more prevalent in developed countries, suggesting that environmental factors are playing a critical

role in their expression. In these countries, 30% of the population may manifest some atopic disease some time in their lives. These syndromes appear to be more frequent in urban areas than in rural areas and among higher socioeconomic classes^{15, 46}. Of all skin diseases affecting children, AD has one of the greatest impacts on the impairment of the child's quality of life²⁷. In hospital studies, cases of chronic AD or repeated episodes of acute AD often achieve the highest morbidity scores on disability measures when compared with other skin diseases^{8, 11}. In adults, atopic hand dermatitis exacerbated by occupational exposure accounts for a considerable loss of working hours⁴⁴.

In this review we will examine cellular and immune

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mechanisms that are thought to play an important role in the pathogenesis of AD. An understanding of the biological bases of AD has important implications in the management as well as the early identification and prevention of this common illness.

Phenotypes and Genotypes in AD

Dry skin and increased susceptibility to cutaneous irritation are always present in AD patients and, thus, AD can be exacerbated following exposure to reduced humidity, excessive sweating and a vast array of irritants, such as wool, acrylic, soaps and detergents. Along with these factors, exposure to environmental allergens can be a relevant flare factor, and a propensity to IgE hyperresponsiveness is present in about 80% of AD patients⁷. However, a direct relationship between immediate or delayed skin test reactivity to allergens and the course of AD is seldom present, and sometimes highly elevated IgE levels do not accompany any clinical manifestation of atopy⁴¹. In contrast to respiratory atopy, specific hyposensitization is not generally helpful in AD patients. Among the environmental factors that may contribute to AD pathogenesis, skin colonization by *Staphylococcus aureus* appears to have some role in disease exacerbation²⁴. Staphylococcal strains can, in fact, release both allergenic compounds and superantigens⁴⁸ and can act as effective immunological adjuvants for increased IgE response to aeroallergens¹⁴. Intense pruritus is the cardinal feature of AD, and the skin damage inflicted by scratching is considered critical for the development and maintenance of the eczema. Moreover, compared with normal controls, patients appear to have a reduced itch threshold and a more prolonged itch duration to different stimuli.

Familial aggregation and twin studies have confirmed a fundamental contribution of genetic factors to the development of atopic disorders². However, the endeavor to finely characterize a genetic background underlying atopy copes with an important degree of genetic heterogeneity, which implies multiple genetic actions at different phenotypic levels⁵. So far, the identification of specific functional polymorphisms in major candidate genes and their correlation to the expression of atopic diseases in different groups of families and unrelated subjects have restricted the search to at least five distinct chromosomal regions². In particular, significant genetic linkages to the atopic phenotype have been found in chromosomal loci coding for components of the antigen-directed mechanisms of IgE responsive-

ness, such as the D region of human leukocyte antigen (HLA) (6p21.3), the α and δ chains of the T cell receptor (TCR) (14q11.2-13) and the β chain of the high affinity receptor for IgE (Fc ϵ RI) (11q13). Two large clusters of polymorphic genes coding for cytokines, i.e. interleukin 3 (IL-3), IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor (GM-CSF) (all in 5q), and interferon γ (IFN- γ) and stem cell factor (SCF) (both in 12q), are thought to exert a critical control not only on B cell isotype switching to IgE, but also on a predominant activation of T helper 2 (Th2) cells, eosinophils and basophils. A gain-of-function mutation in the gene coding the α subunit of the IL-4 receptor (16p11.2-16p12.1) is also considered to be a condition predisposing to atopy²². It is important to underline that some of the polymorphic regions characterized so far, in particular those in chromosomes 12 and 14, include genes coding for transcription factors.

Since genetic predisposition to allergy in AD may be similar to that in patients with respiratory atopy, it is likely that the preferential targeting of the allergic immune response in a given tissue may depend on a series of events, such as the site of initial sensitization to a specific allergen, the ability of T lymphocytes to home differentially to the skin vs the respiratory mucosa, and the programmed response of resident cells, e.g. epithelial cells, to injury and inflammation²⁵. Hence, it is expected that, in addition to genes responsible for the abnormalities shared by all atopic patients, other genes confer specific organ susceptibility. Interestingly, AD patients were found to have polymorphism in the gene coding for mast cell chymase (14q11.2), a serine protease expressed exclusively in cutaneous mast cells, which have a key role in the allergic inflammation of the skin²⁸.

Skin Abnormalities in AD

Skin dryness of AD patients is the consequence of an epidermal permeability barrier dysfunction, which in turn is related to an altered lipid metabolism in keratinocytes³³. In particular, a reduced content of ceramides has been reported in the cornified envelope of both healthy and diseased epidermis. Ceramides serve as the major water-holding molecules in the extracellular space of the cornified envelope, and the barrier function of this complex structure is provided by a matrix of structural proteins bound to ceramides by ester linkages²⁹. Also filaggrin, a precursor of the proteic components of that matrix, is prominently decreased in AD skin⁴⁷. In both human and mouse models it has been

repeatedly shown that a perturbation of the epidermal barrier causes an increased keratinocyte generation of cytokines, including IL-1 α , tumor necrosis factor α (TNF- α), GM-CSF and growth factors⁵⁴. These cytokines can stimulate lipid synthesis and cell proliferation and, thus, contribute to restoring cutaneous homeostasis. However, many of these cytokines are also potent initiators of inflammatory responses³⁷ and can create a microenvironment that favors the emergence of specific immune reactions (Fig. 1). Accordingly, investigations in mice have shown that immune responses induced by sensitization with haptens or aeroallergens through barrier-disrupted skin are strongly associated

with the induction of Th2-dominant immune responses, as observed in AD²³.

If the barrier dysfunction can help to explain the decreased irritancy threshold in the skin of AD patients, it is reasonable to postulate that an impairment in ceramide synthesis can affect skin homeostasis through more intimate mechanisms. In fact, ceramides have received increasing attention over the past decade as modulators of specific biological events. In particular, ceramides seem to differentially down-regulate the activity of protein kinase C (PKC) isozymes. It has been demonstrated that ceramides can compete with the binding of the physiologic ligands, 1,2-diacylglycerols,

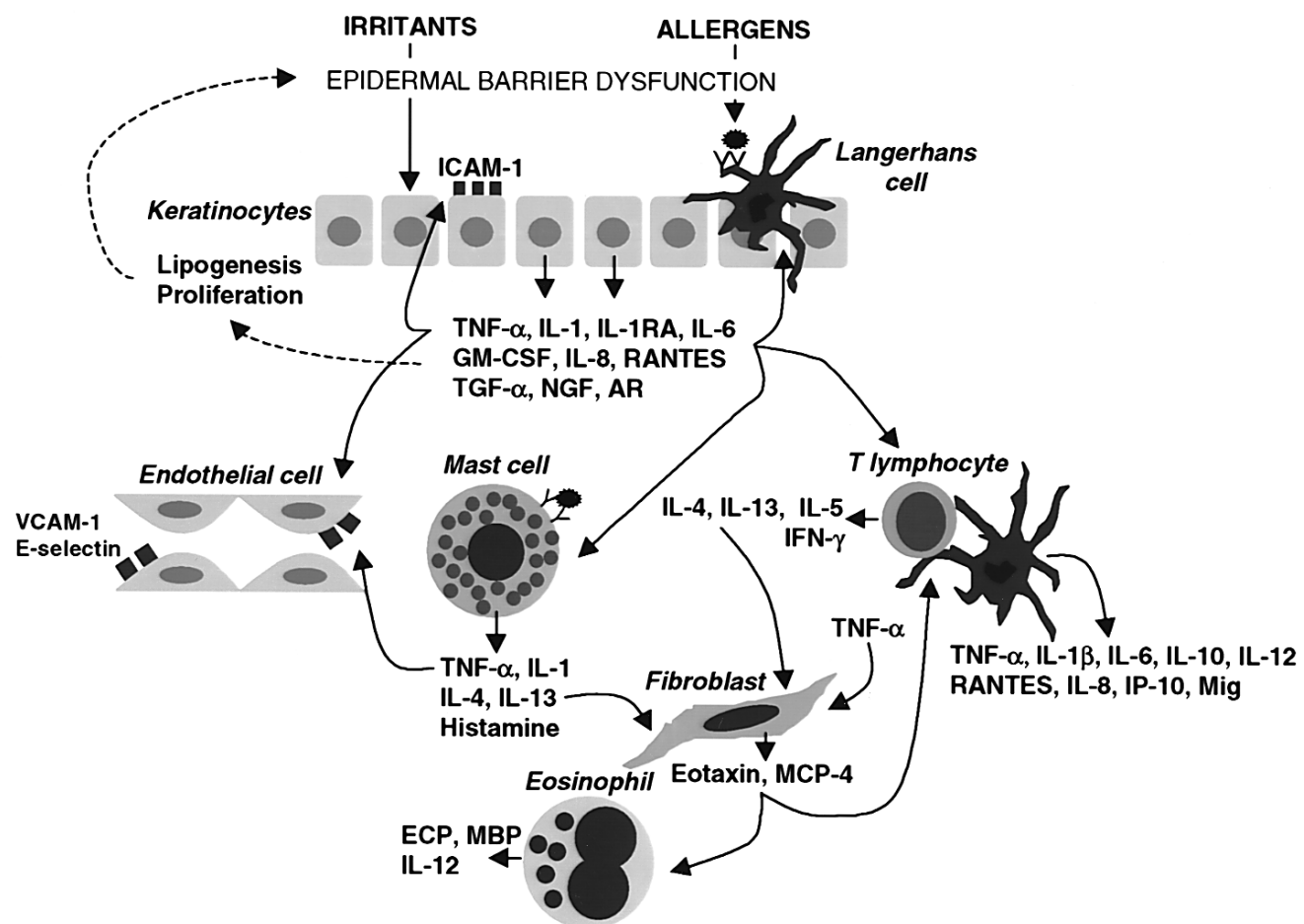


Fig. 1. Overview of cellular and molecular mechanisms underlying atopic dermatitis (AD). Epidermal barrier dysfunction facilitates the skin permeability to both irritants and allergens. These substances are potent stimuli for release of a variety of cytokines and chemokines by keratinocytes. In addition, due to mechanisms aimed to restore cutaneous homeostasis, barrier perturbation *per se* induces keratinocyte synthesis of proinflammatory molecules, active on keratinocyte themselves, dendritic cells (DC), T cells, mast cells and endothelial cells, thus favoring initiation and persistence of inflammatory and immune responses. IgE hyperresponsiveness to environmental allergens is a flare factor of AD and involves both DC and mast cells: mast cells participate in the IgE-mediated immediate hypersensitivity reaction by releasing vasoactive amines and several cytokines. DC can bind IgE through specific membrane receptors, and can efficiently present IgE-bound allergens to T lymphocytes, causing the expansion and effector functions of Th2 cells. The large number of activated DC in AD lesions may also contribute directly to the inflammatory process through the release of inflammatory cytokines and chemokines. Eosinophils are recruited primarily by chemokines released by fibroblasts, and participate to AD pathogenesis by releasing toxic molecules and IL-12, which helps in the generation of Th1 responses. Inflammatory cells have a prolonged survival capacity, which contributes to perpetuation of inflammation

or can interfere with enzyme translocation from the cytoplasm to the membrane, which is a necessary step in PKC activation¹⁸. In turn, PKC activation has a primary role in transmembrane signaling by hormones and growth factors³⁴, so that a defect in ceramide generation could determine a dysregulated, enhanced activation of these intracellular mechanisms of signal transduction, eventually leading to an exaggerated production of proinflammatory cytokines by keratinocytes. Indeed, a series of experimental data leads to conclude that the intrinsic hyperreactivity of AD skin as well as its tendency to develop more pronounced and persistent inflammatory reactions could depend, at least in part, on an abnormal functional activation of epidermal keratinocytes. Compared with keratinocytes from nonatopic controls, keratinocytes cultured from nonlesional skin of AD patients spontaneously synthesize and release higher levels of several proinflammatory cytokines, such as IL-1, TNF- α and GM-CSF. Moreover, these cells display an exaggerated cytokine secretion in response to metabolic activators (phorbol esters and synthetic analogs of diacylglycerols) or proinflammatory cytokines, such as IFN- γ ^{38, 39}. Preliminary data from the authors' laboratory confirm that keratinocytes from AD patients present a dysregulated control over gene transcription, with a more prominent activation of activator protein 1 (AP-1) transcription factors and, consequently, a more efficient binding to gene promoters. Reasonably, altered levels of nuclear PKC isozymes could be implicated in this phenomenon. Enhanced expression of proinflammatory cytokines such as IL-1 β and TNF- α by epidermal cells has also been reported *in vivo* following the application onto nonlesional skin of allergens¹⁹. It is thus possible to speculate that specific targeting of atopic inflammation to the skin could depend not only on the existence of a barrier dysfunction, but also on the constitutive, enhanced propensity to synthesize and release proinflammatory cytokines by AD keratinocytes. The specific pathways through which such functional dysregulation takes place are now under active investigation.

Immunological Abnormalities in AD

Many studies have shown the existence of a variety of perturbations of the immune system in AD patients, even though their real importance is not completely understood. Peripheral blood lymphocytes from patients with AD secrete increased amounts of Th2 cytokines (e.g., IL-4, IL-13, IL-5) and decreased IFN- γ . Moreover, monocytes and B cells from AD patients

express high levels of the IL-4 receptor and of the low affinity IgE receptor (CD23), and peripheral blood B cells from AD patients spontaneously produce high levels of IgE^{21, 42}. A series of data also suggests that AD monocytes are metabolically activated: when collected from the peripheral blood, they are primed for superoxide generation and produce significantly higher levels of GM-CSF compared with monocytes from healthy controls⁴. Monocytes present in the perivascular infiltrate of atopic lesions have been identified as the major source of prostaglandin E₂ (PGE₂) and IL-10³⁵. Both PGE₂ and IL-10 can act on infiltrating T cells to determine an impairment in their IFN- γ production, thus contributing to the establishment of a local Th2 cytokine milieu. A major immunopathogenetic role is thought to involve activated CD4⁺ T lymphocytes with a high propensity to recirculate in the skin thanks to the expression of the skin-homing receptor, cutaneous lymphocyte-associated antigen (CLA).

AD is associated with an abnormal activation and expansion of distinct subpopulations of helper T lymphocytes reactive against allergens in the skin. In particular, the initiation and maintenance of AD are believed to be caused by CD4⁺ T cells belonging to the Th2 subset, since the cytokines predominantly found in skin lesions are IL-4, IL-5 and IL-13, with lowered amounts of IFN- γ . IL-4 expression is most prominent in acute AD, while IL-5 expression is predominant in chronic AD, mostly from lymphocytes but also from eosinophils¹³. A recent characterization of the lymphocytic infiltrate suggested the existence of an IL-13-dominated pattern of cytokine secretion¹, also confirmed by the fact that a strong IL-13 gene expression has been found in the lymphocytic infiltrate of both acute and chronic skin lesions⁵⁰. The proinflammatory action of Th2 cytokines seems pertinent when serum IgE levels are raised, since IL-4 and IL-13 from activated Th2 are strong inducers of IgE production by B cells¹⁰. Because of the reciprocal regulation of Th2 and Th1 responses, the prevalent expansion of CD4⁺ Th2 cells could also contribute to explaining the reduced capacity of patients with severe AD to mount Th1 responses, including the delayed-type hypersensitivity response and protective immunity to viral infections^{26, 31}. According to some authors, the current understanding of Th2 predominance in AD may be an oversimplification. In particular, IFN- γ mRNA has been reproducibly detected in AD lesions and shown to decrease after successful therapy, and IFN- γ -producing allergen-specific Th1 clones have been obtained from chronic skin lesions. Based on experiments performed

on skin lesions elicited by topical application of allergens, a two-phase model of AD pathogenesis has been proposed in which an initiation phase, with a predominant Th2-like inflammatory response and without clinically apparent skin lesions, is switched into a second eczematous phase, dominated by the presence of IFN- γ ¹². In this context, IFN- γ can act as a potent stimulus for the synthesis and secretion of proinflammatory cytokines by keratinocytes, and recent results from the authors' laboratory indicate that keratinocytes from AD skin are particularly responsive to IFN- γ in terms of IL-1, TNF- α and GM-CSF release³⁸. Other T cell responses, such as those against keratinocyte self antigens or microbial superantigens, could provide additional mechanisms for the perpetuation of immune responses in AD skin.

The selection of the type of inflammatory infiltrate that characterizes a specific disease is strictly controlled by the chemokine receptor repertoire displayed by the leukocytes and by the pattern of chemokines released by the tissue. Interestingly, the various cell populations recruited into AD lesions, including Th2 cells, immature DC, monocytes and eosinophils, express receptors for chemokines such as eotaxin, RANTES, monocyte chemoattractant protein 3 (MCP-3) and MCP-4⁴⁵. *In situ* hybridization experiments performed

on skin biopsies soon after challenge with a proper provocation factor have demonstrated a prominent neosynthesis of RANTES and MCP-3 by dermal fibroblasts⁵⁵. It has also been demonstrated that IL-4 is a powerful inducer of eotaxin and MCP-4 in fibroblasts^{32, 40}. Even though the involvement of resident epidermal cell populations in the release of specific chemokines has not been fully characterized so far, the prominent keratinocyte expression of GM-CSF³⁹, RANTES and MCP-1 can provide strong chemotactic signals for T cells.

Role of Dendritic Cells

A prominent skin infiltration by hyperstimulatory dendritic cells (DC) is an important feature of AD⁶. The lesional skin of AD patients exhibits an increased number of cells belonging to the DC lineage (Fig. 2), including epidermal Langerhans cells (LC), dermal DC and a unique population of epidermal CD1a⁺ DC expressing CD1b and/or CD36, which closely resemble DC generated *in vitro* by culturing monocytes with GM-CSF and IL-4^{24, 53}. Such DC can efficiently present IgE-bound allergens to T lymphocytes, since they display an up-regulated expression of both the high affinity (Fc ϵ RI) as well as the low affinity (Fc ϵ RII/CD23)

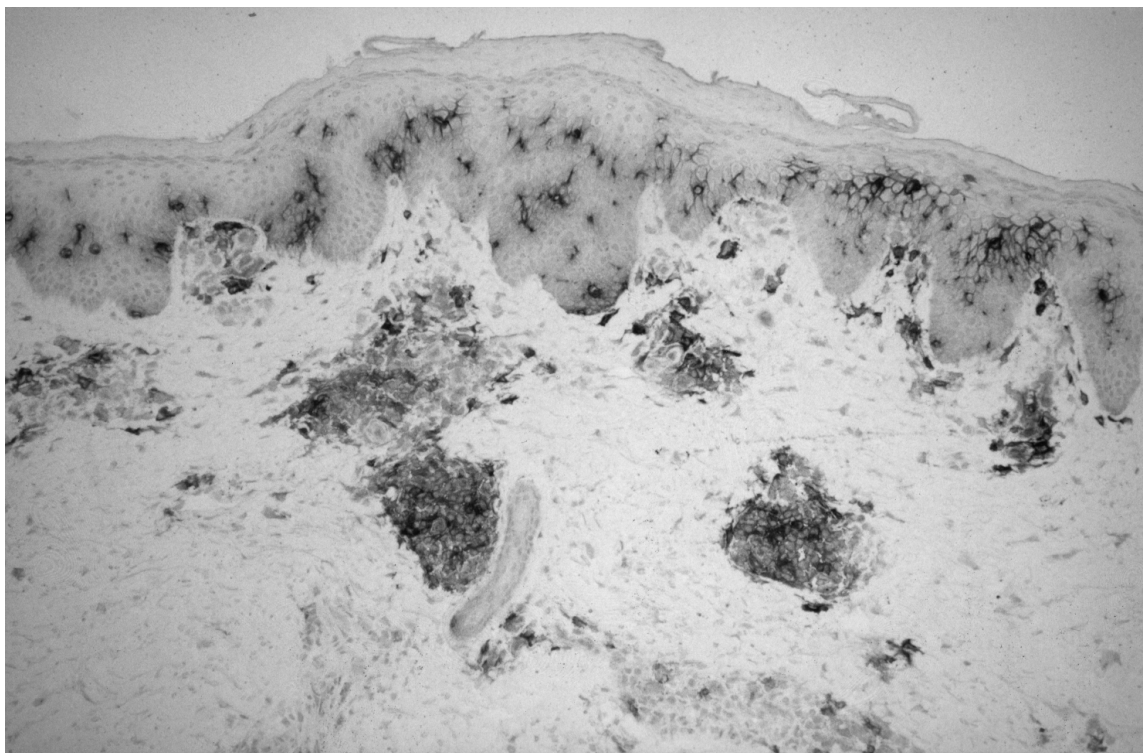


Fig. 2. Lesional skin of atopic dermatitis patients exhibits an increased number of CD1a⁺ cells belonging to the dendritic cell lineage. AD lesional skin was stained with anti-CD1a monoclonal antibody and a three-step avidin-biotin peroxidase complex amplification system

IgE receptors, with the former being the principal IgE-binding receptor. Interestingly enough, the FcεRI expressed by epidermal LC and DC differs from the classical receptor expressed on mast cells because it lacks the β chain, but it is fully functional in transducing an intracellular activation signal and enhancing the allergen internalization and presentation to T cells³⁰. A positive feedback loop of increased IgE production and further aeroallergen presentation may be very important for the amplification of local response to antigens.

Recently, the authors have shown that keratinocytes cultured from AD patients display enhanced GM-CSF gene expression and produce, spontaneously or after stimulation, high amounts of GM-CSF compared with keratinocytes from nonatopic controls. In addition, conditioned medium from stimulated AD keratinocyte cultures could support the differentiation of CD1a⁺ DC from peripheral blood precursors³⁹. A higher release of GM-CSF can considerably contribute to *in situ* LC and DC recruitment, survival and maturation into highly efficient antigen-presenting cells. It is therefore possible to postulate that, as precursors pass through the milieu of AD skin, they are induced to differentiate into DC by GM-CSF and IL-4 provided by activated keratinocytes and mast cells, respectively. In this way, increased concentrations of GM-CSF at the skin level could explain the persistent infiltration of activated DC in AD skin. Finally, the large number of activated DC present in AD may be directly involved in the establishment and perpetuation of inflammation through the release of proinflammatory molecules such as IL-6, IL-1β, TNF-α and an array of chemokines⁹.

Role of Eosinophils and Mast Cells

Various studies point to a major role of eosinophils as potent effector cells by releasing chemical mediators and cytotoxic granule proteins such as eosinophil cationic protein, eosinophil peroxidase, major basic protein and eosinophil-derived neurotoxin/eosinophil protein X. Although eosinophils are not prominent in the AD infiltrate, they can be easily localized in close proximity to mononuclear cells in chronic lesions¹⁰. In acute skin lesions, extensive extracellular deposition of eosinophil major basic protein suggests that degranulation has occurred, and several studies have shown a correlation of serum eosinophil cationic protein concentration with disease activity^{20, 36}. Recently, it has been shown that eosinophils can be actively induced by Th2-type cytokines, including IL-4, to release IL-12,

a cytokine that may contribute to precipitate the later, Th1-dominated phase of the disease¹². Moreover, peripheral blood eosinophilia in early childhood is associated with subsequent development of atopic diseases, which further points to an important role of eosinophil activation³. Strikingly, delayed programmed cell death has been reported to occur for circulating eosinophils in AD, probably mediated by an increased autocrine production of GM-CSF and IL-5⁵¹. Because mature, circulating eosinophils are terminally differentiated cells incapable of proliferation and self-renewal, regulation of eosinophil survival may represent a major mechanism through which functional eosinophils accumulate at inflammatory sites.

Mast cells have been shown to participate in the immediate hypersensitivity reaction, where the cross-linking of the membrane IgE-receptors by allergens leads to the synthesis and release of a variety of proinflammatory mediators (Fig. 1). This release determines the acute weal and flare reaction visible in skin prick tests. In acute lesions, mast cells are normal in number but are in different stages of degranulation. In chronic lesions, there is a significant increase in mast cell number, especially in areas of lymphocytic infiltration in the papillary dermis¹⁷, as to indicate a dependence on the presence of functional T cells. In turn, mast cells contribute to the recruitment of inflammatory cells by releasing preformed TNF-α, which is crucial for endothelial cell activation, and to condition the local cytokine environment toward a Th2 promotion, since they are major sources of IL-4¹⁶.

Conclusions

AD is a complex and heterogeneous disorder whose expression is dependent upon several mechanisms, including antigen-specific immune responses and inflammatory reactions elicited by non-antigenic environmental factors. Increasing evidence suggests that resident skin cells are major players of AD pathogenesis and respond with exaggerated production of proinflammatory mediators to various activation signals. The unfolding of the molecular mechanisms that underlie such responses will permit the development of more efficacious strategies for disease prevention and management.

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