

The role of eosinophils in the pathogenesis of atopic dermatitis – eosinophil granule proteins as markers of disease activity

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Currently, there is a large body of evidence that atopic dermatitis (AD) has an immunologic basis. Atopy-specific helper T cells (Th2-like T cells) may play a pathogenetic role by producing and releasing cytokines relevant for the allergic inflammation, such as IL-4, IL-5, and other growth factors. Eosinophils are believed to be of major importance as effector cells mediating the pathogenetically relevant late-phase reaction which is associated with a significant destruction of the surrounding tissue. Accordingly, a significant preactivation of peripheral blood eosinophils was detected in AD patients, leading to an enhanced susceptibility of these cells to distinct stimuli such as IL-5. Toxic proteins, such as eosinophil cationic protein (ECP), contained in the matrix and the core of secondary granules of eosinophils, may play an important role by propagating the allergic inflammatory process and by modulating the immune response. The pathogenetic role of eosinophils in AD is further supported by the detection of these proteins in the eczematous skin of patients. Furthermore, recent data point to a significant correlation between disease activity and deposition of eosinophil granule content: ECP serum levels were significantly increased in AD patients. In addition, ECP levels correlated with the disease activity. Moreover, clinical improvement was associated with a decrease of both the clinical score and serum ECP levels. These data clearly indicate that activated eosinophils may play a major role in the allergic inflammatory process of AD. Therefore, modulation of eosinophil activation could prove to be an important pharmacologic modality for the treatment of AD.

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The last decade brought an exponential increase of the knowledge dealing with mechanisms of allergic inflammation. In particular, there is increasing evidence that similar mechanisms are involved in the pathogenesis of atopic diseases, e.g. allergic bronchial asthma, allergic rhinitis and conjunctivitis, and atopic dermatitis (AD) (14, 15, 29). Recent studies support the concept of an immunologic basis of these disorders, and specific alterations of T-lymphocyte functions may be of major relevance. As a result of this cell-specific dysregulation, changes of the immune response may be due to an imbalance of the cytokine network (15).

Atopic dermatitis – the skin manifestation of atopy

AD shows a familial disposition with increasing incidence in the last years (14, 29). This inflammatory

skin disease is characterized clinically by extreme pruritus, typical eczematoid morphology and integumental distribution, a chronic relapsing course, and a personal or familial history of atopic diseases. In addition, there is a variety of other stigmata and minor symptoms which are peculiar to this important skin manifestation of atopy.

Although the entity of AD has been well known since the beginning of the century, its pathogenesis is still not clearly understood. However, recent studies offer new insights into the pathophysiology of this complex skin disorder. As demonstrated by several groups in a number of patients, environmental allergens, e.g. those of grass pollen and house-dust mites, induce eczematous reactions when applied to the skin (4, 11, 31). Moreover, it was shown that skin contact with these allergens may result in a general exacerbation of AD (1, 5, 17, 26, 36).

This exacerbation may be due to an allergen-

specific process mediated by Langerhans cells (34) (Fig. 1). As shown recently, these antigen-presenting cells express a low-affinity as well as a high-affinity Fc-receptor for IgE which is normally found on basophils or mast cells. Activation of Langerhans cells, associated with release of interleukin-1 (IL-1), results in activation of an atopy-specific, T-helper lymphocyte subset which, in turn, releases IL-4, IL-5, or other growth factors. IL-4 mediates various immunologic effects, two of which may be of major importance for the pathogenesis of AD: interleukin-4 induces the low-affinity receptor for IgE on Langerhans cells (CD 23); IL-4 regulates IgE synthesis, particularly influencing the switch of IgM to IgE/IgG1. On the other hand, IL-5 represents one of the most important cytokines for eosinophil differentiation and proliferation in man. Moreover, IL-5 activates distinct metabolic functions exclusively in eosinophils. Upon stimulation by environmental allergens, atopy-specific helper T-cell clones derived from the skin or the peripheral blood of patients with allergic bronchial asthma and AD expressed and released a cytokine pattern similar to that of Th2-cells in the mouse system (32, 40).

Eosinophilic granulocytes and AD

It is a well-known feature of AD that patients show elevated blood eosinophil counts (14, 29). The diagnostic importance of this finding, however, has been unclear and a matter of speculation (37). The role of eosinophils in the pathogenesis of allergic diseases has long been controversial (9, 24). It was thought that eosinophils might have a protective role. In contrast, research of the last decade has clearly demonstrated that eosinophils, as a result of their functional capabilities and the release of highly toxic

proteins, are active proinflammatory cells causing various symptoms of allergic inflammation.

An accumulation of eosinophils, however, is rarely found in biopsies of chronic eczematous skin of patients by hematoxylin-eosin staining. This may be explained by investigations of infiltrating cells in the bronchial mucosa of patients with allergic bronchial asthma (2). In some patients the eosinophil infiltrate was not found by hematoxylin-eosin staining, since cells were completely degranulated and seen only by means of immunohistochemistry or electron microscopy. Accordingly, significant deposits of eosinophil-derived MBP and EDN/EPX could be detected in the eczematous skin of patients by immunohistochemical techniques, as a sign of eosinophil degranulation at the site of inflammation (22, 23). Moreover, a correlation between disease activity and deposition of eosinophil granule content could be suggested (23).

Late-phase reactions

Similar findings were obtained in investigating the "late-phase reaction" (LPR) upon provocation with allergens by the skin chamber technique (6). Because of the mode of induction, however, the pattern of eosinophil-derived granule proteins is comparable with, but not identical to, findings in patients with AD. Nevertheless, the experimental LPR, as a model system, represents a useful tool for a better understanding of pathophysiologic principles which may also be important in AD (23). Hence a significant infiltration of basophils and, particularly, of eosinophils in the skin was detected, characteristically within 6–8 h after local allergen challenge. A mononuclear cellular infiltrate was detected after almost 24 h. During this interval most of the eosinophils were degranulated or even completely destroyed (6, 23). As reported recently, a significant increase in the number of activated, EG2-positive eosinophils was observed in LPRs of nasal mucosa within 24 h following intranasal challenge of patients with allergic rhinitis with *Phleum pratense* (8). Moreover, these findings correlated significantly with the expression of mRNA for Th2 cytokines, particularly for IL-5. Almost identical results were obtained in investigating LPRs 24 h following intradermal provocation in atopic patients' skin (10, 20). These findings prove that eosinophil activation and activation of Th2 cells are closely associated.

Patch tests with inhalant allergens in patients with AD

There is a large body of evidence that AD may be provoked by skin contact with allergens (1, 4, 5, 11, 17, 26, 31, 36). Accordingly, eczematous skin reac-

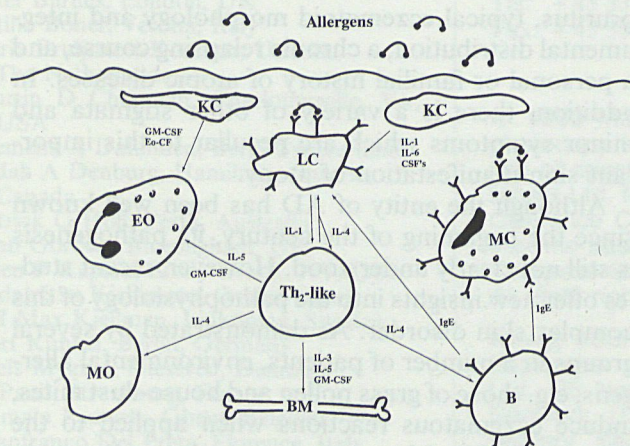


Fig. 1. Possible cytokine interactions and their pathophysiologic role in the pathogenesis of atopic dermatitis: (B = B lymphocyte, EO = eosinophilic granulocyte, BM = bone marrow, KC = keratinocyte, LC = Langerhans cell, MO = monocyte, MC = mast cell).

tions could be induced by patch tests with environmental allergens (house-dust mites, grass pollen) as a model for this mode of triggering AD (3, 4, 11, 31). In these patients, as in LPRs, a significant infiltration of eosinophils in the skin was detected within 2–6 h. By means of monoclonal antibodies against eosinophil toxic proteins, it was shown that these eosinophils were preactivated and that most of their granule content was released into the surrounding tissue. Twenty-four hours after allergen challenge, eosinophils could be detected even in the epidermis. Therefore, it appears likely that eosinophils are involved in the pathogenesis of AD. Toxic granular proteins released from activated eosinophils may play a major role in the propagation of the allergic inflammatory response.

Eosinophil activation *in vitro*

There are various distinct mediators which are capable of activating eosinophils (9, 19, 24), particularly the platelet-activating factor (PAF) and the complement split product C5a. Thus, peripheral blood eosinophils of patients with AD responded with an increased chemotactic activity upon stimulation with PAF which correlated with the disease activity (25). Similar results were obtained for other inflammatory skin diseases. Moreover, eosinophils are specifically triggered by immunomodulating cytokines. As compared with PMN, distinct cytokines are of relevance for the activation of eosinophils (18), first of all the eosinophil-selective IL-5, as well as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and tumor necrosis factor α (TNF α) (19). In atopic diseases, particularly AD peripheral blood eosinophils were significantly preactivated. This preactivation was associated with enhanced susceptibility of the cells to stimulation by the last-named cytokines. Accordingly, isolated granulocytes showed a significantly increased *in vitro* production of oxygen radicals upon stimulation with IL-5 (18). However, future studies have to show which stimuli are the priming agents, or preactivators, *in vivo*.

Eosinophil products: ECP

Toxic proteins, contained in the matrix and the core of secondary granules of eosinophils, may play an important role by propagation of the allergic inflammatory process and by their direct immunomodulatory capabilities: these proteins include major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin/eosinophil protein X (EDN/EPX) (9, 19, 24). Deposition of these proteins, particularly ECP (2), could be detected beneath the basement membrane and among the

damaged epithelial cells in patients with bronchial asthma.

As mentioned above, there is also evidence of eosinophil degranulation in AD because deposits of eosinophil-derived major basic protein have been detected in eczematous skin (22). Among the eosinophil-derived toxic proteins, ECP is well characterized with respect to its biochemistry and function (9, 28, 38). This protein represents a single-chain, highly basic protein ($pI \geq 11$). As a result of differences in degree of glycosylation, at least three forms are known with molecular masses ranging from 18.5 to 22 kDa. ECP is a very cytotoxic agent; it kills parasites, and is important in the eosinophil-mediated killing of schistosomes of *Schistosoma mansoni*. ECP secreted during allergic inflammatory processes, however, is capable of inducing the release of histamine from mast cells and mediates the destruction of epithelial cells and, possibly, of keratinocytes (24).

Elevated levels of ECP have been detected in serum and other body fluids, e.g. sputum, nasal secretion, and BAL, of patients with atopic diseases (30, 38, 39). ECP serum levels in patients were significantly increased (12, 13, 16, 27, 33, 35) (Fig. 2). Comparable results were obtained for children (27, 33) and adults (12, 16, 35) with AD. Children, however, usually had lower serum ECP levels. ECP does not represent a simple measure of blood eosinophilia, since there was no correlation between blood eosinophil count and ECP in patients with AD. Therefore, it appears likely that increased ECP levels may reflect the activation state of the whole eosinophil pool. It is also possible that eosinophils localized at the site of inflammation cause the increase of serum ECP levels and reflect the state of eosinophil activation in the skin since detected by immunohistochemistry. EG₂-positive (activated) eosinophils and free eosinophil granules beneath degranulated eosinophils were found in skin lesions of

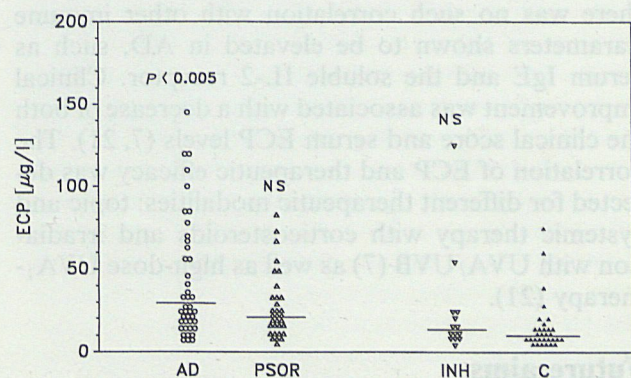


Fig. 2. ECP levels in sera of patients with atopic dermatitis (AD), psoriasis (PSOR), and inhalant allergy (INH), and of normal, nonatopic controls (C) (16). Bars indicate geometric mean.

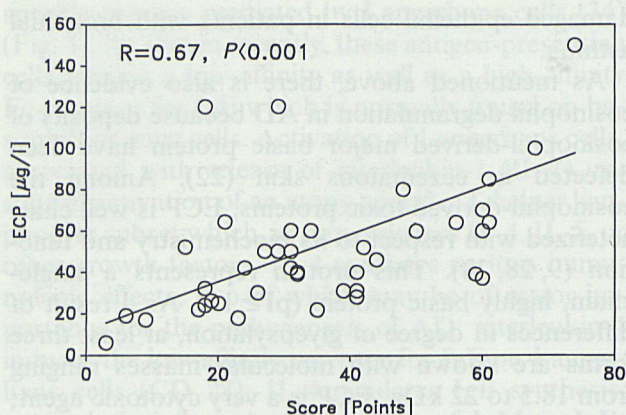


Fig. 3. Correlation of serum ECP and disease activity expressed by clinical score points (7).

patients with AD (35). This is further supported by the finding that patients with low serum IgE and without detectable specific IgE, who were thought to constitute a subgroup of patients with AD, also had increased serum ECP. In contrast, no significant amounts of ECP could be detected in the serum of patients with symptoms of mild to moderate perennial allergic rhinitis (16). However, eosinophil activation in these patients may take place only in the nasal mucosa. Although a few patients with active, plaque-type psoriasis, as an example of an inflammatory skin disorder with a "nonallergic" pathogenesis, showed increased serum ECP levels, there were no significant differences between the psoriasis group and normal controls (16). This indicates that increased serum ECP levels are not a nonspecific sign of systemic inflammation. Nevertheless, ECP is not a suitable parameter of atopic disposition because neither patients with allergic rhinitis nor psoriatic patients with a history of allergic rhinitis were found to have increased levels of ECP.

ECP serum levels, however, may be a helpful tool in monitoring disease activity in AD (7, 12, 35). Accordingly, ECP serum levels significantly correlated with the disease activity in AD, as determined by an established scoring system (7) (Fig. 3). In contrast, there was no such correlation with other immune parameters shown to be elevated in AD, such as serum IgE and the soluble IL-2 receptor. Clinical improvement was associated with a decrease of both the clinical score and serum ECP levels (7, 21). The correlation of ECP and therapeutic efficacy was detected for different therapeutic modalities: topical and systemic therapy with corticosteroids and irradiation with UVA/UVB (7) as well as high-dose UVA₁-therapy (21).

Future aims

Measurement of ECP, for the first time, allows an objective monitoring of the disease activity in AD by

an *in vitro* parameter. The prognostic value for that laboratory parameter is presently under investigation. First results indicate that there is an increase of serum ECP levels before exacerbation of the disease. Therefore, regular determinations of ECP levels could be useful in evaluating the influence of various environmental triggers, such as seasonal increase of pollen count and relevance of food allergens, but also the effect of psychologic factors, before clinical symptoms appear.

In summary, the data clearly support the concept of an active participation of eosinophils in the pathogenesis of AD. Therefore, modulation of eosinophil activation could prove to be an important pharmacologic modality for the treatment of AD.

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