

# The Role of Eosinophils in Atopic Eczema

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The histology of atopic eczema (AE) is characterized by epidermal alterations and a dermal inflammatory infiltrate containing eosinophils. Although tissue eosinophilia is not striking in AE, infiltrating eosinophils in the context with other inflammatory cells are suggestive for an allergic reaction similar to that seen in bronchial asthma, allergic rhinitis, or in allergic gastrointestinal diseases. In this chapter, I summarize our current knowledge regarding the mechanisms of eosinophil skin infiltration as well as the potential role of eosinophils in the pathogenesis of AE.

## 31.1 Evidence for Eosinophil Involvement in Atopic Eczema

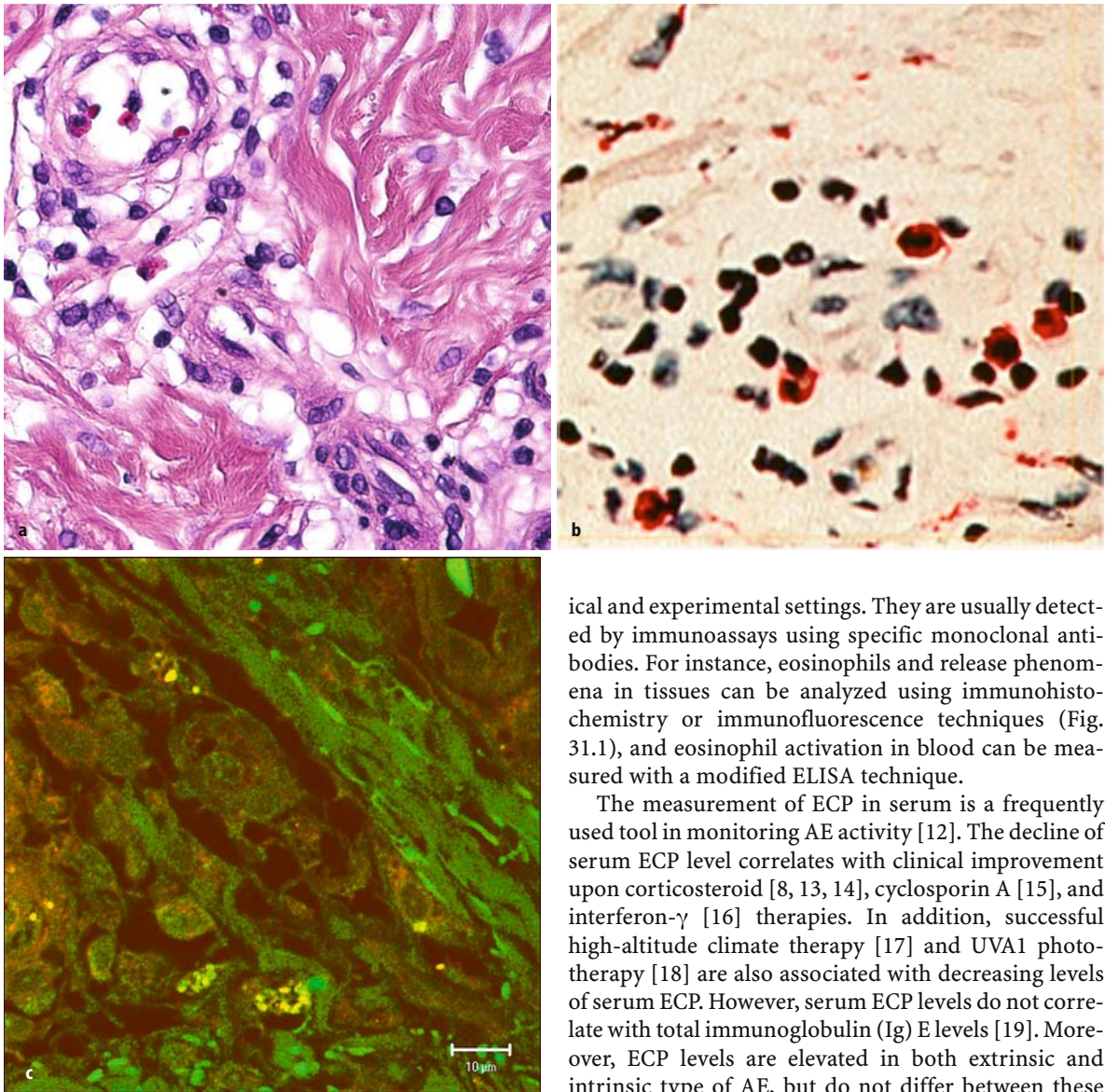
Eosinophils are typically characterized by a bilobar nucleus with highly condensed chromatin and cytoplasm containing two major types of granules, specific and primary granules, and lipid bodies. Specific granules contain a number of cationic proteins that give the eosinophils the unique staining properties. About 120 years ago, Paul Ehrlich described the affinity of a coarsely granular leukocyte for the acid dye eosin and called this cell eosinophil. Due to the characteristic staining property, eosinophils can also be detected in paraformaldehyde-fixed and paraffin-embedded tissues (Fig 31.1). In healthy individuals, eosinophils are almost exclusively limited to the digestive tract [1, 2] and are not present in many other tissues including the dermis. In contrast, eosinophils are part of a mixed perivascular inflammatory infiltrate within the dermis of AE patients [3].

Increased skin eosinophil numbers are particularly present in patients with onset of AE before adulthood [4]. In adults with AD, eosinophil skin infiltration is

often only modest [5], but tissue eosinophilia as well as eosinophilic granule protein deposition can be found in nearly all biopsies of AE lesions with a median eosinophil count of 2.8 cells/mm<sup>2</sup> (range 0 to 90.3) [6]. The maximum deposits of eosinophilic granule proteins are located in the upper dermis, 0.47 to 0.93 mm from the epidermis, whereas in the lower dermis, below a depth of 1.39 mm, no deposition can be detected. Tissue eosinophilia has been shown to be a feature in both acute and chronic stages of AE and correlates with disease severity. In chronic AE, eosinophilia appears to be more pronounced in lesions with marked epidermal hyperplasia compared to those with no or slight hyperplasia. Moreover, a correlation between eosinophilia and the degree of spongiosis was noticed in acute dermatitis or acute exacerbations of chronic AE [6]. Moreover, in a mouse model of AE, tissue eosinophilia correlated with an increase in the thickness of the epidermal and dermal layers and skin hypertrophy was suggested to result from repair processes following cytotoxic effects of eosinophil MBP or ECP [7].

In addition to tissue eosinophilia, blood eosinophilia is present in most patients with AE correlating roughly with the severity [8]. Blood eosinophilia was described to be more pronounced if the AE was associated with respiratory allergic diseases [9] as well as in patients with extrinsic AE compared to those with intrinsic AE [10]. Since some patients exhibit normal blood eosinophil counts despite active AE and since increased eosinophil numbers might be the consequence of additional allergic disorders, the determination of eosinophil number in blood is not a reliable tool in establishing the diagnosis AE.

Besides eosinophils, eosinophil-derived products are present in increased amounts in the blood and the skin of AE patients. In particular, the basic proteins eosinophil cationic protein (ECP), eosinophil-derived



**Fig. 31.1.** Eosinophilic infiltrates in lesional skin of AE demonstrated by eosin-hematoxylin staining (a), immunohistochemistry (anti-ECP; APAAP technique) (b), and immunofluorescence (anti-ECP, confocal microscopy) (c)

neurotoxin (EDN, EPX), and major basic protein (MBP) have been analyzed in clinical studies. Although EDN and ECP might also be synthesized in small amounts by neutrophils [11], all these proteins can be considered as specific eosinophil proteins in most clin-

ical and experimental settings. They are usually detected by immunoassays using specific monoclonal antibodies. For instance, eosinophils and release phenomena in tissues can be analyzed using immunohistochemistry or immunofluorescence techniques (Fig. 31.1), and eosinophil activation in blood can be measured with a modified ELISA technique.

The measurement of ECP in serum is a frequently used tool in monitoring AE activity [12]. The decline of serum ECP level correlates with clinical improvement upon corticosteroid [8, 13, 14], cyclosporin A [15], and interferon- $\gamma$  [16] therapies. In addition, successful high-altitude climate therapy [17] and UVA1 phototherapy [18] are also associated with decreasing levels of serum ECP. However, serum ECP levels do not correlate with total immunoglobulin (Ig) E levels [19]. Moreover, ECP levels are elevated in both extrinsic and intrinsic type of AE, but do not differ between these two groups [8]. Besides ECP, serum EDN [14], serum MBP [20, 21], and urine EPN levels [22, 23] have also been used as markers for monitoring AE activity.

In the absence of eosinophilic-specific surface markers [24], MBP and ECP have also been popular molecular targets in immunohistochemical studies using skin biopsies of patients with AE. These studies demonstrated that eosinophil granule proteins do not only occur inside of eosinophils but also in extracellular spaces, suggesting eosinophil degranulation. Extra-

cellular MBP deposition is primarily localized in the upper dermis and was detected in all biopsies obtained from patients with chronic lesions of their AE [25]. Another striking observation of this study was the near absence of intact eosinophils in the presence of extensive extracellular MBP staining. Intact eosinophils, however, were located predominantly within the perivascular mononuclear cell infiltrate. Interestingly, dermal eosinophil granule protein deposits have also been observed during the cutaneous late phase reaction that precede the maximal expression of clinical symptoms [26]. The presence of mostly disrupted eosinophils in the dermis of AE patients was confirmed by an electron-microscopy study, in which disrupted eosinophils and/or free eosinophil granules were detected in seven out of ten specimens [27, 28]. Various degrees of eosinophil degeneration were observed ranging from intact eosinophils with granule abnormalities, to intact eosinophils with abnormal granules and pseudopod-like extensions, to eosinophils with degenerating cell and/or nuclear membranes to free eosinophil granules in proximity to, or in the absence of eosinophils. It remains to be investigated how the eosinophil cytolysis in AE is initiated.

Taken together, there is clear evidence for eosinophil infiltration and activation of eosinophils in AE skin lesions. In experimental models, the eosinophils are present before clinical symptoms occur. Higher clinical activity correlates with elevated eosinophil numbers and increased release of eosinophil-derived proteins. Clinical improvements due to therapeutic interventions are associated with markedly reduced eosinophilic inflammation. Although these observations make it likely that the eosinophil plays an important pathogenic role in AD, its exact function remains to be determined.

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## 31.2

### Mechanisms Causing Eosinophilia

#### 31.2.1

##### Regulation of Eosinophil Production in the Bone Marrow

Eosinophils are derived from a CD34<sup>+</sup> hematopoietic progenitor cell in the bone marrow. Eosinophils share this progenitor with basophils, defined as the eosinophil/basophil-colony-forming unit (Eo/B-CFU) [29]. In the peripheral blood of atopic individuals, the Eo/B-CFU were elevated and correlated with the severity of

the atopic disease. Allergen exposure of patients with allergic rhinitis during the pollen season caused a decline in the number of eosinophil/basophil progenitors, suggesting that these progenitors are trafficking through the peripheral blood into the local tissues, where they mature [30]. Moreover, the  $\alpha$ -subunit of the interleukin-5 receptor (IL-5R $\alpha$ ) was seen to be upregulated on bone marrow CD34<sup>+</sup> progenitors after allergen challenge [31], indicating increased sensitivity towards the eosinophil differentiation factor interleukin-5 (IL-5) after allergen exposure of patients.

The importance of IL-5 for the generation of eosinophils was evident from studies of IL-5-deficient mice, which were unable to develop eosinophilia upon allergen sensitization and challenge. On the other hand, IL-5-transgenic mice exhibited large eosinophil production in the bone marrow and tissue eosinophilia in multiple organs [32, 33]. Besides IL-5, the cytokines IL-3 and granulocyte/macrophage colony-stimulating factor (GM-CSF) have also been shown to stimulate eosinophil production in the bone marrow [34]. An experimental mouse model of allergic rhinitis indicated that even an apparently isolated allergic response within the nasal mucosa is associated with increased progenitor cell production in the bone marrow, resulting in an IL-5-dependent increase in eosinophil and basophil numbers [35]. In conclusion, there is evidence from human and mouse studies that accelerated eosinophilopoiesis plays a critical role during allergic eosinophilic responses.

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#### 31.2.2

##### Eosinophil Infiltration into the Skin

Under physiological conditions, eosinophils are located in the gastrointestinal tract but not in other tissues [2]. Eosinophil mobilization from the bone marrow was suggested to be under the control of IL-5 and eotaxin, which is an important chemotactic factor for eosinophils, in a selective and concentration-dependent manner [36, 37]. How do eosinophils migrate into tissues, which they usually do not enter? This problem appears to be quite complex and many groups are performing intense research in this field. Under normal conditions, the luminal surface of blood vessels does not express sufficient levels of adhesion molecules to allow leukocytes to adhere. However, their expression is induced by cytokines such as IL-1, IL-4, and tumor necrosis factor (TNF) at the sites of allergic inflamma-

tion. Animal studies have demonstrated that IL-1 and TNF receptor expression on endothelial cells is important in both mediating eosinophil rolling and adhesion to the endothelium [38]. IL-1 $\beta$  release has been detected at sites of allergic reactions in the skin [39]. On the other hand, the cutaneous late phase reaction can be inhibited by soluble IL-1 receptors [40].

Which are the adhesion molecules responsible for the sequential events rolling, adhesion, and transmigration of eosinophils into allergic tissues? The following molecules have been identified as important players: E-selectin, P-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1, as well as the corresponding ligands on eosinophils including L-selectin, P-selectin glycoprotein (PSGL)-1, and integrins ( $\alpha$ 4 $\beta$ 1 and  $\alpha$ 4 $\beta$ 7 integrins) [38]. In the skin of AE the number of eosinophils as well as the deposits of eosinophil granule proteins correlated with VCAM-1 expression and were found to be more pronounced in acute lesions compared to chronic lesions [41]. Also other studies suggested a key role of VCAM-1 for the specific recruitment of eosinophils into inflamed tissues [42–45].

Several chemokines important for eosinophil recruitment are expressed at sites of allergic inflammation. For instance, eotaxin and RANTES are two important chemotactic factors for eosinophils and largely contribute to the movement of eosinophils from the blood to the sites of inflammation [46]. The complement anaphylatoxins C3a and C5a have also been implicated in eosinophil recruitment [47]. Moreover, leukotrienes and prostaglandins, in particular LT $B_4$  and PGD $_2$ , were found to induce eosinophil chemotaxis [48, 49], whereas lipoxin A $_4$  blocked eosinophil trafficking [50]. In contrast to eotaxin, IL-5 alone had only weak chemotactic activity, but might increase eotaxin-mediated chemotaxis [38]. The T helper 2 (Th2) cytokines, IL-4, IL-13, and IL-9 are believed to promote eosinophilia by regulating local IL-5 and/or eotaxin synthesis and/or by suppressing IFN- $\gamma$  production. For instance, IL-4 induces the expression of eotaxin mRNA in dermal fibroblasts in a dose- and time-dependent manner supporting this concept [51]. Moreover, Th2 cytokines induce the expression of the adhesion molecules VCAM-1 and PSGL-1 which may additionally support eosinophil influx into allergic tissues. In contrast, IL-6 and IL-11 seem to inhibit Th2 cytokine expression, VCAM-1 expression, and eosinophilia [52].

Additional studies revealed that eotaxin [53] and

monocyte chemotactic protein (MCP)-3 [54] are important in the early recruitment of eosinophils following allergen challenge, whereas at later time points eosinophil recruitment is largely eotaxin-independent and chemokines such as RANTES, MCP-5, and MIP-1 $\alpha$  play important roles [55]. Several CXC chemokines such as CXCL9, CXCL10, and CXCL12 have also been shown to induce eosinophil chemotaxis. [52]. The biological effects of chemokines are mediated by their interaction with specific receptors that belong to the seven-transmembrane G-protein-coupled receptors [56]. The principal receptor involved in eosinophil attraction seems to be CCR-3 [57]. The major ligands for CCR-3 are eotaxin, eotaxin-2, eotaxin-3, RANTES, MCP-2, MCP-3, and MCP-4 in humans [58].

Eotaxin as well as CCR-3 are expressed in human AE skin lesions [59]. In a mouse model of AE, CCR-3 was found to be essential for eosinophil recruitment into the skin at sites of repeated antigen sensitization with ovalbumin and into the lung [60]. After transmigration of blood vessels, eosinophils enter the extracellular matrix, where they bind to matrix proteins such as fibronectin. This binding is mediated by VLA-4 on eosinophils. Eotaxin decreases the affinity of eosinophil-expressed VLA-4 to its counterligand, the CS-1 region of fibronectin [61]. This de-adhesion seems to be a prerequisite for further tissue migration, in which metalloproteases are involved [62].

Intradermal injection of eotaxin and eotaxin-2 has been shown to cause an eosinophil infiltrate within 1 h, which further increased at 6- and 24-h time points. Surprisingly, eotaxin also recruited neutrophils and macrophages into the skin of atopic and nonatopic individuals [63]. A similar fast recruitment of eosinophils has been seen in sensitized AD patients following patch testing with a relevant allergen. The influx of eosinophils into the dermis started from 2–6 h and reached its maximum 6–24 h after patch testing [64]. A quantification of the infiltrating cells in positive patch test reactions revealed a proportion of eosinophils of 9% [65]. Moreover, atopy patch testing with house dust mite allergens was performed in AE patients sensitized to house dust mites. Eosinophils were detected in post-capillary venules in the dermis 2 h upon allergen challenge, followed by eosinophil infiltration at 6 h, which peaked at 24 and 48 h. The adhesion molecules E-selectin and ICAM-1 were upregulated as eosinophils increased in numbers [66]. That the recruitment of eosinophils into the skin of patients is rapid is also

reflected by the fact that eosinophil numbers in blood can decrease following allergen challenge [67]. Taken together, the recruitment of eosinophils upon an adequate trigger is rapid and involves the increased expression of adhesion molecules including VCAM-1 and specific chemotactic factors such as eotaxin [68].

### 31.2.3

#### Delayed Eosinophil Apoptosis

Eosinophilia and high IL-5 expression are often associated in chronic allergic diseases such as bronchial asthma or atopic dermatitis. In addition to increased production of eosinophils, inhibition of eosinophil apoptosis by IL-5 appears to play an important role at sites of allergic inflammation [69]. Delayed eosinophil apoptosis as a mechanism of tissue eosinophil accumulation has been demonstrated in nasal polyps [70]. In addition to IL-5, IL-3, and GM-CSF are also known to increase eosinophil viability *in vitro* [71, 72]. Recently, CCR3-reactive chemokines such as eotaxins have been demonstrated to prolong eosinophil survival [73].

Purified blood eosinophils from AE patients that were cultured *ex vivo* had a reduced death kinetic compared to normal eosinophils [74]. This observation might reflect that eosinophils were exposed to survival factors *in vivo* before isolation. However, the intracellular mechanisms, which mediate increased *in vitro* survival in the absence of survival cytokines remain to be investigated. It is possible that antiapoptotic proteins of the Bcl-2 family play a role [75–77]. Eosinophils from AE patients have also been demonstrated to be resistant to Fas-induced apoptosis, a phenomenon which was not related to decreased Fas receptor surface expression [78]. Although additional mechanisms might play a role [79], these data support the idea that eosinophils from AE patients express increased amounts of antiapoptotic proteins.

All the mentioned studies in AE were performed using blood eosinophils. Whether delayed eosinophil apoptosis occurs in the skin of AD patients has not been demonstrated. Since eosinophil cytolysis was present in about 70% of the cases [27], it is possible that unknown death triggers operate in the skin of these patients and kill the cells even in the presence of increased amounts of antiapoptotic proteins.

## 31.3

### Activation of and Immunoregulation by Eosinophils

#### 31.3.1

##### Activation of Eosinophils

As mentioned earlier in this article, the release of eosinophil basic proteins at the inflammatory sites suggests eosinophil activation. Since the eosinophil granule proteins may also cause tissue damage, the process of eosinophil activation needs to be tightly controlled. There have been a number of studies describing eosinophil activation mechanisms. Hematopoietins, such as IL-3, IL-5, and GM-CSF [80] increase functional responses of eosinophils to various agonists, including lipid mediators, complement factors, and chemokines [80–83]. This effect of hematopoietins, called „priming,“ is also observed in other granulocyte subtypes [84]. Recently, IL-2 has also been reported to act as a priming factor in CD25<sup>+</sup> eosinophils [85]. That effector cells of the immune system do not immediately release toxic proteins upon stimulation might be part of a safety mechanism, which prevents accidental degranulation.

Second signals that trigger functional responses after previous priming of eosinophils are provided by a number of various agonists, including lipid mediators, complement factors and chemokines [80, 86]. The same factors might also release preformed cytokines from eosinophils [87, 88]. According to their physiological function in host defense surveillance of mucosal surfaces, eosinophils can also be activated by IgG, IgA, or soluble IgA [89]. Although the high-affinity IgE receptor had been proposed as a stimulus for activation [90], recent studies did not obtain evidence for functional high-affinity IgE receptors on human eosinophils [91, 92].

#### 31.3.2

##### Immunoregulatory Functions

Eosinophils express a variety of receptors for immunoglobulins, cytokines, chemokines, and other chemotactic factors that upon activation result in degranulation and the release of inflammatory mediators, e.g., cationic proteins, leukotrienes, and immunoregulatory cytokines [62]. Due to the production and the release of cytokines, eosinophils appear to have immunoregulatory properties. Although eosinophils are terminally

differentiated cells, their capacity to generate cytokines can be quite intriguing. Eosinophils are able to produce a wide spectrum of cytokines, including TNF, transforming growth factor, IL-1, IL-3, IL-4, IL-5, IL-8, and GM-CSF [62]. By secretion of these cytokines eosinophils are capable of enhancing the inflammatory processes, including T cell differentiation, but also to initiate tissue repair processes. IL-13 has recently been described to be expressed by peripheral blood eosinophils derived from patients with atopic diseases, including AE, and as being released upon stimulation with eotaxin [87]. Eosinophil-derived IL-13 was functional, as it increased the surface expression of the low-affinity IgE receptor on purified B cells. Besides IL-13, RANTES and eotaxin have also been shown to be able to release IL-4 and IL-10 from eosinophils [50].

Besides cytokines, eosinophils contain lipid bodies, which play a role in the generation of eicosanoid mediators [93]. They are a major source of the cysteinyl leukotriene LTC<sub>4</sub> and its active metabolites LTD<sub>4</sub> and LTE<sub>4</sub>. The generation of LTC<sub>4</sub> by blood eosinophils of AE patients was enhanced when compared with healthy controls and did not depend on the presence or absence of associated bronchial asthma [94]. In asthma, a specific polymorphism within the promoter of the LTC<sub>4</sub> synthase has been identified that may contribute to increased LTC<sub>4</sub> synthesis [95]. Released leukotrienes may amplify the inflammatory cascade, for instance by acting as chemotactic factors or by triggering the release of cytotoxic proteins. Eosinophils have also been described as antigen-presenting cells. However, compared to professional antigen-presenting cells, they are relatively inefficient in activating T cells [96].

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### 31.4 Eosinophils as a Therapeutic Target

Eosinophils or factors participating in the development of eosinophilia, such as chemokines and cytokines, in particular IL-5, are interesting therapeutic targets in AE. Glucocorticoids, cyclosporin A and tacrolimus significantly inhibited IL-5 production by peripheral blood mononuclear cells from atopic patients [97]. Glucocorticoids have been shown to suppress IL-5 synthesis by targeting CD4<sup>+</sup> T cells activated via the T cell receptor or by IL-2 [98]. The reduction of IL-5 expression by corticosteroids was associated with

both reduced eosinophil production and increased eosinophil apoptosis [99, 100]. Cyclosporin A and tacrolimus have also been shown to be clinically effective in AE. Both drugs inhibited cytokine production of T cells, including IL-5, confirming the critical role of IL-5 and T cells in the pathogenesis of AE [10]. In a recent study, a decrease of eosinophils and Th2 cytokine-expressing T cells in lesional skin after treatment with topical tacrolimus was observed [101]. Also the beneficial clinical effect of phototherapy such as UVA irradiation was associated with a marked decrease of CD4<sup>+</sup> T cells and eosinophils [102].

Because of the pivotal role of IL-5 for eosinophilia and its selective activity on eosinophils and basophils, specific neutralization of this cytokine is a promising strategy in the treatment of eosinophilic diseases [103]. For instance, the administration of a neutralizing anti-IL-5 antibody to ovalbumin-sensitized mice during repeated allergen challenge prevented the development of airway hyperresponsiveness [104]. In patients with bronchial asthma, the therapy with an anti-IL-5 antibody was associated with a reduction of eosinophil numbers in both blood and sputum, although it had no effect on bronchial hyperreactivity [105]. In contrast, anti-IL-5 antibody therapy was clinically effective in patients with eosinophilic dermatitis [106].

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### 31.5 Conclusion

Despite the progress in understanding the immunology of AE, the pathogenesis of still remains unclear. The presence of eosinophils in the inflammatory infiltrate of AE has long been known. Eosinophil numbers as well as eosinophil granule protein levels in the peripheral blood are elevated in most AE patients and appear to correlate with disease activity. Moreover, eosinophil granule proteins, which were shown to possess cytotoxic activities, are deposited in AE skin lesions. These observations point to a potential important role of eosinophils in the pathogenesis of AE. Furthermore, AE is associated with increased production of Th2 cytokines including IL-5, which specifically acts on eosinophils, resulting in accelerated eosinophilopoiesis, chemotaxis, cell activation, and delayed apoptosis. Therefore, IL-5 is an interesting target for therapy of AE.

## References

1. Kato M, Kephart GM, Talley NJ, Wagner JM, Sarr MG, Bonno M, McGovern TW, Gleich GJ (1998) Eosinophil infiltration and degranulation in normal human tissue. *Anat Rec* 252:418–425
2. Straumann A, Simon HU (2004) The physiological and pathophysiological roles of eosinophils in the gastrointestinal tract. *Allergy* 59:15–25
3. Mihm MC, Soter NA, Dvorak HF, Austen KF (1976) The structure of normal skin and the morphology of atopic eczema. *J Invest Dermatol* 67:305–312
4. Steigleder GK, Inderwisch R (1975) Eosinophilic leucocytes in the skin lesions of psoriasis and atopic dermatitis. *Arch Dermatol Res* 254:253–255
5. Braun-Falco O, Burg G (1974) Celluläres infiltrat und Capillaren bei Neurodermitis diffusa. *Arch Derm Forsch* 249:113–124
6. Kiehl P, Falkenberg K, Vogelbruch M, Kapp A (2001) Tissue eosinophilia in acute and chronic dermatitis: a morphometric approach using quantitative image analysis of immunostaining. *Br J Dermatol* 145:720–29
7. Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha RS (1999) Roles of Th1 and Th2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 103:1103–1111. Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha RS (1999) Roles of Th1 and Th2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 103:1103–1111
8. Kagi MK, Joller-Jemelka H, Wüthrich B (1992) Correlation of eosinophils, eosinophilic cationic protein and soluble interleukin-2 receptor with the clinical activity of atopic dermatitis. *Dermatology* 185:88–92
9. Uehara M, Izukura R, Sawai T (1990) Blood eosinophilia in atopic dermatitis. *Clin Exp Dermatol* 15:264–266
10. Akdis CA, Akdis M, Simon D, Dibbert B, Weber M, Gratzl S, Kreyden O, Disch R, Wüthrich B, Blaser K, Simon H-U (1999) T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis. *J Invest Dermatol* 113:628–634
11. Sur S, Glitz DG, Kita H, Kujawa SM, Peterson EA, Weiler DA, Kephart GM, Wagner JM, George TJ, Gleich GJ, Leiferman KM (1998) Localization of eosinophil-derived neurotoxin and eosinophil cationic protein in neutrophilic leukocytes. *J Leukoc Biol* 63:715–722
12. Czech W, Krutmann J, Schopf E, Kapp A (1992) Serum eosinophil cationic protein (ECP) is a sensitive measure for disease activity in atopic dermatitis. *Br J Dermatol* 126:351–355
13. Halmerbauer G, Frischer T, Koller DY (1997) Monitoring of disease activity by measurement of inflammatory markers in atopic dermatitis in childhood. *Allergy* 52:765–769
14. Taniuchi S, Chihara J, Kojima T, Yamamoto A, Sasai M, Kobayashi Y (2001) Serum eosinophil-derived neurotoxin may reflect more strongly disease activity in childhood atopic dermatitis than eosinophil cationic protein. *J Dermatol Sci* 26:79–82
15. Caproni M, Agata AD, Cappelli G, Fabbri P (1996) Modulation of serum eosinophilic cationic protein levels by cyclosporin in severe atopic dermatitis. *Br J Derm* 135:336
16. Stevens SR, Hanifin JM, Hamilton T, Tofte SJ, Cooper KD (1998) Long-term effectiveness and safety of recombinant human interferon gamma therapy for atopic dermatitis despite unchanged serum IgE levels. *Arch Dermatol* 134:799–804
17. Wakugawa M, Nakagawa H, Yamada N, Tamaki K (1996) Chronologic analysis of eosinophil granule protein deposition and cell adhesion molecule expression in mite allergen-induced dermatitis in atopic subjects. *Int Arch Allergy Immunol* 111:S5–11
18. Krutmann J, Czech W, Diepgen T, Niedner R, Kapp A, Schopf E (1992) High-dose UV A1 therapy in the treatment of patients with atopic dermatitis. *J Am Acad Dermatol* 26:225–230
19. Kim TY, Park HJ, Kim CW (1997) Eosinophil cationic protein (ECP) level and its correlation with eosinophil number or IgE level of peripheral blood in patients with various skin diseases. *J Dermatol Sci* 15:89–94
20. Ott NL, Gleich GJ, Peterson EA, Fujisawa T, Sur S, Leiferman KM (1994) Assessment of eosinophil and neutrophil participation in atopic dermatitis: comparison with the IgE-mediated late phase reaction. *J Allergy Clin Immunol* 94:120–128
21. Wassom DL, Loegering DA, Solley GO, Moore SB, Schooley RT, Fauci AS, Gleich GJ (1981) Elevated serum levels of the eosinophil granule major basic protein in patients with eosinophilia. *J Clin Invest* 67:651–661
22. Breuer K, Kapp A, Werfel T (2001) Urine eosinophil protein X (EPX) is an in vitro parameter of inflammation in atopic dermatitis in the adult age. *Allergy* 56:780–784
23. Tischendorf FW, Brattig NW, Lintzel M, Buttner DW, Burchard GD, Bork K, Muller M (2000) Eosinophil granule proteins in serum and urine of patients with helminth infections and atopic dermatitis. *Trop Med Int Health* 5:898–905
24. Prussin C, Metcalfe DD (2003) IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 111:S486–494
25. Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venencie PY, Gleich GJ (1985) Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis: comparison with onchocerciasis. *N Engl J Med* 313:282–285
26. Leiferman KM, Fujisawa T, Gray BH, Gleich GJ (1990) Extracellular deposition of eosinophil and neutrophil granule proteins in the IgE-mediated cutaneous late phase reaction. *Lab Invest* 62:579–589
27. Cheng JF, Ott NL, Peterson EA, George TJ, Hunkee MJ, Gleich GJ, Leiferman KM (1997) Dermal eosinophils in atopic dermatitis undergo cytolytic degeneration. *J Allergy Clin Immunol* 99:683–692
28. Leiferman KM (2001) A role for eosinophils in atopic dermatitis. *J Am Acad Dermatol* 45:S21–24
29. Denburg JA, Telizyn S, Messner H, Lim B, Jamal N, Ackerman SJ, Gleich GJ, Bienenstock J (1985) Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. *Blood* 66:312–318
30. Linden M, Svenson C, Andersson M, Greiff L, Andersson E, Denburg JA, Persson CG (1999) Circulating eosinophil/basophil progenitors and nasal mucosal cytokines in seasonal allergic rhinitis. *Allergy* 54:212–219

31. Cyr MM, Denburg JA (2001) Systemic aspects of allergic diseases: the role of the bone marrow. *Curr Opin Immunol* 13:727–732
32. Dent LA, Strath M, Mellor AL, Sanderson CJ (1990) Eosinophilia in transgenic mice expressing interleukin 5. *J Exp Med* 172:1425–1431
33. Foster P, Hogan P, Ramsay AJ, Matthaei KI, Young IG (1996) Interleukin-5 deficiency abolishes eosinophilia, airway hyperreactivity and lung damage in a mouse asthma model. *J Exp Med* 183:195–201
34. Nashinakamura R, Miyajima A, Mee PJ, Tybulewicz VLJ, Murray R (1996) Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood* 88:2458–2464
35. Saito H, Howie K, Wattie J, Denburg A, Ellis R, Inman MD, Denburg J (2001) Allergen-induced murine upper airway inflammation: local and systemic in murine experimental allergic rhinitis. *Immunology* 104:226–234
36. Palframan RT, Collins PD, Severs NJ, Rothery S, Williams TJ, Rankin SM (1998) Mechanisms of acute eosinophil mobilization from the bone marrow stimulated by interleukin-5: the role of specific adhesion molecules and phosphatidylinositol 3-kinase. *J Exp Med* 188:1621–1632
37. Palframan RT, Collins PD, Williams TJ, Rankin SM (1998) Eotaxin induces a rapid release of eosinophils and their progenitors from the bone marrow. *Blood* 91:2240–2248
38. Broide D, Sriramarao P (2001) Eosinophil trafficking to sites of allergic inflammation. *Immunological Reviews* 179:163–172
39. Bochner BS, Charlesworth EN, Lichtenstein LM (1990) Interleukin-1 is released at sites of human cutaneous allergic reactions. *J Allergy Clin Immunol* 86:830–839
40. Mullarkey M, Leiferman KM, Peters MS, Caro I, Roux ER, Hanna RK, Rubin AS, Jacobs CA (1994) Human cutaneous allergic late-phase response is inhibited by soluble IL-1 receptor. *J Immunol* 152:2033–2041
41. Wakita H, Sakamoto T, Tokura Y, Takigawa M (1993) E-selectin and vascular adhesion molecule 1 as critical adhesion molecules for infiltration of T lymphocytes and eosinophils in atopic dermatitis. *J Cutan Pathol* 21:33–39
42. Dobrina A, Menegazzi R, Carlos TM, Nardon E, Cramer R, Zacchi T, Harlan JM, Patriarca P (1991) Mechanisms of eosinophil adherence to cultured vascular endothelial cells. *J Clin Invest* 88:20–26
43. Moser R, Fehr J, Bruijnzeel LB (1992) IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J Immunol* 149:1432–1438
44. Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Luscinskas FW, Gimbrone MA, McIntyre BW, Bochner BS (1992) IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 148:1086–1092
45. Schnyder B, Lugli S, Feng N, Etter H, Lutz RA, Ryffel B, Sugamura K, Wunderli-Allenspach H, Moser R (1996) IL-4 and IL-13 bind to a shared heterodimeric complex on endothelial cells mediating vascular adhesion molecule-1 induction in the absence of the common  $\gamma$  chain. *Blood* 87:4286–4295
46. Elsner J, Kapp A (1999) Regulation and modulation of eosinophil effector functions. *Allergy* 54:15–26
47. DiScipio R, Daffern P, Jagels MA, Broide DH, Sriramarao P (1999) C3a and C5a mediate the rapid activation dependent conversion of rolling eosinophils to firmly adherent eosinophils in vivo. *J Immunol* 162:1127–1136
48. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K (2001) Prostaglandin D2 selectively induces chemotaxis in T-helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 193:255–262
49. Tager AM, Dufour JH, Goodarzi K, Bercury SD, von Adrian UH, Luster AD (2000) BLTR mediates leukotriene (4)-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. *J Exp Med* 192:439–446
50. Bandeira-Melo C, Bozza PT, Dias BL, Cordeiro RS, Jose PJ, Martins MA, Serhan CN (2000) Lipoxin (LX) A4 and aspirin-triggered 15-epi-LXA4 block allergen-induced eosinophil trafficking. *J Immunol* 164:2267–2271
51. Mochizuki M, Bartels J, Mallet AI, Christophers E, Schröder JM (1998) IL-4 induces eotaxin: A possible mechanism of selective eosinophil recruitment in helminth infection and atopy. *J Immunol* 160:60–68
52. Dombrovicz D, Capron M (2001) Eosinophils, allergy and parasites. *Curr Opin Immunol* 13:716–720
53. Rothenberg ME, MacLean JA, Pearlman E, Luster AD, Leder P (1997) Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J Exp Med* 185:785–790
54. Ying S, Tabora-Barate L, Meng Q, Humbert M, Kay MB (1995) The kinetics of allergen-induced transcription of messenger RNA for monocyte chemotactic protein-3 and RANTES in the skin of human atopic subjects: relationship to eosinophil, T cell, and macrophage recruitment. *J Exp Med* 181:2153–2159
55. Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, Martinez-A C, Dorf M, Bkerke T, Coyle AJ, Gutierrez-Ramos JC (1998) The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J Exp Med* 188:157–167
56. Sallusto F, Mackay CR, Lanzavecchia A (2000) The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 18:593–620
57. Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, Newman W, Gerard C, Mackay CR (1996) Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med* 183:2349–2354
58. Homey B, Zlotnik A (1999) Chemokines in allergy. *Curr Opin Immunol* 11:626–634
59. Yawalkar N, Ugucioni M, Schärer J, Braunwalder J, Karlen S, Dewald B, Braathen LR, Baggiolini M (1999) Enhanced expression of eotaxin and CCR-3 in atopic dermatitis. *J Invest Dermatol* 113:43–48
60. Weillie M, Bryce PJ, Humbles AA, Laouini D, Yalcindag A, Alenius H, Friend DS, Oettgen HC, Gerard C, Geha RS (2002) CCR-3 is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of allergic skin inflammation. *J Clin Invest* 109:621–628
61. Masumoto A, Hemler ME (1993) Multiple activation states



- of VLA-4. Mechanistic differences between adhesion to CS-1/fibronectin and to vascular cell adhesion molecule-1. *J Biol Chem* 268:228–234
62. Gleich GJ (2000) Mechanisms of eosinophil associated inflammation. *J Allergy Clin Immunol* 105:651–663
63. Menzies-Gow A, Ying S, Sabroe I, Stubbs VL, Soler D, Williams T, Kay AB (2002) Eotaxin and eotaxin-2 induce recruitment of eosinophils, basophils, neutrophils, and macrophages as well as features of early- and late-phase allergic reactions following cutaneous injection in human atopic and nonatopic volunteers. *J Immunol* 169:2712–2718
64. Bruynzeel-Koomen CAFM, van Wichem DF, Spry CJF, Venge P, Bruynzeel PLB (1988) Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Br J Dermatol* 118:229–238
65. Mitchell EB, Chapman MD, Pope FM, Crow J, Jouhal SS, Platts-Mills TAE (1982) Basophils in allergen-induced patch test sites in atopic dermatitis. *Lancet I*: 127–130
66. Walker C, Kagi MK, Ingold P, Braun P, Blaser K, Bruynzeel-Koomen CA, Wüthrich B (1993) Atopic dermatitis: correlation of peripheral blood T cell activation, eosinophilia and serum factors with clinical severity. *Clin Exp Allergy* 23:145–153
67. Niggemann B, Beyer K, Wahn U (1994) The role of eosinophils and eosinophil cationic protein in monitoring oral challenge tests in children with food sensitive atopic dermatitis. *J Allergy Clin Immunol* 94:963–971
68. Simon D, Braathen LR, Simon HU (2003) Eosinophils and atopic dermatitis. *Allergy* 59:561–570
69. Simon HU (1998) Eosinophil apoptosis in allergic diseases – an emerging new issue. *Clin Exp Allergy* 28:1321–1324
70. Simon HU, Yousefi S, Schranz C, Schapowal A, Bachert C, Blaser K (1997) Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. *J Immunol* 158:3902–3908
71. Simon HU (2000) Eosinophil apoptosis—pathophysiologic and therapeutic implications. *Allergy* 55:910–915
72. Simon HU, Blaser K (1995) Inhibition of programmed eosinophil death: a key pathogenic event for eosinophilia? *Immunol Today* 16:53–55
73. Shinagawa K, Trifilieff A, Anderson GP (2003) Involvement of CCR3-reactive chemokines in eosinophil survival. *Int Arch Allergy Immunol* 130:150–157. (2003) Involvement of CCR3-reactive chemokines in eosinophil survival. *Int Arch Allergy Immunol* 130:150–157
74. Wedi B, Raap U, Lewrick H, Kapp A (1997) Delayed eosinophil programmed cell death in vitro: a common feature in inhalant allergy and extrinsic and intrinsic dermatitis. *J Allergy Clin Immunol* 100:536–543
75. Dibbert B, Daigle I, Braun D, Schranz C, Weber M, Blaser K, Zangemeister-Wittke U, Akbar AN, Simon HU (1998) Role for Bcl-xL in delayed eosinophil apoptosis mediated by granulocyte-macrophage colony-stimulating factor and interleukin-5. *Blood* 92:778–783
76. Ogawa K, Hashida R, Miyagawa M, Kagaya S, Sugita Y, Matsumoto K, Katsunuma T, Akasawa A, Tsujimoto G, Saito H (2003) Analysis of gene expression in peripheral blood eosinophils from patients with atopic dermatitis and in vitro cytokine-stimulated blood eosinophils. *Clin Exp Immunol* 131:436–445
77. Ploetz SG, Dibbert B, Abeck D, Ring J, Simon HU (1998) Bcl-2 expression by eosinophils in a patient with hypereosinophilia. *J Allergy Clin Immunol* 102:1037–1040
78. Wedi B, Raap U, Kapp A (1999) Significant delay of apoptosis and Fas resistance in eosinophils of subjects with intrinsic and extrinsic type of atopic dermatitis. *Int Arch Allergy Immunol* 118:234–235
79. Hebestreit H, Dibbert B, Balatti I, Braun D, Schapowal A, Blaser K, Simon HU (1998) Disruption of Fas receptor signaling by nitric oxide in eosinophils. *J Exp Med* 187:415–425
80. Takafuji S, Bischoff SC, de Weck AL, Dahinden CA (1991) IL-3 and IL-5 prime normal eosinophils to produce leukotriene C4 in response to soluble agonists. *J Immunol* 147:3855–3861
81. Rothenberg ME, Owen WF, Silberstein DS, Soberman RJ, Austen KF, Stevens RL (1987) Eosinophils cocultured with endothelial cells have increased survival and functional properties. *Science* 237:645–647
82. Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P, Kay AB (1992) Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* 79:2952–2959
83. Tomioka K, MacGlashan DW, Lichtenstein LM, Bochner BS, Schleimer RP (1993) GM-CSF regulates human eosinophil responses to F-Met peptide and platelet activating factor. *J Immunol* 151:4989–4997
84. Dahinden CA, Zingg J, Maly FE, de Weck AL (1988) Leukotriene production in human neutrophils primed by recombinant human granulocyte/macrophage colony-stimulating factor and stimulated with the complement component C5a and fMLP as second signals. *J Exp Med* 167:1281–1295
85. Simon HU, Plötz S, Simon D, Seitzer U, Braathen LR, Menz G, Straumann A, Dummer R, Levi-Schaffer F (2003) Interleukin-2 primes eosinophil degranulation in hypereosinophilia and Wells' syndrome. *Eur J Immunol* 33:834–839
86. Simon HU, Weber M, Becker E, Zilberman Y, Blaser K, Levi-Schaffer F (2000) Eosinophils maintain their capacity to signal and release eosinophilic cationic protein upon repetitive stimulation with the same agonist. *J Immunol* 165:4069–4075
87. Schmid-Grendelmeier P, Altzauer F, Fischer B, Bizer C, Straumann A, Menz G, Blaser K, Wüthrich B, Simon HU (2002) Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol* 169:1021–1027
88. Yousefi S, Hemmann S, Weber M, Hoelzer C, Hartung K, Blaser K, Simon HU (1995) IL-8 is expressed by human peripheral blood eosinophils. *J Immunol* 154:5481–5490
89. Bochner BS (2000) Systemic activation of basophils and eosinophils: markers and consequences. *J Allergy Clin Immunol* 106:S292–302
90. Gounni AS, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M (1994) High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 367:183–186
91. Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, Gleich GJ (1999) Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 162:6901–6911

92. Seminario MC, Saini SS, MacGlashan DW, Bochner BS (1999) Intracellular expression and release of FcεRIα by human eosinophils. *J Immunol* 162:6893–6900
93. Bandeira-Melo C, Bozza PT, Weller PF (2002) The cellular biology of eosinophil eicosanoid formation and function. *J Allergy Clin Immunol* 109:393–400
94. Schauer U, Trube M, Jäger R, Gieler U, Rieger CH (1995) Blood eosinophils, eosinophil-derived proteins, and leukotriene C4 generation in relation to bronchial hyperreactivity in children with atopic dermatitis. *Allergy* 50:126–132
95. Sanak M, Simon HU, Szczeklik A (1997) Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 350:1599–1600
96. Mawhorter SD, Kazura JW, Boom WH (1994) Human eosinophils as antigen-presenting cells: relative efficacy for superantigen- and antigen-induced CD4+ T-cell proliferation. *Immunology* 81:584–591
97. Mori A, Suko M, Nishizaki Y, Kaminuma O, Matsuzaki G, Ito K, Etoh T, Nakagawa H, Tsuruoka N, Okudaira H (1994) Regulation of interleukin-5 production by peripheral blood mononuclear cells from atopic patients with FK506, cyclosporinA and glucocorticoid. *Int Arch Allergy Immunol* 104:S32–35
98. Mori A, Kaminuma O, Suko M, Inoue S, Ohmura T, Hoshino A, Asakura Y, Miyazawa K, Yokota T, Okumura Y, Ito K, Okudaira H (1997) Two distinct pathways of interleukin-5 synthesis in allergen-specific human T-cell clones are suppressed by glucocorticoids. *Blood* 89:2891–2900
99. Meagher LC, Cousin JM, Seckl JR, Haslett C (1996) Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J Immunol* 156:4422–4428
100. Oehling AG, Akdis CA, Schapowal A, Blaser K, Schmitz M, Simon HU (1997) Suppression of the immune system by oral glucocorticoid therapy in bronchial asthma. *Allergy* 52:144–154
101. Simon D, Conus S, Vassina E, Braathen LR, Simon HU (2003) Immunopharmacological effects of topical tacrolimus in atopic dermatitis. *J Eur Acad Derm Venerol* 17: S156
102. Breuckmann F, von Kobyletzki G, Avermaete A, Pieck C, Kreuter A, Brockmeyer NH, Altmeyer P, Gambichler T (2002) Mononuclear cells in atopic dermatitis in vivo: immunomodulation of the cutaneous infiltrate by medium-dose UVA1 phototherapy. *Eur J Med Res* 7:315–322
103. Simon HU (2002) The neutralization of Interleukin-5 as a therapeutic concept in allergic inflammation. *Sarcoidosis Vasc Diffuse Lung Dis* 19:25–28
104. Hamelmann E, Oshiba A, Loader J, Larsen GL, Gleich G, Lee J, Gelfand EW (1997) Anti-interleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am J Respir Crit Care Med* 155:819–825
105. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ (2000) Effects of an interleukin-5 blocking antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 356:2144–2148
106. Plötz SG, Simon HU, Darsow U, Simon D, Vassina E, Yousefi S, Hein R, Smith T, Behrendt H, Ring J (2003) Use of anti-interleukin-5 antibody in hypereosinophilic syndrome with eosinophilic dermatitis. *N Eng J Med* 349: 2332–2337