The Role of Eosinophils in Atopic Eczema 31

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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^2 (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

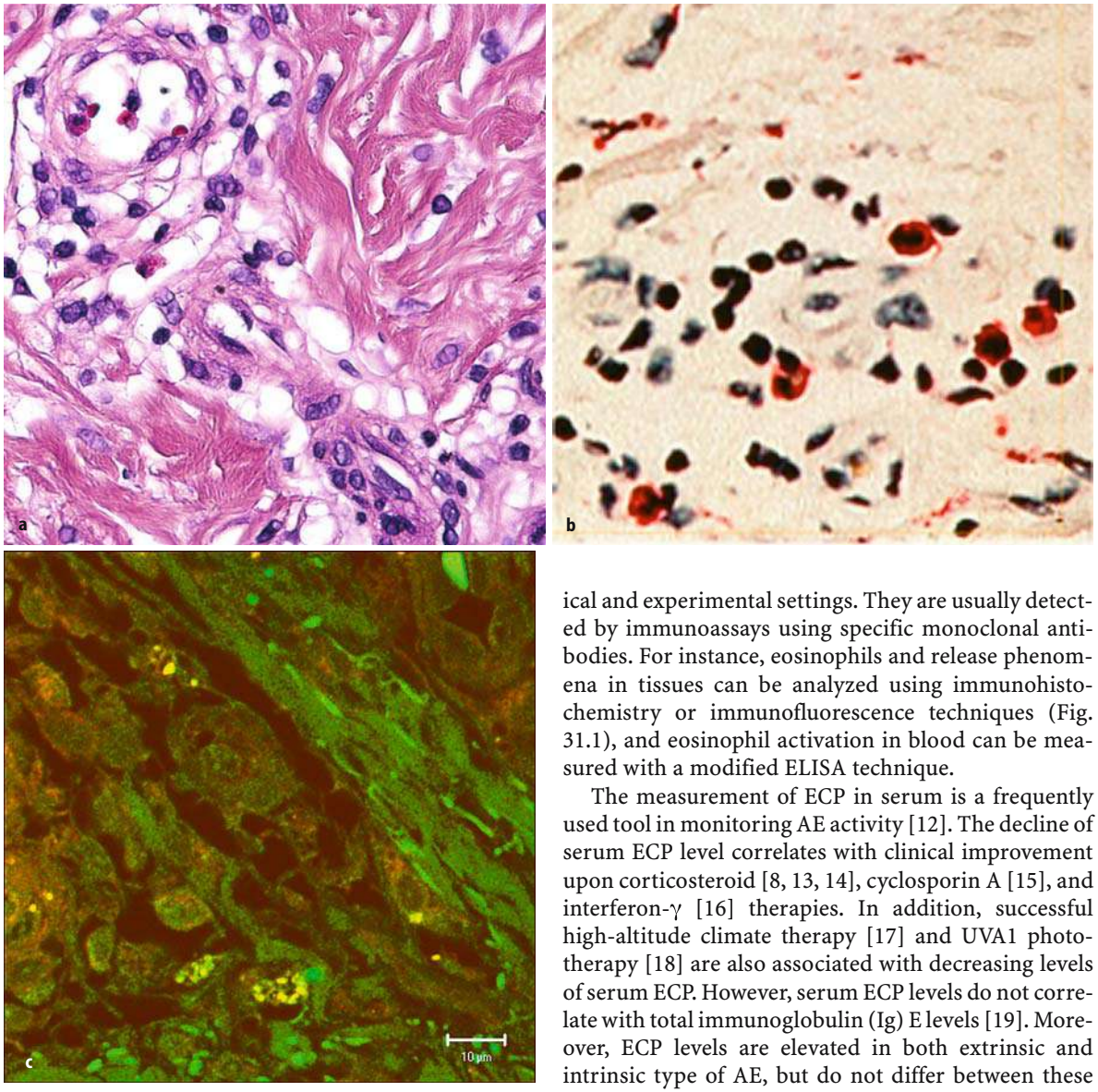

Fig. 31.1. Eosinophilic infiltrates in lesional skin of AE demonstrated by eosin-hematoxylin staining (**a**), immunohistochemistry (anti-ECP; APAAP technique) (**b**), and immunofluorescence (anti-ECP, confocal microscopy) (**c**)

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 clinical 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-γ [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.

31.2 Mechanisms Causing Eosinophilia

31.2.1 Regulation of Eosinophil Production in the Bone Marrow

Eosinophils are derived from a $CD34^+$ 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 α-subunit of the interleukin-5 receptor (IL-5Rα) was seen to be upregulated on bone marrow $CD34^+$ 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.

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-1β 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 (α4β1 and α4β7 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 LTB_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-γ 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-1α 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 postcapillary 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 $CD25^+$ 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_4 and its active metabolites LTD_4 and LTE_4. The generation of LTC_4 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_4 synthase has been identified that may contribute to increased LTC_4 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].

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 $CD4^+$ 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 $CD4^+$ 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].

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.

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