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Regulation and modulation of eosinophil effector functions

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The presence of peripheral blood eosinophilia and activated eosinophils in the inflammatory tissue are characteristic features of allergic diseases such as allergic asthma, rhinoconjunctivitis, and atopic dermatitis (1–10). Moreover, eosinophils are believed to be of major importance in other inflammatory diseases. These include connective-tissue diseases of unknown origin such as bullous dermatoses and hypereosinophilic syndromes (11–15). Tissue damage and propagation of inflammation are thought to be mediated by the interaction between Th2-like T cells (16, 17), antigen-presenting cells (18), and eosinophils (1). In this process, eosinophils are activated by several cytokines and inflammatory mediators, leading to invasion of eosinophils at the site of inflammation and to tissue damage by the release of toxic granule proteins and release of reactive oxygen species (19–24).

Cytokines, chemokines, and inflammatory mediators, such as GM-CSF, interleukin (IL)-5, eotaxin, platelet-activating factor (PAF), C5a, and C3a (25–29), are responsible for the activation of human eosinophils (Fig. 1). Under the high shear forces present in the blood flow, eosinophils first become tethered and then roll along the vessel surface (30, 31). When local signals such as cytokines or chemokines are released in their vicinity, the cells are arrested, develop firm adhesion, and then migrate across the endothelium. Thereafter, cells orientate their locomotion along a gradient of a chemotactic factor by extending lamellipodia in the direction of the higher concentration of the chemotactic factor (30). Migration requires the coordination of a cycle of cytoskeletal proteins such as actin, entailing the formation of adhesive contacts at the leading edge of the cell, and breaking adhesive contacts, and cytoskeleton-dependent retraction at the trailing edge (32–34). In a previous study,

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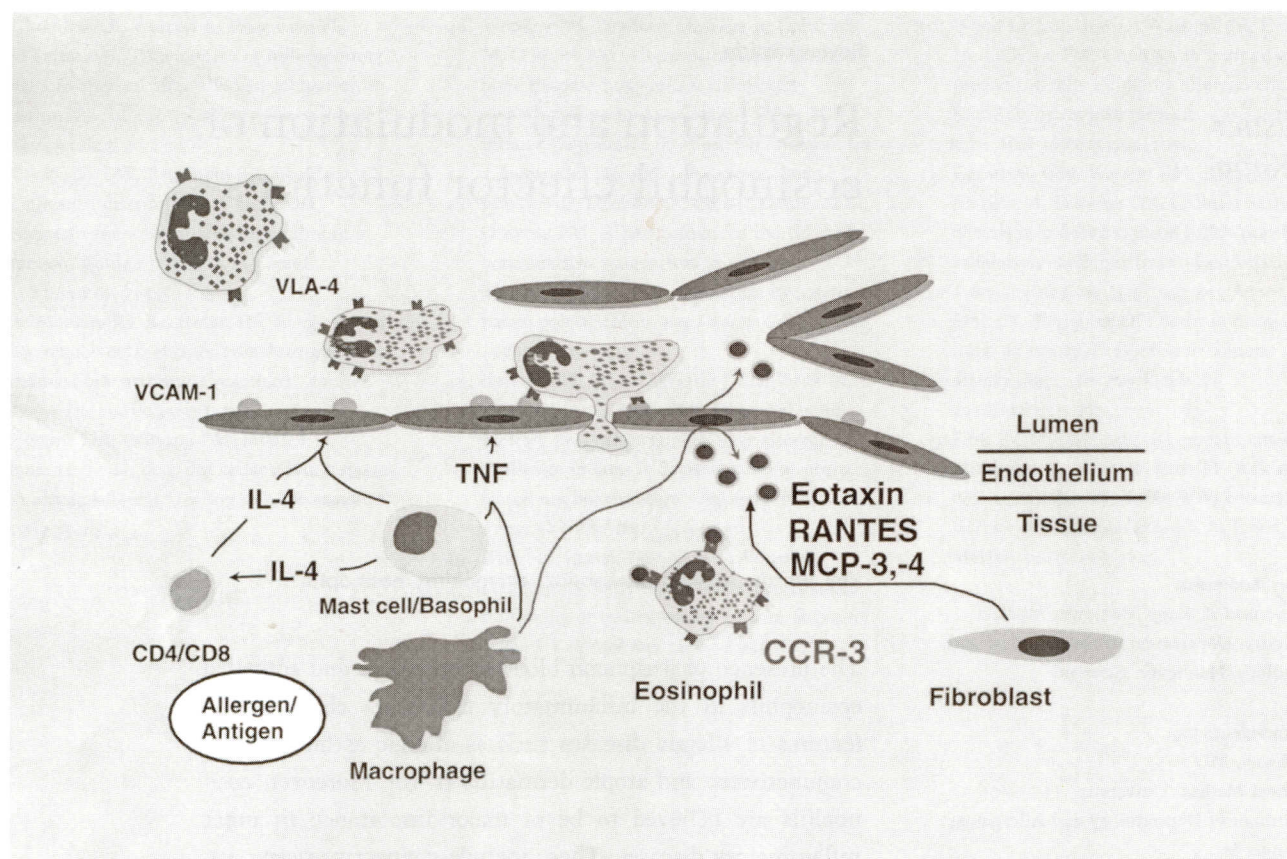


Figure 1. Model of eosinophilic inflammation. Attraction to and activation of eosinophils at site of inflammation and release of reactive oxygen species, leading to tissue damage and propagation of inflammatory response, are mediated by chemokines and cytokines.

we could demonstrate that intracellular Ca^{2+} fluxes in eosinophils after stimulation with C_5a , PAF, and RANTES represent an important step in the activation leading to directed migratory response and actin polymerization (35a).

At the site of inflammation, eosinophils release toxic proteins such as eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil-derived neurotoxin (EDN), and reactive oxygen species, leading to tissue damage of the host (19, 20, 24, 36–38). Reactive oxygen species are generated by the NADPH oxidase that can be activated by a number of different soluble and particulate agents. Intracellular Ca^{2+} seems to play a central role in the modulation of the respiratory burst in eosinophils (37). The activation results in a reduction of molecular oxygen to the potentially toxic oxygen species superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2), with NADPH serving as the electron donor. The increase in oxygen consumption is termed the respiratory burst (39–41) and is a major cause of tissue damage and propagation of the inflammatory response by acting as a competence signal in T cells, inducing early gene expression, particularly IL-2, as well as cell proliferation (19, 20, 37, 38, 42–44).

Until now, there has been no specific therapy to hinder the eosinophil response in allergic and nonallergic diseases. The treatment of patients with steroids, dapsone, and H_1 -receptor antagonists has been reported in eosinophilic diseases; however, these compounds have also been effective on other cells (45). Therefore, agents that would be able to inhibit or antagonize mediator-induced eosinophil activation seem to be of interest as a new therapeutic strategy. In this review, we will focus on the functional properties and the modulation of human eosinophils in inflammation. We will then consider whether modulation of eosinophil effector functions might be successful as a future therapeutic strategy for diseases that are accompanied by activated eosinophils.

Surface receptors on human eosinophils

General surface molecules

Many of the interactions between inflammatory cells and their environment are mediated by surface molecules which bind both intracellular and extracellular ligands and thus

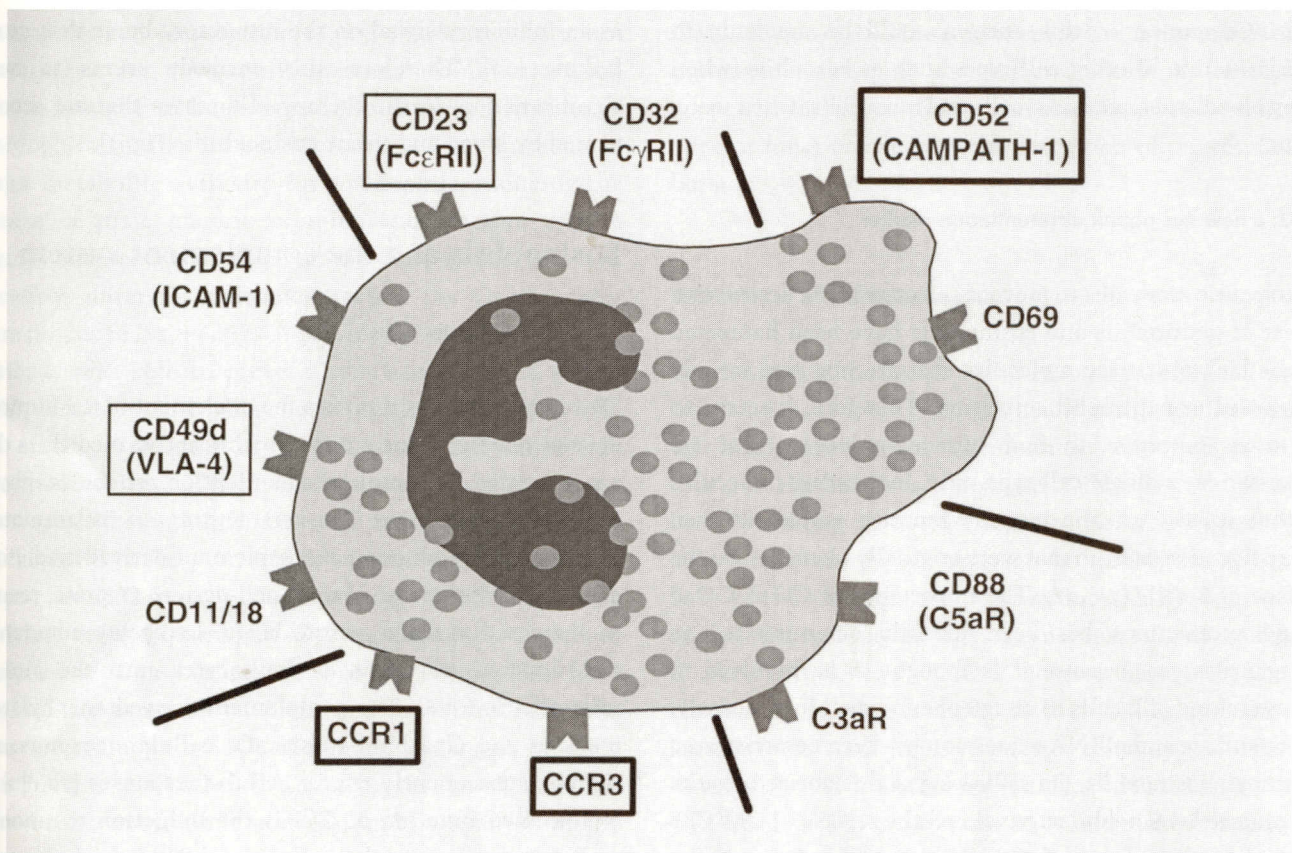


Figure 2. Surface molecules on human eosinophils. Surface markers have been used to distinguish neutrophils from eosinophils and might indicate different functional properties of these cells. Summary of most important functional surface molecules is shown. Note that surface molecules indicated by box are expressed on eosinophils only.

transfer signals across the membrane. These signals may result in changes in cytoskeletal morphology, gene expression, and cellular differentiation and secretion. Eosinophils share some surface molecules with other granulocytes such as adhesion molecule receptors (34, 46–51), Fc receptors (52–54), cytokine receptors, chemokine receptors (55–59), and complement receptors (60). Surface markers have been used to distinguish neutrophils from eosinophils and might indicate different functional properties of these cells. A summary of the most important functional surface molecules is given in Fig. 2.

CD69 on eosinophils

An interesting molecule on the surface of eosinophils is the CD69 antigen, an activation antigen that is also found on activated neutrophils, lymphocytes, and natural killer (NK) cells (61–63). CD69 is not constitutively expressed on fresh eosinophils, but culture of eosinophils with GM-CSF, IL-3, or IL-5 induces CD69 expression. Moreover, eosinophils in bronchoalveolar lavage fluid from patients with asthma and pulmonary eosinophilia also express CD69 (61, 62). More

recently, ligation of CD69 was shown to induce apoptosis and cell death in human eosinophils cultured with GM-CSF and IL-13 (63). Therefore, this antigen might play an important role in eosinophil removal *in vivo* (64).

Fc_ε-receptors on eosinophils

Controversial results have been reported for Fc_ε-receptor expression on human eosinophils (52, 65–69). The constitutive expression of the low-affinity IgE receptor (Fc_ε-RII/CD23) has been demonstrated at mRNA level on human eosinophils; however, flow cytometric analysis shows only limited and heterogeneous expression of Fc_ε-RII/CD23 receptors (52, 66). In addition, of most interest is the observation that eosinophils also express the high-affinity IgE receptor (Fc_ε-RI) that is involved in degranulation and antigen presentation of mast cells and Langerhans' cells (18, 69). However, studies in which flow cytometry or immunostaining was used did not reveal the expression of Fc_εRI protein on human eosinophils (70). Only mRNA of Fc_εRI in eosinophils was detected in several studies (70). Therefore, it is doubtful whether eosinophils express Fc_εRI, because

mRNA detection of this antigen could be due only to contamination of other cell types such as basophils (when using blood) or Langerhans' cells and mast cells (when using tissue).

CD52: a new eosinophil differentiation marker

Therapeutic strategies to prevent selectively the destructive power of neutrophils and eosinophils have been hampered by the lack of surface molecules that are involved in only eosinophil or neutrophil activation. Therefore, it seems to be more important to find surface molecules that are expressed on a single cell type only. Interesting molecules in that respect are the recently reported surface antigen CD40 ligand and CD40 that were originally identified on the surface of B cells (71, 72). The importance of CD40-CD40 ligand interactions has been recently demonstrated in allergic diseases, because it is thought to be involved in the switching of B cells to an IgE phenotype. More recently, CD40 surface and mRNA expression has been demonstrated in human eosinophils. Cross-linking of this antigen results in enhanced eosinophil survival and the release of GM-CSF (72). Another molecule that has become of interest is the adhesion molecule CD49d that is expressed on the surface of eosinophils (48, 49, 51). It has been demonstrated that CD49d/VCAM-1 interaction plays a predominant role in controlling antigen-induced eosinophil recruitment into the tissue. However, the anti-CD49d mAb alone was not able to prevent eosinophil invasion of inflammatory tissue (48, 49, 51). Another surface molecule that has been found to distinguish eosinophils from neutrophils is the surface antigen CD9 (73). Flow cytometric analysis clearly shows that this antigen is also expressed on neutrophils (personal communication).

In a recent study, we have attempted to find a surface molecule expressed on eosinophils, but not on neutrophils. We could demonstrate that CD52 is a GPI-anchored molecule expressed on the surface and at mRNA level by human eosinophils, but not on neutrophils (60). The CD52 antigen first identified on T cells and macrophages is an unusually good target for complement-mediated attack; therefore, anti-CD52 mAbs have been widely used for removal of T cells from donor bone marrow to prevent graft-versus-host disease in man (74-76). Anti-CD52 mAbs are therefore suitable to distinguish eosinophils from neutrophils as a simple marker molecule in flow cytometry, and also to enrich neutrophils from hypereosinophilic patients. Furthermore, the importance of the CD52 molecule on human eosinophils has been shown by its function

as an inhibitory signal on the respiratory burst after cross-linking (60). Therefore, this antibody seems to be a significant tool in the therapy of diseases that are accompanied by blood and tissue eosinophilia (Fig. 3).

Eosinophils and the complement system

C3a activates eosinophils

The complement system is a major element of the humoral defense reaction. One group of highly active mediators that are generated by complement activation are the anaphylatoxins C3a, C4a, and C5a (77). During an inflammatory process, local production of complement-derived mediators, e.g., due to the release of mast-cell-derived tryptase, results in increased vascular permeability, leukocyte adherence, and directed migration of leukocytes into the site of inflammation (78). The complement-derived anaphylatoxins C3a and C5a bind to specific cellular receptors and thereby subsequently trigger cellular responses (79, 80). A well-known function of C3a is the induction of smooth-muscle contraction, the release of histamine from mast cells, and the activation of guinea-pig platelets (77). The role of C3a in the modulation of leukocyte functions, in contrast to its chemical analog C5a, is poorly understood and controversial. Earlier studies reported that C3a induced granule release, chemotaxis, and aggregation of neutrophils (81, 82). However, study of most of the earlier reported effects of C3a has been hampered due to the contamination of C3a preparations with C5a. Previous studies revealed biologic effects of purified C3a, in addition to C5a, in the activation of the respiratory burst in human eosinophils (26, 83). This activation was coupled specifically to the C3a receptor. Stimulation of eosinophils by C3a involves pertussis toxin-sensitive G_i -proteins and leads to a transient rise in $[Ca^{2+}]_i$. Therefore, C3a plays an important role as a potent mediator of the microbicidal and tissue-destructive power of eosinophils and may take part in the pathophysiology of diseases with complement activation and activated eosinophils, such as inflammatory skin diseases (26, 83).

C5a receptor on human eosinophils

C5a seems to be the most potent proinflammatory mediator derived from the complement system (84, 85). Besides inducing the release of granule enzymes and reactive oxygen species, C5a also acts as a chemotaxin for

neutrophils and eosinophils (26, 35, 83). Since C3a is not chemotactic for eosinophils (personal communication), C5a is thought to be responsible for the infiltration of eosinophils in the tissue. Moreover, C5a represents a major metabolite activator for eosinophils, inducing the release of toxic granule proteins and reactive oxygen species that cause damage to the host tissue (28, 37). Recently, different antibodies against the C5a receptor were produced (84, 86, 87). A polyclonal anti-C5a receptor antibody was able to prevent zymosan-activated serum (ZAS)-induced neutrophil chemotaxis, and the monoclonal anti-C5a receptor mAb S5/1 specifically inhibited C5a-induced intracellular calcium transients in human neutrophils (86, 87). In contrast to the well-characterized neutrophil C5a receptor, which was recently identified as a 350-residue G protein-coupled receptor, there are only a few data on the eosinophil C5a receptor (88). Scatchard analyses of C5a binding to human eosinophils have implied the existence of high- and low-affinity binding sites on eosinophils (89). Moreover, cyanogen bromide treatment of ^{125}I -labeled C5a-receptor complexes obtained from neutrophils and eosinophils resulted in the appearance of different ^{125}I -labeled C5a-receptor fragments (89).

A previous study demonstrated that the anti-C5a receptor mAb S5/1 detected homogeneous C5a receptor expression on the surface of human eosinophils and completely inhibited several eosinophil effector functions such as the release of reactive oxygens and chemotaxis (60). The human eosinophil C5a receptor is homogeneously expressed not only on normal eosinophils from healthy donors but also on hypodense and normodense eosinophil subpopulations which were obtained from patients with hypereosinophilia (60). According to the inhibitory effect of the S5/1 mAb on C5a-induced eosinophil effector functions, a single receptor type, identified by this mAb, mediates C5a effects in these cells. In addition, the inhibitory effect of the S5/1 mAb on C5a functions may enable a new experimental approach to the treatment of diseases that have been associated with C5a-mediated activation (60) (Fig. 3).

Eosinophils are activated by cytokines and chemokines

Cytokines

Eosinophils are produced in the bone marrow under the stimulation of differentiation factors, such as IL-3, IL-5, and

GM-CSF (90–93). These cytokines are also involved in prolonged eosinophil survival in culture by abrogating apoptosis. Moreover, they are involved in several eosinophil effector functions such as the activation of the respiratory burst (27, 54, 90).

Chemokines activate eosinophils

Besides several mediators such as C5a, C3a, PAF, and 5-oxo-eicosanoids (26, 28, 83, 94, 95), a new family of chemotactic cytokines, now termed "chemokines", have become interesting in the last decade because of their restricted target-cell specificity (25, 96–100). So far, four chemokine subfamilies are known and can be distinguished on the basis of the arrangement of the amino-acid cysteine in the amino-terminal region: CXC chemokines, CC chemokines, C chemokines, and CXXC chemokines (96, 97). The chemokine subclasses differ in their biologic activity to stimulate different kinds of effector cells (25, 96). Chemokines of the CXC chemokine family, such as IL-8, ENA-78, GRO- α , and GCP-2, activate mainly neutrophils (25, 96, 101–109). Chemokines of the CC chemokine family, such as RANTES, MIP-1 α , MCP-2, MCP-3, MCP-4, eotaxin, and eotaxin-2, activate mainly eosinophils (25, 27, 96, 110–114). In contrast to these CC chemokines, the effect of the recently cloned CC chemokines AMAC-1, leukotactin-1, and exodus-1, -2, and -3 on eosinophils has not been investigated (115–119).

A more recent study has revealed the importance of the CC chemokine profile in the skin of human atopic subjects (23). Intradermally, timothy grass-pollen extract allergen challenge results in a significant increase in mRNA⁺ cells for MCP-3, which peaked at 6 h and progressively declined at 24 and 48 h. This parallels the kinetics of total (MBP⁺ cells) and activated (EG2⁺ cells) eosinophil infiltration (23). The allergen-induced expression of cells mRNA⁺ for RANTES is also observed at 6 h, reaches the maximum at 24 h, and decreases at 48 h. Moreover, the number of mRNA⁺ cells for RANTES parallels the kinetics of infiltration of CD3⁺, CD4⁺, and CD8⁺ T cells, whereas the number of CD68⁺ macrophages was still increasing at 48 h. Therefore, these data suggest that MCP-3 seems to be involved in the regulation of the early eosinophil response to specific allergen, whereas RANTES may be involved in the later accumulation of T cells and macrophages (23). Moreover, eosinophil recruitment occurred more rapidly in allergic subjects than in nonallergic subjects after intradermal injection of RANTES (120).

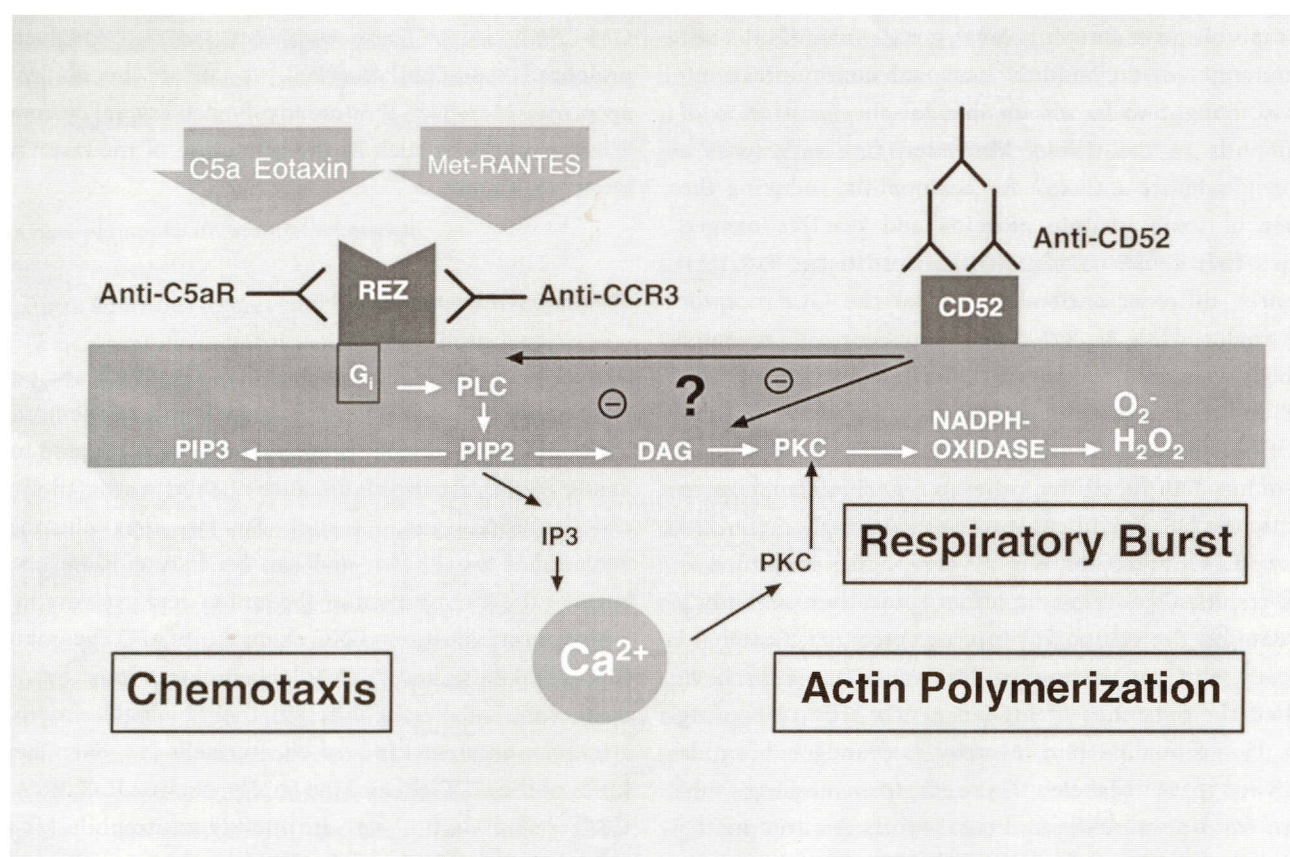


Figure 3. Modulation of eosinophil effector functions. Effector mechanisms of human eosinophils are modulated by different monoclonal antibodies (against C5aR, CCR3, and CD52) or chemokine receptor antagonist (Met-RANTES).

Chemokine receptors

According to desensitization experiments, binding studies, and mRNA detection, normal eosinophils seem to express two CC chemokine receptors, CCR1 and CCR3 (57, 58, 121–127). MIP-1 α , RANTES, and MCP-3 have been identified as ligands for CCR1, and RANTES, MCP-3, MCP-4, and eotaxin as ligands for CCR3. Recently, desensitization experiments with eotaxin-2 and eotaxin, and binding studies with an anti-CCR3 mAb showed that both CC chemokines act through the CCR3 (128–130). The usage of monoclonal antibodies against human eotaxin revealed that certain leukocytes, as well as respiratory epithelium, were intensely immunoreactive, and eosinophil infiltration occurred at sites of eotaxin upregulation (123). Moreover, increased levels of mRNA of human eotaxin were found in normal small bowel and colon and at lower levels in other organs (58, 131). The importance of CCR3 has recently been demonstrated in a study of patients with allergic asthma. Eotaxin (source: epithelial and endothelial cells) and CCR3 mRNA and protein were significantly elevated in bronchial mucosal biopsies from patients with allergic asthma compared with controls (29).

Moreover, differential expression of chemokine receptors and chemotactic responsiveness of naive T cells (CXCR4), most memory/activated T cells (CXCR3), Th0 (CXCR3), Th1 (CXCR3 and CCR5), and Th2 (CCR3, CCR4, CCR5) CD4-positive cells could be demonstrated (132–135).

Recent studies demonstrated that CC chemokines are not only potent activators of eosinophil chemotaxis but are also involved in the activation of the respiratory burst (27, 35, 125, 130, 136, 137). The CC chemokines eotaxin, eotaxin-2, MCP-3, MCP-4, and RANTES are potent activators of eosinophil effector functions with clearly distinct profiles of activity. These studies underline the importance of eotaxin as a potent activator of the respiratory burst (eotaxin=eotaxin-2>MCP-3=MCP-4>RANTES), actin polymerization, and chemotaxis (eotaxin=eotaxin-2>RANTES>MCP-3=MCP-4 (35, 125, 130).

Chemokine receptor antagonists and inhibitory anti-CCR3 monoclonal antibodies (mAbs)

Since these chemokines are of major importance in attracting and activating eosinophils, agents that can

antagonize or block eosinophil activation are of interest. It is well known that the NH₂-terminal region is critical for the biologic activity of chemokines, and it has been shown that modifications of the corresponding region of chemokines profoundly alter the effect of the chemokines on leukocytes (138–141). Previously, potent chemokine receptor antagonists, such as Met-RANTES, AOP-RANTES, RANTES (3–68), MCP-2 (6–76), and others, were constructed by deletion or extension of amino acids or by chemical modification on the NH₂-terminal residue of CC chemokines (139, 140, 142–147). Interestingly, the leukocyte activation marker CD26 that possesses dipeptidyl peptidase IV activity has now been shown to truncate RANTES (1–68) into RANTES (3–68) (148). Extension of recombinant human RANTES by the retention of the initiating methionine resulted in the production of a potent antagonist inhibiting RANTES-induced [Ca²⁺]_i transients in the promonocytic cell line THP-1, as well as chemotaxis of THP-1 cells and human T cells (142). Moreover, deletion of the NH₂-terminal residue of MCP-1, MCP-3, and RANTES resulted in a competitive inhibition of chemotaxis and enzyme release in the THP-1 cell line and monocytes after stimulation with the native form of chemokines (139). Furthermore, the chemokine receptor antagonist AOP-RANTES can inhibit macrophage infection by HIV (144, 145). Besides the modification of chemokines to produce potent chemokine receptor antagonists, a potent selective nonpeptide CXCR2 antagonist has recently been found to inhibit IL-8-induced neutrophil migration (108). Furthermore, in the past few months, several new virally encoded chemokines have been described which can modify both the host immune and antiviral responses (149). In this context, blockade of chemokine activity by a soluble chemokine-binding protein from vaccinia virus has been demonstrated (150). Whereas the effect of these chemokine antagonists is well documented on the THP-1 cell line, monocytes, and lymphocytes, we have no data about the effect of these compounds on eosinophil effector functions.

More recently, we showed that Met-RANTES can specifically inhibit eosinophil effector functions including chemotaxis, transient [Ca²⁺]_i release, actin polymerization, and release of reactive oxygen species after stimulation with RANTES, MCP-3, and eotaxin (126). Met-RANTES seems to be able to antagonize the response of eosinophils through the CCR1 at lower concentrations and CCR3 at higher concentrations (126). Furthermore, it has been demonstrated that the mouse anti-CCR3 mAb, 7B11, is selective for human CCR3 and can block chemotaxis and calcium flux in human eosinophils induced by RANTES, MCP-3, and

eotaxin (151). Moreover, chemotaxis and stimulation of adhesion were abrogated completely by pretreatment of eosinophils with anti-CCR3 mAb (31). In addition, we could demonstrate that this anti-CCR3 mAb can inhibit the release of reactive oxygen species after stimulation with eotaxin and eotaxin-2 (130). Lastly, the chemokine receptor antagonist Met-RANTES and the anti-CCR3 mAb, 7B11, inhibit eosinophil but not neutrophil effector functions, indicating a more target-specific way of inhibition than other drugs such as steroids and antihistamines. Therefore, these observations point to a potential new therapeutic approach to prevent the invasion and destructive power of eosinophils in diseases that are accompanied by eosinophil infiltration such as allergic asthma, allergic rhinitis, and connective-tissue diseases (Fig. 3).

Eosinophils are a major source of cytokines and chemokines

Recent studies have demonstrated that eosinophils can also synthesize cytokines and chemokines, such as IL-6, IL-8, RANTES, and IL-16 (152–157). Thus, eosinophils represent a source of cytokines that are chemoattractants for neutrophils and lymphocytes as well as eosinophils. Therefore, eosinophils could contribute cytokines to enhance the recruitment of additional populations of lymphocytes and eosinophils. Other important eosinophil-derived cytokines include TGF- β , which might be important for the interaction with fibroblasts in wound healing and connective-tissue diseases such as scleroderma (15, 158). IL-10 and IL-12 from eosinophils might be able to suppress and activate T cells (17, 159). Therefore, eosinophils are not only potent effector cells due to their capacity to secrete toxic proteins and reactive oxygens but are also responsible for the propagation of the inflammatory response due to the release of chemokines and cytokines.

Eosinophils and apoptosis

Hypereosinophilic diseases such as allergic asthma and atopic dermatitis are accompanied by an elevated number of eosinophils in the peripheral blood or in the inflamed tissue, possibly due to enhanced eosinophil survival. *In vitro* studies have demonstrated that cytokines such as IL-3, IL-5, and GM-CSF result in prolonged eosinophil survival by eliminating apoptosis or programmed cell death (160–162). Apoptosis is an ordered process of fundamental biologic importance characterized by cell morphologic changes, DNA fragmentation, and loss of nucleoli. Aged apoptotic

eosinophils were recognized and ingested as intact cells by autologous macrophages. The mechanism of eliminating apoptosis by IL-3, IL-5, and GM-CSF has recently been demonstrated [163]. The antiapoptotic effect of these cytokines seems to be associated with interactions of their β -receptor with tyrosine kinases, particularly by sequential activation of Lyn and Syk tyrosine kinases [164]. Recent studies have demonstrated that cross-linking of CD95 (Fas antigen) and CD69 induce apoptosis of human eosinophils [63, 64, 160, 161, 165, 166]. Moreover, the steroid dexamethasone has been shown to induce apoptosis of human eosinophils while inhibiting neutrophil apoptosis [160, 167]. Therefore, apoptosis of human eosinophils may contribute to the resolution of inflammatory reactions in which eosinophil accumulation is a prominent feature. In this context, a previous study has shown that eosinophil apoptosis is markedly delayed in the so-called atopic diseases irrespective of allergen sensitization, suggesting that this effect is mediated by the autocrine production of growth factors by eosinophils [163].

Concluding remarks

Eosinophils are predominant effector cells not only in allergic diseases such as allergic asthma, allergic rhinoconjunctivitis, and atopic dermatitis but also in connective-tissue diseases. Attraction to and activation of eosinophils at the site of inflammation and release of reactive oxygen species leading to tissue damage and propagation of the inflammatory response are mediated by chemokines and cytokines. This review focuses on the activation and modulation of eosinophil effector mechanisms. Moreover, it suggests new therapeutic strategies (chemokine receptor antagonists and monoclonal antibodies) to prevent the invasion and destructive power of eosinophils in diseases that are accompanied by eosinophil activation.

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