

Review article

Cytokine-regulated accumulation of eosinophils in inflammatory disease

The role of cytokines in the accumulation of eosinophil granulocytes in inflamed tissue has been studied extensively during recent years, and these molecules have been found to participate throughout the whole process of eosinophil recruitment. Haematopoietic cytokines such as IL-3, IL-5 and GM-CSF stimulate the proliferation and differentiation of eosinophils in the bone marrow, and the release of mature eosinophils from the bone marrow into the blood is probably promoted by IL-5. Priming of eosinophils in the blood following, for example, allergen challenge is performed mainly by IL-3, IL-5 and GM-CSF. An important step in the extravasation of eosinophils is their adhesion to the vascular endothelium. Adhesion molecules are upregulated by, e.g. IL-1, IL-4, TNF- α and IFN- γ and the same cytokines may also increase the affinity of adhesion molecules both on eosinophils and endothelial cells. Finally, a number of cytokines have been shown to act as eosinophil chemotactic factors, attracting the cells to the inflammatory focus in the tissue. Some of the most important eosinophil chemoattractant cytokines are IL-5, IL-8, RANTES, eotaxin, eotaxin-2, eotaxin-3, MCP-3, MCP-4 and TNF- α . Th2 cells, mast cells and epithelial cells are important sources of proinflammatory cytokines, but in recent years, the eosinophils have also been recognized as cytokine-producing and thereby immunoregulatory cells. The aim of this paper is to review the role of cytokines in the process of eosinophil recruitment in asthma, allergy and ulcerative colitis.

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The eosinophil granulocyte is a white blood cell whose main function is probably to kill invading parasites. In the absence of parasites, however, activated eosinophils may cause tissue destruction and inflammation (1, 2). Indeed, a number of inflammatory diseases are associated with eosinophilia; for example, asthma, allergic rhinitis, atopic skin diseases, idiopathic hypereosinophilic syndrome (HES) and inflammatory bowel disease (3, 4).

The eosinophils are produced in the bone marrow, where they develop from primitive stem cells that divide and differentiate until they acquire the typical features of eosinophils, namely a bilobed nucleus and distinct granules of varying sizes (5). The mature eosinophils enter the blood stream, where they remain for about 25 h before migrating into the tissue (6). In circulation, they constitute 1–3% of all white blood cells, but the eosinophils are primarily tissue cells, and there are approximately 500 times as many eosinophils in tissues as in the blood (2).

Activated eosinophils are able to phagocytose particles, such as bacteria, but their main killing mechanism is the

release of toxic granule proteins and production of oxygen free radicals (5). There are four major eosinophil granule proteins, namely eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN) and eosinophil peroxidase (EPO). The Charcot–Leyden crystal (CLC) protein is found mainly in the plasma membrane of the cells. MBP and CLC protein are also found in basophils, whereas ECP, EPO and EPX/EDN are specific for eosinophils. These proteins are able to kill both mammalian cells and nonmammalian cells with varying efficiency. Eosinophils also produce cytokines and mediators such as leukotriene C₄ (LTC₄) and platelet activating factor (PAF) (7). The mechanisms behind the accumulation of the eosinophils at sites of parasite invasion and inflammation are beginning to be unravelled and show an intricate network of various principles. It is the purpose of this paper to review the recent findings in this regard with particular emphasis on the role of cytokine molecules and the mechanisms operative in allergy/asthma and ulcerative colitis.

Table 1. Cytokine receptors (R) on human eosinophils

IL-2R α (CD25), β (CD122) and γ (CD132) chains
IL-3R α chain (CDw123)
IL-5R α chain (CDw125) + common β chain (CDw131)
GM-CSFR α chain (CD116)
IL-4R α (CD124) and γ (CD132) chains
IL-9R
IL-13R (CD213a)
IFN- γ R (α -CDw119)
TNF- α R (TNFR I = CD120a, TNFR II = CD120b)
CXCR-2 (on activated eosinophils)
CXCR-4 (on eosinophils after glucocorticoid treatment)
CCR-1
CCR-3
TGF- β R

Cytokine receptors of importance for eosinophil recruitment

Cytokine receptors expressed on human eosinophils are listed in Table 1. Interleukin (IL)-3, IL-5 and granulocyte macrophage-colony stimulating factor (GM-CSF) share a common β chain and have their own cytokine-specific α chains. The IL-5 receptor is fairly specific, since it is only expressed on eosinophils and basophils, while receptors for IL-3 and GM-CSF are present on many haematopoietic cells (8). IL-5 has a crucial role in the proliferation, differentiation, survival and activation of eosinophils. IL-9 enhances eosinophil expression of the IL-5 receptor and also stimulates eosinophil differentiation and survival (9). Elevated expression of IL-9 mRNA has been detected in the bronchial mucosa of atopic asthmatics and allergen-induced cutaneous late-phase reactions (10).

The chemokine receptors are named CCR or CXCR, according to what kind of chemokines they bind. Among the CXC receptors, the receptor for IL-8 (CXCR-2) has been detected on activated eosinophils (11), although this is not a generally accepted finding (12). CXCR-4 is normally not expressed on eosinophils, but has been found on the surface of these cells after treatment with glucocorticoids. Upregulation of CXCR-4 makes the cell susceptible to stromal cell-derived factor-1 α (SDF-1 α), which is constitutively expressed in almost all tissues. Therefore, SDF-1 α may cause unspecific eosinophil migration to noninflamed vascular tissues rather than specifically to inflammatory sites (13). Important for eosinophil migration are the CC-receptors. Chemokines binding to CCR-1 include MIP-1 α , RANTES and MCP-3. CCR-3 is the principal receptor for eotaxin, eotaxin-2 and eotaxin-3, and also binds RANTES and MCP-2, -3 and -4 (14, 15). These chemokines stimulate the migration of eosinophils, basophils, T cells and/or monocytes, but not of neutrophils (16–18).

The receptor for IL-2 consists of three subunits (α , β and γ), all of which have been detected on eosinophils (19–21). The γ chain is a common subunit shared by the receptors for IL-2, IL-4 and IL-7. The effect of IL-2 on eosinophil accumulation will be discussed later.

Table 2. Cytokine production by eosinophils

Cytokine	Stimuli (<i>in vivo</i> or <i>in vitro</i>)	References
IL-1 α	HES	26
IL-2	Asthma	27–29
IL-3	IgA, IgE or TNF- α	24, 30
IL-4	Freshly isolated, normal eos	24
IL-5	HES or eosinophil cystitis	31
IL-6	IFN- γ	32
IL-8	GM-CSF, PAF, IgA, IgG or TNF- α	24, 33
IL-10	Freshly isolated, normal eos	24, 29
IL-13	GM-CSF, eotaxin	25, 29
TGF- α	HES or oral squamous carcinoma	34
TGF- β	IgA, IgE or TNF- α	24
GM-CSF	IgA, IgE or TNF- α	24
RANTES	IgA, IgE, TNF- α , allergen or IFN- γ	24, 35
Eotaxin	TNF- α , IL-5 or IL-3	36–38
MIP-1 α	HES	39
TNF- α	HES and healthy	39

Cytokine production by eosinophils

In recent years, a number of cytokines have been found to be synthesized, stored and released by human eosinophils. This suggests that the eosinophils may regulate allergic inflammation in a more complicated way than just by releasing tissue-damaging proteins. The release of inflammatory cytokines could amplify and prolong the allergic response and enhance tissue damage. On the other hand, eosinophils may be involved in tissue repair by, for example, promoting collagen synthesis through release of transforming growth factor (TGF)- α and - β (3, 22). Cytokine production by eosinophils can be triggered by chemotactic agonists such as C5a or fMLP (23), inflammatory cytokines such as tumour necrosis factor (TNF)- α , interferon (IFN)- γ (17), IL-3, IL-5 or GM-CSF (24), FcR-related stimuli (24), other cell-associated substances and extracellular matrix proteins (3). Eotaxin is a potent releaser of IL-13 from eosinophils (25). Table 2 lists some of the cytokines that are produced by eosinophils.

Eosinophil recruitment in allergic diseases

The present knowledge of the mechanisms of eosinophil recruitment is based mainly on research on allergic diseases and asthma, but many of these mechanisms may be operative in different inflammatory disorders. The recruitment of eosinophils to inflamed tissue is a multi-stage process that can be divided into the following events:

1. Priming of the eosinophils in the circulation.
2. Rolling along the endothelial cells.
3. Firm adhesion to the endothelium.
4. Trans-endothelial diapedesis.
5. Chemotaxis into the inflammatory site.

Eosinophil priming

Peripheral blood eosinophils of normal, healthy subjects are relatively refractory to activation. However, they can change into an activation-sensitive, or primed, phenotype after interaction with certain cytokines. Eosinophils from patients with allergy and asthma exhibit increased migratory responses (40, 41), adhesiveness (42) and degranulation (43) compared with eosinophils from normal subjects. The priming is likely to be caused by IL-3, IL-5 and GM-CSF in the peripheral blood. These cytokines have been detected in the blood of allergic subjects (44, 45), and priming has been mimicked *in vitro* by use of recombinant IL-3, IL-5 and GM-CSF, resulting in enhanced adhesion to epithelial cells (46), increased migratory responses to chemoattractants (47, 48) and increased degranulation (49). Priming of eosinophils can also induce responses to factors such as IL-8, which have no effect on unprimed eosinophils (48). One mechanism underlying this change in responsiveness may be the induction of receptors on the cell surface (12). In addition to the haematopoietic cytokines, TNF- α has been shown to prime eosinophils for migratory responses to PAF (50, 51), and eotaxin facilitates eosinophil migration into the lung by increasing the adhesion to endothelial cells (52).

Eosinophil rolling and adhesion

Rolling, the initial contact of circulating eosinophils with the blood vessel wall prior to extravasation, is mediated by selectins. The reversible adhesion between the selectins and their ligands makes the eosinophils move slowly along the vascular endothelium. The expression of E- and P-selectins on the endothelial cells can be upregulated by IL-1 and TNF- α , while L-selectin is constantly expressed on the eosinophils (53, 54). The slow movement along the endothelium helps the eosinophils to adhere firmly by interaction of integrins and their ligands. Rolling is also believed to activate the integrin receptors on the eosinophils, which results in high-affinity binding to their ligands (53). A transient arrest of rolling eosinophils may be induced by IL-8, and shifted into firm long-term arrest by eotaxin. These chemokines transduce signals that activate integrins (55). The molecules that are most important for firm adhesion of eosinophils to the endothelial cells are CD11b/CD18 and VLA-4 on the eosinophils, and their ligands intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on the endothelial cells. Expression of adhesion molecules is stimulated by cytokines. For example, IL-1 and TNF- α induce ICAM-1 and VCAM-1 expression on endothelial cells. Studies on human bronchial epithelial cells revealed an important role for TNF- α in priming of eosinophil adhesion: together with $\alpha_5\beta_1$ -integrin as a costimulatory molecule, it potentiated C5a activation of CD11b/CD18 on eosinophils in contact with epithelial cells (56). Some

Table 3. Eosinophil adhesion molecules and their ligands

	Eosinophil receptor	Ligand	Ligand upregulated by
Selectins	L-selectin (CD62L)	MAdCAM-1, CD34	
Integrins	VLA-4 (CD49d/CD29)	VCAM-1, fibronectin	IL-1, TNF- α , IL-4, IL-13
	VLA-6 (CD49f/CD29)	Laminin	
	CD11a/CD18	ICAM-1,-2,-3	IL-1, TNF- α , IFN- γ , eotaxin, IL-5
	CD11b/CD18	ICAM-1, fibrinogen, C3bi	
Ig-like	CD11c/CD18	Fibrinogen, C3bi	
	$\alpha 4\beta 7$	MAdCAM-1, VCAM-1, IL-1, TNF- α , IL-4	
	$\alpha d\beta 2$	VCAM-1	
	ICAM-1/-3, PECAM-1 (CD31)	PECAM-1, $\alpha v\beta 3$	
Carbohydrate	PSGL-1 (CD162)	P-selectin	Histamine, leukotrienes
	Sialyl Lewis X	E-selectin	Histamine, leukotrienes
Others	CD44	Hyaluronate	

cytokines are selective inducers for certain adhesion molecules, such as IL-4 for VCAM-1 and IFN- γ for ICAM-1 (57). IFN- γ also stimulates expression of galectin-9 in human fibroblasts, a molecule required for eosinophil adhesion to these cells (58). Increased expression of ICAM-1 and VCAM-1 has been found in biopsy samples from patients with allergic asthma (57). Thus, the adhesion of eosinophils to endothelial cells is promoted by the activation of both the endothelial cells and the eosinophils. Eosinophil adhesion molecules and their ligands are listed in Table 3.

Eosinophil diapedesis and chemotaxis

Eosinophils from the blood of normal individuals can adhere to IL-1- or TNF- α -activated endothelial cells, but they are unable to migrate through this layer. In contrast, eosinophils from allergic or asthmatic individuals do not only adhere to the endothelial cells, but also pass spontaneously through the cell layer. This capacity to transmigrate can be induced by pretreatment of eosinophils from normal individuals with IL-3, IL-5 or GM-CSF (59). Thus, priming of the eosinophils is a prerequisite for diapedesis. The expression of VCAM-1 and ICAM-1 on the endothelial cell is also necessary for eosinophil transmigration. A study on eosinophil migration across human umbilical vein endothelial cells (HUVECs) under laminar flow condition showed that shear stress is critical for eosinophil diapedesis together with eosinophil activation through CCR3 (60). IL-4-stimulated HUVECs produced eotaxin-3, which when expressed on the endothelial cell surface supported eosinophil transmigration through the cell layer.

The process of transendothelial migration potentiates the expression of CD69, HLA-DR and ICAM-1, and increases the survival of the eosinophils (61).

Eosinophils then migrate into the tissue in response to chemotactic factors produced locally at the inflammatory site (62–68). This migration is a movement directed towards chemoattractant gradients, and is characterized by adhesion/de-adhesion to extracellular matrix proteins. Chemokines such as eotaxin-2 facilitate detachment from luminal VCAM-1 and shift the integrin usage in eosinophils from β_1 -integrin towards β_2 -integrin-dominated interactions, with enhanced adhesion to bovine serum albumin (BSA) as a result (69). In response to a chemoattractant, the concentration of cytoplasmic Ca^{2+} rises and the cell undergoes polarization. A flat extension of the cytoplasm, called a lamellipod, is formed at the front of the moving cell, and a tail called uropod is formed at the rear. The nucleus is located closer to the rear than to the front (70, 71). In polarized locomoting cells there is always a calcium gradient with higher Ca^{2+} in the tail than in the front. When cells turn, the intracellular Ca^{2+} concentration rises transiently and then falls most rapidly in the new direction of locomotion. These Ca^{2+} -signals arise from internal Ca^{2+} stores released in response to inositol 1,4,5-triphosphate (IP_3). Diacylglycerol (DAG), which is co-produced with IP_3 , has an inhibitory effect on Ca^{2+} signals, probably through protein kinase C. The eosinophil moves by extending the lamellipod that adheres to the substrate, and constricting the uropod while de-adhering from the substrate (70–72). The actin system comprises the motor of the cell, and actin polymerization and depolymerization provide the cell with contractile forces. These forces are probably induced by phosphorylated myosin II interacting with actin filaments (73). Many signal transduction mechanisms, e.g. calcium transients, pH changes, protein phosphorylation reactions, phospholipid turnover, lipoxygenase activity, GTP-binding proteins, and others, have been proposed as mediators of these events (70). A study on eotaxin-induced responses revealed that ligand-induced internalization of CCR3 is required for eosinophil shape-change and actin polymerization (74).

Eosinophil chemotactic factors

Several factors have been suggested as being chemotactic for eosinophils. PAF, complement factor C5a, LTB_4 and f-Met-Leu-Phe were some of the first-identified eosinophil chemoattractants (75). All of these mediators, however, act on both eosinophils and neutrophils. Recently, PAF and C5a have been found to be important for the transmigration of eosinophils across epithelial cells (76). Increasing interest is being paid to the chemotactic properties of cytokines. IL-3, IL-5, IL-13 and GM-CSF have been recognized as activators of eosinophil function, including migration (75, 77, 78). Chemokines such as eotaxin, eotaxin-2 and -3 (38, 79–81), RANTES (82) and IL-8 (83) have also been found to be eosinophil chemoattractants. Chemokines bind to

the intracellular matrix via adhesion to proteoglycans. In this way a fixed gradient of chemokines is formed within the tissue (84). Recent studies on eosinophil recruitment have mainly been focused on the eotaxins, but the recruitment of eosinophils is most probably carried out not by one single factor but by many different factors acting in concert. Our finding that antibodies to IL-5, RANTES and IL-8 were all able to inhibit the chemotactic activity in bronchoalveolar lavage (BAL) fluid from pollen-allergic subjects (85) suggests that these cytokines depend on each other for their function. Experiments with recombinant cytokines in the same study showed interaction between IL-5 and RANTES, and to a certain extent, IL-8. The presence of IL-5 was essential, especially for unprimed eosinophils. *In vivo*-primed eosinophils were not as dependent on IL-5 as the normal, unprimed eosinophils, and a combination of RANTES and IL-8 was potently chemoattractant even in the absence of IL-5. We also found that the basal, unstimulated migration of eosinophils, which reflects their level of *in vivo* activation, determined their response to different cytokines. The more the eosinophils were activated *in vivo*, the less they responded to IL-5, indicating that the cells activated *in vivo* had been exposed to IL-5 in the circulation. A similar observation was made by Liu et al. on BAL-eosinophils after Ag challenge. They found that there was a striking reduction in membrane IL-5R α on these eosinophils, which were also refractory for further IL-5 stimulation (86). In our study, the correlation between *in vivo* activation and the response to RANTES was the reverse, in accordance with the finding that IL-5 enhanced the migration towards RANTES. It also indicates that the *in vivo*-activated eosinophils had not been exposed to RANTES in the circulation, and that RANTES probably is produced locally, at the inflammatory site. IL-8 is a CXC chemokine, which is chemotactic to neutrophils as well as to *in vivo*-primed eosinophils (87). In our experiments, only eosinophils from symptomatic allergic subjects responded to IL-8.

Apart from the priming abilities of IL-5, the presence of this cytokine appears essential for the response of eosinophils to low concentrations of chemoattractants. We found that RANTES and eotaxin were both potent eosinophil chemoattractants at concentrations of 10^{-7} mol/l. However, concentrations of that magnitude are unlikely to occur *in vivo* (64), and the eosinophils may require IL-5 for a response to the physiological concentrations of chemoattractants, even if these are increased after allergen challenge.

Interleukin-2

Since the publication by Rand et al. (19), IL-2 has been regarded as a potent chemoattractant for eosinophils. However, we found that anti-IL-2 enhanced the migration of eosinophils towards chemoattractants, a finding

that was supported by the subsequent experiments with recombinant IL-2, where this cytokine inhibited both the basal migration stimulated by albumin and the chemotaxis towards RANTES, PAF, eotaxin and IL-8 (88). In the majority of our experiments we observed that eosinophils from allergic donors were less sensitive to IL-2 than normal eosinophils. We also showed that priming of eosinophils with IL-5 *in vitro* made the cells completely insensitive to IL-2, which may explain the lower sensitivity of eosinophils from allergic donors to IL-2. Thus, IL-5 may contribute to eosinophil accumulation not only by acting as a chemokinetic and chemotactic factor, but also by eliminating the inhibitory effect of IL-2 on eosinophil migration. IL-2 is one of the cytokines that have been shown to be synthesized and released by eosinophils (27, 28), and we speculate that IL-2 normally acts as an autocrine inhibitor of eosinophil migration (28), whereas in allergy this inhibitory effect is abolished by IL-5 priming, allowing increased migration of eosinophils towards chemotactic factors.

The effect of human serum albumin on eosinophil migration

Albumin is necessary for eosinophil migration towards low-molecular-weight chemotactic factors, such as cytokines, in the Boyden chamber assay (89, 90). The inhibitory effect of IL-2 on the migration of eosinophils towards chemotactic factors may therefore depend entirely on the inhibition of albumin stimulation of the cell. The mechanisms underlying the dependence on albumin in the Boyden chamber system are obscure. The effect of albumin on the cells is reversible, and washing of the cell suspension after albumin incubation abolishes the stimulation. It has been speculated that albumin does nothing but provide the eosinophils with a favourable physico-chemical environment (91), or that albumin diminishes the adhesiveness of the cells and hence prevents them from sticking to the filter. We showed that albumin is actively involved in eosinophil function by inducing intracellular signalling through a PI_3 kinase-dependent pathway (92), a stimulation that may also trigger the response of eosinophils to chemotactic factors. Incubation with albumin caused downregulation of adhesion molecules, such as CD49d and CD49f. These molecules are able to bind to extracellular matrix proteins, and lower adhesiveness may facilitate eosinophil migration through the tissue. This is in agreement with the observation that antibody blocking of CD49d increased the chemokinetic migration of eosinophils stimulated by IL-5 (93). Data on BSA suggested that during chemotaxis, matrix proteins might activate eosinophils via binding with integrins to facilitate PAF-induced chemotaxis (94). Albumin also caused an increase in forward scatter, which may be interpreted as a sign of activation of the cells. Interaction of eosinophils with albumin was found to result in morphological changes and a higher degree of degranulation after

cytokine stimulation (95). Thus, albumin seems to have active influence on eosinophil functions in several ways. This is important to keep in mind, since albumin is commonly used in eosinophil migration assays, and has so far been regarded as an inert protein with no capacity to activate cells. Our finding that albumin generates intracellular signalling may give reason to question earlier results on signal transduction and migration of eosinophils. It may also have *in vivo* implications. Large increases in the concentration of albumin have been observed in BAL fluid of allergic asthmatics after allergen challenge due to plasma leakage into the inflamed tissue (96, 97), and the concentration of albumin correlated with the eosinophil number in the BAL fluid. It is, however, uncertain whether the purified albumin is identical with physiological albumin, and we can only speculate on the role of albumin in eosinophil migration *in vivo*.

Eosinophil progenitors in allergic disease

There have been reports of increased numbers of circulating eosinophil progenitors in allergic rhinitis. These progenitors can, just like the mature cells, acquire an inflammatory phenotype, expressing IgERs and producing their own growth factors such as IL-5 and GM-CSF. CD34⁺ hemopoietic progenitor cells stimulated by IL-5 express CCR3 and respond to the chemotactic activity of eotaxin (98, 99). Hence, the production of progenitors in the bone marrow is increased upon allergen stimulation, and these immature cells may be recruited to the airways in an already activated state (100, 101).

Eosinophil recruitment into the asthmatic airways

Chronic mucosal inflammation has an important part in the pathogenesis of asthma, which is a disease characterized by reversible airway obstruction and hyper-responsiveness to external and endogenous stimuli (102, 103). Histopathological findings include massive infiltration by activated lymphocytes and eosinophils in and around the bronchial epithelium, dilatation of blood vessels, loss of surface lining epithelium, collagen deposition beneath the basement membrane of the epithelium, mucosal oedema and hypertrophy of both submucosal glands and bronchial smooth muscle (102). Activation of T lymphocytes and subsequent eosinophil recruitment and secretion may contribute to epithelial cell damage and possibly also to bronchial hyper-responsiveness in asthma although the role of eosinophils in this respect has been questioned recently (104, 105). The presence of eosinophils and their products in the airways has been found to be positively correlated to the disease severity and the development of airway hyper-reactivity (106, 107). The moderate numbers of neutrophils indicate that selective recruitment mecha-

nisms are operative (106). The presence of enhanced levels of IL-5 in sera of allergic-asthmatic patients gives rise to selective eosinophil growth and differentiation, resulting in eosinophilia (108, 109). Selective priming of eosinophils in the circulation and specific adhesion molecules, such as VLA-4 (110), may also result in eosinophil but not neutrophil recruitment, even if some of the chemotactic activities produced during inflammation are able to attract both types of granulocytes. In addition, a number of chemotactic factors have been defined as eosinophil specific, e.g. RANTES, eotaxin and MCP-3. We found that BAL fluid obtained from patients with birch-pollen allergy during the season exhibited increased eosinophil chemotactic activity compared with pre-season BAL fluid from the same patients. We identified IL-5, IL-8 and RANTES as the main eosinophil chemotactic agents in the BAL fluid (85, 111). Others have shown that the elevated numbers of eosinophils in BAL fluid after allergen-challenge correlates with the levels of eotaxin in the fluid (112).

Eosinophil recruitment in ulcerative colitis

Ulcerative colitis is an inflammatory disease, the aetiology of which has not yet been established. The inflammation is manifested as oedema, congestion, spontaneous bleeding and erosions of the colorectal mucosa. Infiltration of the mucosa by activated lymphocytes, macrophages, neutrophils and eosinophils is a characteristic feature of the inflammatory condition, reminiscent of allergy. Furthermore, the inflammation in ulcerative colitis is recurrent and paroxysmal, another similarity to inflammatory diseases with an allergic component, such as bronchial asthma (4, 66). An association has actually been suggested between allergy and ulcerative colitis on the basis of the finding of a higher prevalence of allergic symptoms in patients with the latter disease than in control subjects (113). The neutrophil infiltration is, however, more pronounced in ulcerative colitis than in allergy, where eosinophil infiltration predominates. The involvement of neutrophils in the pathogenesis of ulcerative colitis has been studied extensively (114–117), but there is also increasing interest in the role of eosinophils in this disease. In normal, noninflamed intestine there is a baseline level of eosinophils resident in the lamina propria, which may be regulated by eotaxin together with IL-5 (118). During intestinal inflammation, the eosinophil numbers are highly increased. Morphological and immunohistochemical studies have revealed activation of intestinal eosinophils in inflammatory bowel disease (119–121), and increased intestinal release of ECP, EPO and EPX/EDN has been found in ulcerative colitis (122). The basal mechanisms of eosinophil recruitment to inflamed intestinal mucosa are probably similar to those in allergy and asthma, but the importance of different cytokines and adhesion molecules may vary between the diseases.

Evidence of activated peripheral blood eosinophils has been provided in both ulcerative colitis and Crohn's disease, another inflammatory bowel disease characterized by eosinophil infiltration. Blood eosinophils from patients with active inflammatory bowel disease (IBD) had an increased propensity to release ECP *in vitro* than eosinophils from patients during remission (123). Increased levels of ECP and EPO were only found in serum from patients with widespread active disease and not with proctitis, which only affects a limited part of the intestine, nor collagenous colitis, which is characterized by mild inflammation (124, 125). In patients with quiescent Crohn's disease, the blood eosinophils showed an increased response to various chemotactic factors compared to blood eosinophils from healthy donors (126). However, in contrast to studies on allergic patients, the role of IL-5 in priming of the blood eosinophils is not as obvious. First of all, there is no enhanced IL-5 synthesis of circulating lymphocytes in IBD. Furthermore, in the latter study, the activated eosinophils were not desensitized to IL-5, nor did they have an increased responsiveness to RANTES. This indicates that in addition to IL-5 other factors may be involved in the priming of blood eosinophils in IBD.

Just like in the asthmatic lung, there is an increased expression of ICAM-1 and VCAM-1 in the intestinal mucosa of patients with inflammatory bowel disease (127). Studies on mice suggest that $\alpha 4\beta 7$ -molecules also may be important for eosinophil recruitment in intestinal inflammation (128).

With the objective to study locally produced chemotactic factors, we examined perfusion fluids obtained from intestinal segments in patients with ulcerative colitis. There was an elevated chemotactic activity for eosinophils in these fluids compared with perfusion fluids from control patients. The importance of this activity is suggested by the findings of positive correlations between the activity and the levels of ECP and EPO in the perfusion fluids, indicating that the chemotactic activity was accompanied by activated eosinophils (129).

The eosinophil chemotactic activity could be partially inhibited by antibodies to IL-5 and TNF- α , but an important part of the activity in the perfusion fluid remained even after addition of combinations of different anti-cytokines. This persistent, and so far unidentified, activity also proved to be insensitive to *in vivo* steroid treatment. In contrast, the cytokine-mediated activity was inhibited by treatment of the patients with steroid enema. RANTES, eotaxin, MCP-3 and GM-CSF seemed to be of minor importance in this system, even though they are not entirely without influence, since antibodies to these cytokines inhibited the activity of 18–23% of the individual perfusion fluids (129). The expression of TNF- α is strongly enhanced in ulcerative colitis (130, 131), and biological therapies based on antibodies against

TNF- α have been found to improve the course of the disease (132). Therefore, our finding that TNF- α may be one of the most important chemoattractant cytokines in the inflamed intestine is very interesting. TNF- α has also been shown to stimulate eosinophil migration *in vitro* (51).

Even if eotaxin seemed to be of minor importance in our study, findings of elevated levels of eotaxin mRNA in intestinal lesions of patients with IBD (38), and large increases of intestinal eosinophil numbers in eotaxin transgenic mice compared to wild type mice (128) suggest that there may be a role for eotaxin also in the increased recruitment of eosinophils to inflamed mucosa. However, the authors of the latter study point out that IL-5 and eotaxin are likely to cooperate with other factors in the pathogenesis of eosinophilic diseases, in accordance with our study on perfusion fluids.

Apoptosis

Even though extravasation of eosinophils is the principal cause of the eosinophilia found in inflamed tissues, a lower elimination rate of these cells may also contribute to eosinophil accumulation. Normally, eosinophils are eliminated from the tissue by programmed cell death, apoptosis. However, evidence of delayed apoptosis has been obtained in nasal polyp tissue from subjects with nonatopic asthma (133) and in cultured peripheral blood

eosinophils from patients with atopic dermatitis (134) or asthma (135).

Apoptosis can be induced in response to specific ligands that engage the 'death-receptor' CD95 (Fas/APO-1) (136, 137) or by withdrawal of survival factors. Apoptotic stimuli lead to release of cytochrome C from mitochondria, resulting in the activation of caspase 9 and other effector caspases, which degrade key cellular substrates (138). The death of the cell is thought to result from the proteolytic degradation of cellular proteins that in turn leads to the characteristic features of apoptosis including chromosome condensation, reduction of the cellular volume and DNA fragmentation. The cell is then recognized and removed by phagocytes (139).

Inhibitors of eosinophil apoptosis

A number of eosinophil survival factors have been identified, the most important being IL-3, IL-5, IL-13 and GM-CSF (78, 140). The IL-3/IL-5/GM-CSF receptor β subunit interacts with cytoplasmic tyrosine kinases to induce phosphorylation of several cellular substrates. Lyn and Syk are essential for the activation of the antiapoptotic pathways induced by cytokines (141). Prolonged survival of eosinophils is dependent on the expression of antiapoptotic genes of which Bcl-x_L appears to be the most important one in eosinophils stimulated by IL-5 (142). Other members of the

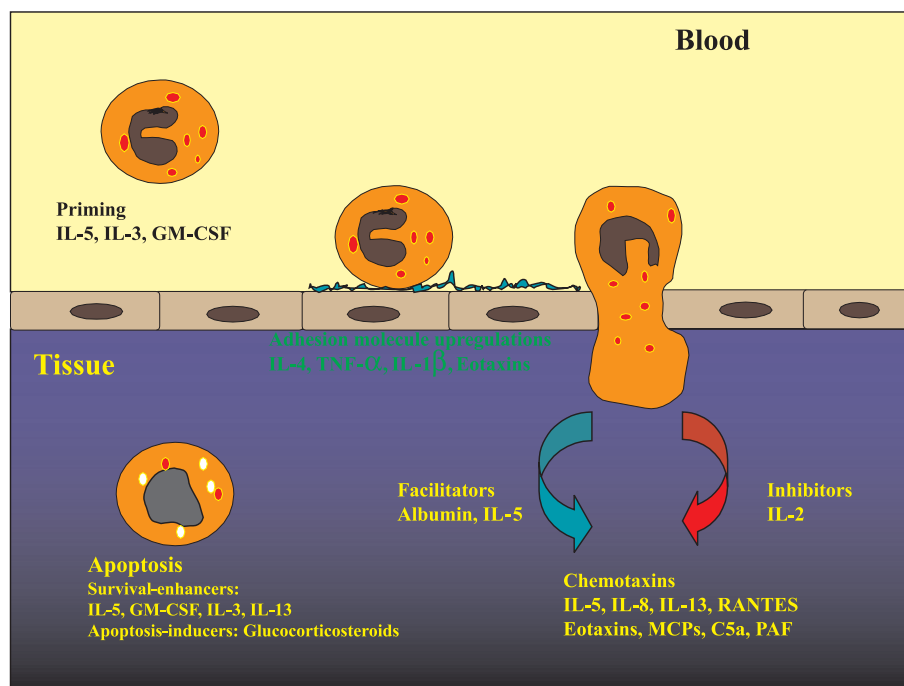


Figure 1. Cytokine participating in the process of eosinophil recruitment from the blood into the tissue.

Bcl-family such as Bax and Bcl-x_s promote apoptosis, and it is thought that the relative levels of these molecules are crucial in determining whether a cell will survive or become apoptotic.

Resistance to CD95 is a phenomenon that has been observed in nasal polyps and could result in unlimited expansion of eosinophils. The mechanisms of this resistance are unknown, but nitric oxide (NO) seems to prevent the effect of CD95-mediated apoptosis. This is an interesting observation since increased levels of NO are present within allergic inflammatory sites (143).

Adhesion of eosinophils via β_1 and β_2 integrin alters a variety of eosinophil functions including degranulation and apoptosis (110). For example, eosinophil adhesion to fibronectin resulted in the prolongation of eosinophil survival and downregulation of Fas antigen expression on cultured eosinophils (144).

Induction of eosinophil apoptosis by drugs

Glucocorticoids comprise the most used therapy for suppression of inflammation in, for example, asthma and exacerbations of ulcerative colitis. Although their precise mechanisms of action remain to be explored, glucocorticoids cause reduction of circulating eosinophils (145).

This reduction depends, at least in part, on cytokine suppression through inhibition of the potent inflammatory transcription factor NF- κ B (146) and on induction of eosinophil apoptosis (147). Glucocorticoid treatment also leads to increased levels of IL-12 that promotes eosinophil apoptosis (148).

Theophylline is used in asthma therapy because of its relaxing effect on bronchial smooth muscle. More recent studies have revealed that this drug also has anti-inflammatory properties (149). Theophylline has been shown to accelerate spontaneous apoptosis in eosinophils and neutrophils (150) and to shorten the IL-5-induced eosinophil survival (151).

Conclusion

A number of cytokines contributing to the accumulation of eosinophils to sites of inflammation have been described. Cytokines participate throughout the whole process of eosinophil recruitment, and a hypothesis of their actions is shown in Fig. 1. This hypothesis is based mainly on experimental data, but is supported by clinical studies on allergy, asthma and to a lesser extent inflammatory bowel disease.

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