

Mechanisms of eosinophil and basophil migration

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Eosinophils and basophils are closely related leukocytes in both their biologic character and the diseases with which they are associated. It is therefore appropriate that the mechanisms involved in their tissue localization should be discussed together. Eosinophils are easier to purify and more readily identified in tissue than basophils. As a result, more is known about mechanisms of eosinophil migration, and this review will devote more attention to them.

Leukocyte migration from the bone marrow to the blood and then to other tissues is one of the central events of the leukocyte function, and it is essential to the integrity of the immune system. This is illustrated by leukocyte adhesion deficiency disease (LAD) type 1 in which the inability of neutrophils to enter tissue because of defective expression of the leukocyte integrins leads to severe, life-threatening infections and death (3). A striking feature of allergic inflammation is the accumulation of activated eosinophils and, in some circumstances, basophils without increased numbers of neutrophils. Because eosinophils and basophils generally make up only a small proportion of the total blood leukocyte count, this accumulation suggests a selective process of leukocyte migration.

In the hope that identification of selective pathways will lead to selective inhibition of migration without the risk of immunosuppression, the mechanisms involved in the tissue localization of eosinophils and basophils have been the subject of intense study over the last three decades. Neutrophil migra-

tion through the endothelium has been shown to be a process involving sequential steps in which the cells are initially lightly tethered to the endothelium and roll along its surface. This is followed by cell activation, mediated by a soluble chemotactic stimulus, which allows a firmer bond to develop between the leukocyte and the endothelial cell, resulting in successful adhesion and transmigration (52, 92, 103). The steps occur in series; thus, each is essential for transmigration to occur. This means that selectivity can be introduced at each of the steps, resulting in considerable diversity in the pattern of signals at any one inflammatory site. It also means that migration can be modulated at each of the steps, offering a range of targets for pharmacologic inhibition. While this staged migration has not been proven to occur with eosinophils and basophils, we can assume that the process is the same.

The receptors and mediators involved in leukocyte migration have, to a large extent, been characterized. A family of adhesion receptors termed "selectins" are thought to mediate the initial attachment and rolling step, a number of chemotactic mediators have been implicated in the activation step, and integrins expressed on leukocytes binding to adhesion receptors belonging to the immunoglobulin superfamily are implicated in the firmer adhesion step (11). The role of each of these families of adhesion receptors in eosinophil and basophil migration, as well as the chemotactic mediators involved, will be discussed in turn.

Selectin-mediated interactions

The selectins

The selectins comprise three receptors, L-selectin, P-selectin (formerly GMP-140), and E-selectin (formerly ELAM-1) (79). E- and P-selectin are expressed on the endothelium, while L-selectin is expressed by all leukocytes. P-selectin is also expressed on platelets. Whereas E-selectin expression is induced on endothelial cells as a result of cytokine-stimulated gene transcription and new protein synthesis (12), P-selectin is stored in cytoplasmic Weibel-Palade bodies and translocated within minutes to the cell surface after stimulation of the endothelium by various mediators including thrombin, leukotriene (LT)C₄, and histamine (29, 56). L-selectin is constitutively expressed and shed on cell activation (42). The selectins have a common structure characterized by an *N*-terminal calcium-dependent (C type) lectin domain, which binds sugars consisting of a family of sialylated, fucosylated glycosaminoglycans typified by the carbohydrate moiety sialyl Lewis X (92). Adjacent to the lectin domain is an epithelial growth factor-like domain and between two and nine (depending on the selectin) repeated units related to complement-binding proteins such as decay-accelerating factor (DAF) (38, 97). Although the adhesion function of the selectins requires binding through sugars, the backbone on which the sugars are presented is important in providing increased specificity and affinity of binding. Ligands for L-selectin and P-selectin have been characterized, and they consist of heavily *O*-glycosylated, mucin-like glycoprotein receptors, in which large amounts of *O*-linked sugars are presented on a rigid protein backbone (7, 51, 81, 88). The selectin receptors and their known ligands are summarized in Table 1.

Eosinophil/selectin interactions

Eosinophil adhesion to cytokine-stimulated, cultured human umbilical vein endothelial cells (HUVEC) can be inhibited by blocking monoclonal antibodies (mAbs) against E-selectin and L-selectin (44, 115). In addition, eosinophils can adhere specifically to COS cells transfected with an E-selectin cDNA (Walsh GM and Wardlaw AJ, unpublished data), to tissue culture plates coated with either E or P-selectin, and to CHO cells transfected with the P-selectin cDNA (101). Eosinophil expression of L-selectin is similar to neutrophil, and, as with neutrophils, L-selectin is shed on eosinophil activation, *in vitro* with chemotactic mediators and *in vivo* (91). Eosinophil binding to E- and P-selectin does not appear to be affected by the state of activation of the cells. Therefore, superficially at least, eosinophils ap-

Table 1. Eosinophil/basophil adhesion receptors and their counterstructures (ligands)

Eosinophil/basophil receptor	Endothelial receptor	Matrix protein
Integrin		
VLA-4 ($\alpha 4 \beta 1$)	VCAM-1	Fibronectin
VLA-6 ($\alpha 6 \beta 1$)		Laminin
$\alpha 4 \beta 7$	MadCAM-1	Fibronectin
LFA-1	ICAM-1, ICAM-2	
Mac-1	ICAM-1	Fibrinogen
Immunoglobulin-like		
PECAM	PECAM	
ICAM-3 (binds LFA-1)		
ICAM-1 (after cytokine stimulation)		
Selectins		
L-selectin	GlyCam-1, CD34	
Carbohydrate		
PSGL-1	P-selectin	
E-selectin ligand	E-selectin	
Others		
CD44		Hyaluronate

ICAM-1: intercellular cell adhesion molecule-1; VCAM-1: cell adhesion molecule-1; PECAM: platelet endothelial cell adhesion molecule; LFA-1: lymphocyte function associated receptor; PSGL-1: P-selectin glycoprotein ligand-1; MadCAM-1: mucosal addressin cell adhesion molecule-1; GlyCAM-1: glycosylated cell adhesion molecule-1.

pear to be very similar to neutrophils in their selectin interactions. There is, however, some evidence of subtle but important differences between these two cell types in this regard. Whereas neutrophils express large amounts of sialyl Lewis X, eosinophils express relatively little of this sugar moiety. The pattern of inhibition of eosinophil adhesion to HUVEC by a panel of L-selectin antibodies was different between eosinophils and neutrophils (44). In addition, neutrophils bound much more avidly than eosinophils to purified E-selectin (17), whereas eosinophils may bind more avidly than neutrophils to P-selectin (101).

We have attempted to study eosinophil adhesion in a more physiologic setting than HUVEC by using the frozen-section Stamper-Woodruff (S-W) assay (94) to investigate eosinophil adhesion to nasal polyp endothelium. Nasal polyps are readily available; they are large pieces of tissue, in which the blood vessels can be readily identified, and they are highly eosinophilic. The S-W assay has been used extensively to characterize lymphocyte homing to high endothelial venules, but it is less often used to study adhesion of other leukocytes. It involves layering the cells onto sections of tissue and allowing adhesion to occur on a rotating platform. We initially investigated the expression of endothelial adhesion molecules in nasal polyps by immunohistochemical means. We found substantial expression of ICAM-1, E-selectin, and P-selectin with, on average, about 30% of blood vessels stained. VCAM-1 expression was either very weak or absent (Table 2). We then investigated eosinophil adhesion to nasal polyp endothelium. Eosinophils adhered in a specific manner

Table 2. Expression of adhesion proteins on nasal polyp endothelium

Adhesion protein	% total blood vessels staining positive	Stain intensity (range)
P-selectin	45.2±7.9	3.5 (3–4)
E-selectin	22.3±4.7	2 (1–3)
ICAM-1	43.5±7.5	2 (2–4)
VCAM-1	5.3±2.8	1 (0–1)

Adhesion protein expression was determined immunohistochemically and expressed as mean percentage of total blood vessels which stained positive for each adhesion protein ± SEM for nasal polyps from four patients. Stain intensity was expressed on scale of 0–4: 0) no staining; 1) staining just visible; 2) staining clearly seen but weak; 3) strong staining; 4) very strong staining.

to endothelial cells with minimal adhesion to background structures. Adhesion was dose and temperature dependent, room temperature being optimal. Using blocking mAbs, we found that antibodies against ICAM-1, VCAM-1, E-selectin, L-selectin, VLA-4, and LFA-1 had no effect, while an antibody against Mac-1 had a significant although modest inhibitory effect. Most striking, however, was the almost total inhibition observed with an antibody against P-selectin. Similar inhibition was seen with an IgG-P-selectin chimeric molecule that binds to the P-selectin ligand and blocks P-selectin interactions (26). Because P-selectin is stored in Weibel-Palade granules within the endothelial cell, eosinophils may have bound to intracellular P-selectin.

However, no eosinophil binding was observed to normal skin endothelium, in which P-selectin expression, determined immunohistochemically, was similar to that observed in nasal polyps. In addition, immunofluorescence with confocal microscopy showed membrane staining of P-selectin in nasal polyps, unlike the granular pattern suggestive of intracellular staining in normal skin. This suggests that, in allergic inflammation, P-selectin is constitutively expressed on airway endothelium, and that P-selectin is the principal receptor involved in the tethering stage of eosinophil adhesion to vascular endothelium (96).

Basophil/selectin interactions

Basophil selectin interactions have been less well studied. They express L-selectin, which is shed on activation, and have a more neutrophil-like than eosinophil-like pattern of sialyl Lewis X expression. Antibodies against E-selectin inhibit adhesion of basophils to cytokine-activated HUVEC (15).

In vivo inhibition of eosinophil/selectin interactions

There are few, if any, published studies on the effects of mAbs against P- and L-selectin on eosinophil or basophil migration into sites of allergic inflamma-

tion. However, some work has been done with anti-E selectin mAbs. Several studies have been done on wild caught cynomolgous monkeys with a natural sensitivity to *Ascaris* antigen (31). Inhalation challenge of *Ascaris* antigen in these animals resulted in an airway inflammatory response and increased bronchial hyperresponsiveness (BHR). Different responses were observed depending on whether antigen challenge was single, in which case an early and sometimes dual (early and late) response was observed, or multiple over several days, in which case a marked eosinophilia and increased BHR were seen. The prechallenge state of the animal was also important in determining the response. Some animals had a prechallenge airway eosinophilia and markedly increased BHR. After a single allergen challenge, these animals developed a late response which was characterized by increased numbers of airway neutrophils. Other animals had a relative paucity of eosinophils in their bronchoalveolar lavage (BAL) prechallenge, but they developed a marked airway eosinophilia and increased BHR after multiple allergen challenge. The airway eosinophilia and increased BHR after multiple antigen challenge were inhibited by an mAb against ICAM-1, but not E-selectin (113). In contrast, the airway neutrophilia generated by a single challenge was inhibited by anti-E-selectin, but not anti-ICAM-1 (32). This suggests that, in this model at least, BHR was associated with an airway eosinophilia which was mediated in part by ICAM-1, whereas the airway neutrophilia was associated with the development of a late response and mediated by E-selectin.

Expression of selectins in allergic inflammation

Expression of E-selectin in allergic inflammation has been studied with variable results. To some extent, expression is organ dependent. Normal skin endothelium has a low background expression of E-selectin. Expression was increased after allergen challenge, the kinetics of upregulation broadly corresponding to the pattern observed *in vitro* with HUVEC (47). Upregulation of E-selectin expression was observed after allergen challenge of skin biopsy organ cultures, suggesting that cells resident in the skin were able to generate the mediators (tumour necrosis factor (TNF)- α from mast cells would be a likely candidate) causing increased expression (53). In both the upper and lower airways, a more complex pattern of expression is observed, with substantial constitutive staining of E-selectin in the normal airway. This has made it more difficult to show an increase in expression of these receptors after allergen challenge or in clinical disease. In a study by Bentley et al., E-selectin expression was increased in the airways of intrinsic asthmatic patients, but not

extrinsic asthmatic patients, as compared with non-asthmatic controls (9).

Similar findings have been reported by Montefort et al. (62). In the Bentley study, no increases in E-selectin expression were seen after aerosol allergen challenge. However, in another study, E-selectin expression was increased after segmental allergen challenge through a bronchoscope (60). P-selectin expression has not been addressed by the above studies, but a recent study reported as an abstract showed expression of P-selectin on the luminal surface of skin endothelium by immunoelectron microscopy 1 h after allergen challenge; this expression had dissipated by 6 h after challenge (67). Another study reported the translocation of P-selectin to the endothelial cell membrane for up to 1 h after the injection of the neuropeptides substance P, vasoactive intestinal polypeptide, and calcitonin gene related peptide (90).

Therefore, in summary, while eosinophils could potentially use all three selectins, our evidence points toward a prominent role for P-selectin in the tethering stage of eosinophil migration in allergic inflammation. There is tantalizing evidence of subtle but important differences between eosinophils and neutrophils in their selectin interactions. Basophils have been less well studied but, on the basis of their expression of sialyl Lewis X, they may have a more neutrophil-like interaction with the selectins.

Integrin and immunoglobulin family adhesion interactions

Eosinophil and basophil integrin interactions

Eosinophils express several integrins that can bind to immunoglobulin family receptors expressed on the endothelium (Table 1). Eosinophil adhesion to unstimulated HUVEC is enhanced about twofold by stimulation with platelet-activating factor (PAF) and other eosinophil-active inflammatory mediators, and this enhancement is almost totally inhibited by mAbs against the leukocyte integrin Mac-1 binding to an unidentified endothelial ligand (41, 50). As compared with adhesion to unstimulated HUVEC eosinophil and basophil adhesion to TNF- α or interleukin (IL)-1, stimulated HUVEC is markedly increased, and this enhancement can be inhibited by mAbs against ICAM-1 and VCAM-1 on the endothelium and LFA-1, Mac-1, and VLA-4 on the leukocyte (15, 23, 106, 115).

Eosinophil transmigration through endothelial cells has also been investigated with artificial models of the blood vessel wall. These studies have shown that eosinophil transmigration through HUVEC was increased by cytokine stimulation of the endothelium, and that eosinophils from allergic

donors showed an increased migration capacity consistent with the concept that eosinophils from subjects with allergic disease are activated (65). Similarly, *in vitro* culture of peripheral blood eosinophils from normal donors with granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-3, and IL-5 increased their migration capacity. The receptors that mediate transmigration of eosinophils through IL-1- or TNF- α stimulated HUVEC appear to be primarily the leukocyte integrins LFA-1 and Mac-1 on the eosinophil binding to ICAM-1 and possibly other, unidentified ligands on the endothelium (25). Antibodies against VLA-4 and VCAM-1 did not inhibit IL-1-stimulated transmigration. In contrast, eosinophil migration through IL-4-stimulated HUVEC was partly inhibited by anti-VLA-4 antibodies, as well as by antibodies against the leukocyte integrins (64). The observation that VLA-4 mediated eosinophil and basophil adhesion and transmigration to HUVEC was very exciting because neutrophils do not express this receptor. Therefore, this could be an eosinophil- and basophil-selective adhesion pathway of relevance to allergic inflammation. This hypothesis was strengthened by the observation that IL-4 selectively induced expression of VCAM-1 on HUVEC (100), and that, as mentioned above, eosinophil, but not neutrophil, adhesion and transmigration through HUVEC were enhanced by IL-4 in a VLA-4-dependent manner (83). In addition, IL-4-transgenic mice had eosinophilic inflammation of the conjunctiva (98). In contrast, however, the expression of VCAM-1 in allergic inflammation was weak, and the IL-4-associated eosinophilia in transgenic mice did not appear to be associated with increased VCAM-1 expression on the endothelium.

In vivo inhibition of integrin/immunoglobulin/eosinophil interactions

Animal models of allergic inflammation have provided further information on the relative importance of integrins and their ligands in eosinophil migration. Basophils have not been studied. As mentioned earlier, in the cyanomologous monkey model, anti-ICAM-1 was effective at inhibiting eosinophil migration into the airways and the development of BHR. It is, of course, possible that the antibody was working on other cell types rather than blocking eosinophil migration directly. In the same multiple-allergen-challenge model, anti-Mac-1 mAb inhibited the development of BHR and reduced the levels of eosinophil cationic protein (ECP) in the BAL fluid but did not inhibit the airway eosinophilia. This suggests that Mac-1 is not essential for eosinophil migration, but that Mac-1 may be involved in triggering eosinophil activation and mediator release (114).

In a sheep model of allergen challenge, the anti-

VLA-4 mAb HP1/2 did inhibit the late response to allergen challenge and the development of BHR when given both intravenously and by inhalation. Interestingly, there was little difference between the antibody-treated animals and control animals in the recruitment of eosinophils into BAL, dissociating the airway eosinophilia from the physiologic effects of allergen challenge. One explanation suggested by the authors for this apparent discrepancy was that anti-VLA-4 treatment inhibited eosinophil activation and mediator release in a manner similar to that observed with the anti-Mac-1 treatment in the monkey model. In support of this hypothesis, they observed that eosinophils treated *in vitro* with anti-VLA-4 mAb released less eosinophil peroxidase after PAF stimulation than control eosinophils. Consistent with the observation that eosinophil migration was not VLA-4 dependent in this model, no increase in VCAM-1 expression was seen on the airway endothelium (1).

A different pattern has been observed in guinea pigs and mice. In guinea pigs, the anti-VLA-4 mAb HP1/2 inhibited the migration of eosinophils into the skin both after injection of chemotactic factors and after passive cutaneous anaphylaxis. Expression of VCAM-1 on skin endothelium was not investigated (112). In sensitized mice, anti-VLA-4 mAb inhibited eosinophil infiltration into the trachea after ovalbumin challenge (69). Anti-VCAM-1 mAb was also effective, and VCAM-1 expression was strongly induced by antigen challenge. Neither the VCAM-1 expression nor the eosinophil infiltration was IL-4 dependent. Unlike the monkey model, anti-ICAM-1 and anti-LFA-1 mAbs were ineffective at inhibiting eosinophil migration.

Lastly, aerosolized anti-VLA-4 mAb inhibited allergen-induced BAL eosinophilia and BHR in rabbits sensitized to house-dust mite (57). These studies point to an important role for VLA-4, Mac-1, ICAM-1, and possibly VCAM-1 in eosinophil recruitment and activation in allergic inflammation, suggesting that suitably designed adhesion receptor antagonists may be therapeutically effective. They also suggest that there are important species differences in the adhesion receptors used by eosinophils to migrate into antigen-challenged airways.

Expression of integrins and immunoglobulin family adhesion receptors in allergic inflammation

The expression of ICAM-1 and VCAM-1 in allergic inflammation has been investigated by several groups. Normal skin endothelium has a low background expression of ICAM-1, and this expression is increased after allergen challenge (48). VCAM-1 expression is weak in both normal and allergen-challenged skin. Good constitutive staining of

ICAM-1 has been observed in the normal human airway, with increased expression of ICAM-1 in the bronchi of intrinsic, but not extrinsic, asthmatic patients (9). VCAM-1 expression is weak in both the upper and lower airways in biopsies from normal subjects and patients with rhinitis and asthma, although there is a suggestion that expression is elevated in nasal biopsies from subjects with seasonal allergic rhinitis, as compared with normal subjects. There is also evidence of increased expression of VCAM-1 after allergen challenge. For example, in the study by Bentley et al. (9), although no significant increases in ICAM-1 and VCAM-1 expression were seen in the bronchi after aerosol allergen challenge, there was a trend toward an increase in VCAM-1 expression with a good correlation between numbers of airway eosinophils after allergen challenge and expression of VCAM-1, suggesting that VCAM-1 may be involved in eosinophil recruitment after allergen challenge, if not in clinical disease. In support of this, a modest increase (9 to 26%) in the number of blood vessels expressing VCAM-1 was observed after allergen challenge (by applying paper discs to the nasal mucosa) in subjects with allergic rhinitis (68). ICAM-1-like E-selectin expression was increased after segmental allergen challenge through a bronchoscope (60). In perennial rhinitis, an increase in endothelial expression of ICAM-1 and VCAM-1 was observed over normal controls, although VCAM-1 was relatively weakly expressed (61).

In general, therefore, there is evidence of increased expression of endothelial ICAM-1, both in clinical disease and after allergen challenge, although this is less marked in the airways than the skin because of background expression in normal or unchallenged subjects. VCAM-1 expression is generally weak in allergic inflammation with limited evidence of higher expression than that of nonallergic subjects in clinical disease, but with more evidence of increased expression after allergen challenge.

Eosinophil adhesion to extracellular matrix

After migration through the endothelium, the eosinophil and the basophil come into contact with the proteins of the extracellular matrix. This is not simply a mesh in which the leukocytes are supported, but a complex network of large fibrillar proteins that have a profound influence on cellular function, mainly through adhesive contacts with integrin receptors expressed both on the surface of resident cells such as fibroblasts and on the epithelium, as well as on leukocytes migrating from the peripheral blood. Tissue eosinophils have an activated phenotype. They express activation receptors such as CD69 (33), gen-

erate mRNA for a number of cytokines that do not appear to be generated by normal peripheral blood eosinophils (22), and express the secreted form of ECP recognized by the mAb EG2 (6, 10). Whether basophils also become activated as they migrate into inflammatory sites is not so clear. The mechanism of eosinophil activation is not well understood. *In vitro* studies suggest that the process of migration through the endothelium itself may result in eosinophil activation (104). In addition, locally generated chemotactic mediators may be involved. Interaction with the extracellular matrix may result in "outside in" signalling through integrin receptors, leading to eosinophil and basophil priming and mediator release (36).

There have been relatively few studies of such interactions. Dri et al. (24) studied the production of superoxide by eosinophils resting on different surfaces, including endothelial cells and a number of matrix proteins, after stimulation with soluble mediators. They found that the nature of the surface influenced the amount of superoxide produced; for example, endothelial cells inhibited superoxide production and fibrinogen primed eosinophils for enhanced superoxide generation after stimulation by f-MLP. Anwar et al. (4) demonstrated enhancement of calcium ionophore-stimulated LTC₄ generation by eosinophils adhering to fibronectin, as compared with bovine serum albumin (BSA)-coated surfaces, and Neeley et al. (70) have reported that VLA-4 mediated interaction with fibronectin resulted in increased f-MLP-induced eosinophil degranulation. In contrast, Kita et al. (43) found that adherence to fibronectin and laminin inhibited EDN release stimulated by PAF, C5a, and IL-5, but not by PMA (43). The secretagogue used is obviously important in determining the effects of matrix proteins on eosinophil degranulation. When eosinophils were cultured for several days on plasma fibronectin, they had greater survival than eosinophils cultured on BSA or plastic as a result of autocrine generation of GM-CSF and IL-3. Cytokine release and survival were inhibited by anti-VLA-4 mAb (5).

We have now extended these observations to show that tissue fibronectin is considerably more effective than plasma fibronectin at supporting eosinophil survival. This is consistent with the concept that survival is a result of triggering through $\alpha 4/\beta 1$ (VLA-4) because tissue fibronectin contains more of the alternatively spliced 111CS region that contains the binding site for VLA-4 (66). In addition to antibodies against VLA-4, survival was also inhibited by antibodies against $\alpha 4/\beta 7$ and Mac-1, suggesting that survival on fibronectin may be due to triggering through multiple integrin receptors. Eosinophils can adhere to laminin through $\alpha 6\beta 1$ (30), and our preliminary data suggest that laminin also promotes

eosinophil survival. As well as being important to eosinophil cytokine generation in disease, the interaction between eosinophils and fibronectin may be a homeostatic mechanism by which eosinophils survive for prolonged periods in tissue before undergoing apoptosis and removal.

Eosinophil and basophil chemotaxins

It has long been felt by investigators working in the field of allergic inflammation that an essential component of eosinophil and basophil migration is an effective and selective chemoattractant. Numerous candidates have been suggested over the years, perhaps most notably the eosinophil chemotactic factor of anaphylaxis (ECF-A) tetrapeptides, but all have so far failed to live up to expectations because of either weak activity, lack of selectivity, or lack of evidence for their generation during the allergic process. Histamine has been reported to have *in vitro* chemotactic activity (18), but, like the ECF-A tetrapeptides, we found it to be less effective than PAF and C5a (109). Histamine does increase cytosolic calcium in human eosinophils (74), and the H₁-antagonist cetirizine appears to modulate eosinophil migration both *in vitro* and *in vivo*. *In vitro*, it inhibits eosinophil chemotaxis and adhesion to endothelium; *in vivo*, it inhibits allergen-induced eosinophil migration into skin windows, but not into tissue. Some of these activities are shared by other antihistamines. The mechanism involved is unclear (86). Because histamine challenge given to establish the histamine PC₂₀ does not result in airways inflammation, one can conclude that histamine is not an effective *in vivo* chemoattractant. Cyclo-oxygenase-derived lipid mediators, including prostaglandin (PG)D₂, are not chemotactic for eosinophils, at least *in vitro*.

In contrast, a number of both 5- and 15-lipoxygenase-derived mediators, including LTB₄ and various DiHETEs (63, 84), have been shown to have chemotactic activity. However, LTB₄ is much more active on guinea pig eosinophils than human cells, and the DiHETEs shown to have activity by Morita et al. (63) were generated only when exogenous arachidonic acid was present. Intriguingly, inhaled LTE₄ generated airway eosinophilia, although it is not clear whether this was a direct or indirect effect (49). Dexamethasone does not inhibit eosinophil chemotaxis (46). Eosinophil and basophil chemoattractants are summarized in Table 3.

In recent years, the chemotactic potential of cytokines, particularly growth factors and chemokines, has been explored in some detail. GM-CSF, IL-3, and IL-5, as well as being eosinopoietic, enhance the effector function of mature eosinophils in terms of adhesion to HUVEC, prolonged survival, mediator

Table 3. Eosinophil (eos) and basophil (baso) chemoattractants

Type	Effectiveness (unprimed)	eos/baso	Comments
<i>Lipids</i>			
PAF	High	eos/baso	Nonselective
LTB ₄	Low	eos	Nonselective
LTE ₄	Not known	eos	Activity reported <i>in vivo</i> only
18(s), 15(s), DiHETE	Moderate	eos	Part of guinea pig ECF-A
<i>Low-mol.-wt. peptides</i>			
C5a	High	eos/baso	Nonselective
f-MLP	Low	eos/baso	Nonselective
<i>Growth factors</i>			
IL-5	Low	eos/baso	Active only on cells from
IL-3	Low	eos/baso	normal subjects; effective priming
GM-CSF	Low	eos/baso	agents
IL-2 (75)	Low	eos	Probably mainly
LCF (76)	Moderate	eos	chemokinetic
<i>Chemokines</i>			
<i>C-C family (not active neutrophils)</i>			
RANTES	High	eos/baso	Active <i>in vivo</i> and <i>in vitro</i>
MIP1 α	Low/moderate (eos)	eos/baso	Histamine releasers
MCP-3	Moderate	eos/baso	Histamine releasers
Eotaxin	High	eos	Only characterized in guineapigs at present; active <i>in vivo</i> and <i>in vitro</i>
<i>C-X-C family</i>			
IL-8	Low	eos/baso	Only active in primed cells
GRO α / β / γ		baso	Histamine releasers

release, and mobility (27, 54, 73, 107, 108). IL-5 and IL-3 are also active on mature basophils (13, 16, 35, 89, 116), but inactive on neutrophils, and are therefore relatively selective. There is abundant evidence that these cytokines are generated in allergic inflammation (76, 77, 105). In the Boyden chamber, IL-3, IL-5, and GM-CSF are chemotactic for eosinophils from normal subjects but less active on eosinophils from allergic subjects, possibly as a result of *in vivo* desensitization (87, 111). Although active at low concentrations, in our hands at least, they were only weakly effective, as compared with well-established eosinophil chemoattractants such as PAF. They appear to be much more active at priming eosinophils *in vitro* for enhanced chemotactic responses to suboptimal concentrations of lipid and low-mol.-wt. chemoattractants such as LTB₄ and f-MLP that are otherwise weakly active on unprimed normal eosinophils. This priming effect appears to be relevant *in vivo* because eosinophils from allergic subjects with mild to moderate eosinophilia are much more responsive in the Boyden chamber assay to chemotactic stimuli such as PAF than eosinophils from normal subjects (110). This effect is also seen *in vivo*, as shown by Henocq & Vaargaftig, who showed that subcutaneous PAF induced a neutrophil infiltration in normal subjects, but an eosinophil-rich infiltration in allergic subjects (34). It is possible that a selective priming stimulus from growth factors active on mature eosinophils

and basophils combining with a nonselective but highly effective chemoattractant results in selective migration.

Consistent with this distinction between a priming stimulus from the growth factors and a chemotactic stimulus from mediators such as PAF is the different nature of the growth factor and chemoattractant receptors, the former being heterodimeric receptors related to other members of the growth factor receptor family, and chemoattractant receptors being among the G-protein-linked, seven-transmembrane, "serpentine" receptors (59). Presumably, these two types of receptor are linked to distinct signal transduction mechanisms which result in different effector functions.

Despite the relative lack of activity of IL-5 in the Boyden chamber, inhalation of IL-5 in animals and man leads to eosinophil recruitment (37, 99), and antibodies against IL-5 have been effective in inhibiting eosinophil migration into the lung of allergen-challenged guinea pigs (55, 102). Antibody-mediated inhibition occurs even when the anti-IL-5 antibody is given at the time of the challenge; therefore, it is not solely working through an effect on eosinopoiesis. A possible explanation of the apparent discrepancy between the *in vivo* and *in vitro* findings is that IL-5 is a more effective chemoattractant *in vivo* than *in vitro*. Alternatively, IL-5 priming of adhesion to vascular endothelium may be important in eosinophil transmigration after allergen challenge. A third

Table 4. Summary of adhesion receptors and chemotactic mediators potentially involved in eosinophil and basophil migration in allergic inflammation

Step	Receptor/chemoattractant
Tethering	Eosinophils: all selectins? P-selectin primarily Basophils: all selectins
Activation	PAF/IL-5 and IL-3/chemokines
Transmigration	LFA-1/Mac-1/VLA-4 binding to ICAM-1/VCAM-1/?other
Extracellular matrix	$\alpha 4\beta 1/\alpha 6\beta 1/\alpha 4\beta 7/\text{Mac-1}$

possibility is that anti-IL-5 inhibits eosinophil release from the bone marrow.

An exciting development in the area of eosinophil and basophil migration is the identification of relatively cell-specific chemotactic peptides termed "chemokines" or "intercrines" (72). Chemokines are 6–10-kDa proteins that are 20–55% similar in amino acid sequence. They are basic polypeptides that are strongly bound to heparin and are highly effective leukocyte chemoattractants. The family share a common four-cysteine motif and are divided into two subfamilies by the position of the first two of the conserved cysteines. In the C-X-C subfamily, the cysteines are divided by an amino acid; in the C-C family, the cysteines are adjacent (82). The C-X-C family, typified by IL-8, are particularly active on neutrophils and monocytes, although IL-8 has chemotactic activity for primed eosinophils and is both a basophil chemotaxin and a secretagogue (19, 21, 28, 85). The C-C family, typified by RANTES, as well as being chemotactic for monocytes and lymphocytes, are potent basophil chemotaxins and histamine releasers, as well as being chemotactic for eosinophils (Table 3) (14, 20, 39, 40, 45). As well as being active *in vitro*, RANTES has been shown to result in eosinophil accumulation in the skin of dogs after intradermal injection (58). Eotaxin is interesting because it was originally identified in BAL fluid from allergen-challenged guinea pigs and therefore is of direct relevance to allergic disease.

Chemokines are generated by several cell types including airway epithelial cells (93). Like other chemoattractants, the chemokine receptors are members of the G-protein-linked, seven-transmembrane-receptor family (71). There appears to be a sharing of receptors by different chemokines. For example, eotaxin inhibits binding of RANTES to eosinophils. In basophils, as well as having chemotactic activity, chemokines can cause basophil degranulation (2). The relative role of the chemokines in controlling eosinophil and basophil migration remains to be determined; however, they fit all the criteria of important chemotactic mediators in allergic inflammation because they are selective, effective, and active *in vivo*. Early reports suggest that they also appear to be generated in allergic disease (8).

Summary and conclusions

Considerable progress has been made in our understanding of the molecular mechanisms involved in eosinophil and basophil migration into sites of allergic inflammation. It is clearly a staged process, each stage offering a level of control over the cell specificity and degree of migration. On the basis of current evidence, the various receptors and mediators involved are summarized in Table 4. Once in the tissues, eosinophils may persist for several days or weeks, surviving under the influence of locally generated cytokines, and this persistence may also partly explain the selective tissue accumulation of eosinophils and basophils. Understanding the molecular mechanisms involved in leukocyte migration may lead to the discovery of selective and effective antagonists to treat allergic disease by preventing cell migration. Results in a number of animal models already suggest that this approach may be successful. The development of drugs that can be tested in the clinic is awaited with much interest.

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