Rostrum

Molecular basis for selective eosinophil trafficking in asthma: A multistep paradigm

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Asthma is characterized by a 50- to 100-fold increase in the number of eosinophils relative to neutrophils in the bronchial mucosa. This increase is not the result of a single molecular event but of the cumulative and sequential effects of several approximately 4-fold increases in selective eosinophil versus neutrophil migration, occurring at a number of stages in the life cycle of the eosinophil. These steps include (1) effects on the bone marrow, mediated principally by IL-5, which result in a 4-fold increase in circulating eosinophils, (2) selective tethering of eosinophils to venular endothelium through the combined effects of P-selectin/P-selectin glycoprotein ligand 1 and very late activation antigen-4/vascular cell adhesion molecule-1, which has the potential for an up to 10-fold increase in eosinophil versus neutrophil adhesion, (3) selective chemotaxis under the influence of CC chemokines, and (4) prolonged survival, again mediated by IL-5. These events are integrated and directed by allergen-specific T_H2 lymphocytes through the generation of IL-5, IL-4, and IL-13. The implications of this multistep process are that antagonists of IL-5, very late activation antigen-4, P-selectin glycoprotein ligand 1, and CCR3 as well as IL-4 and IL-13 each have the potential to markedly inhibit eosinophil recruitment in asthma. (J Allergy Clin Immunol 1999;104:917-26.)

Key words: *Eosinophils, migration, adhesion, chemotaxis, asthma*

Abbreviations used

BAL:	Bronchoalveolar lavage
BHR:	Bronchial hyperresponsiveness
ECF-A:	Eosinophil chemotactic factor of anaphylaxis
FSA:	Frozen section assay
GC:	Glucocorticoid
HUVEC:	Human umbilical vein endothelial cell
ICAM:	Intercellular adhesion molecule
MCP:	Monocyte chemotactic protein
MIP:	Macrophage inflammatory protein
NPE:	Nasal polyp endothelium
PAF:	Platelet-activating factor
PSGL:	P-selectin glycoprotein ligand 1
PT:	Pertussis toxin
VCAM:	Vascular cell adhesion molecule
VLA:	Very late activation antigen

It has been appreciated for many decades that allergic diseases such as asthma are characterized by increased numbers of eosinophils in the affected tissue.[1] The increase in eosinophils has a degree of selectivity, in that it usually occurs without an increase in neutrophils. This observation is one of the cornerstones of the current hypothesis suggesting a central role for eosinophil-derived mediators in causing asthma and related allergic diseases. This review attempts to summarize, in terms of a coherent framework, the fruits of the major research effort that in recent years has attempted to explain the molecular basis for selective eosinophil migration (Fig 1). This has been undertaken in the expectation that it will result in increased understanding of the pathogenesis of asthma and identification of targets for therapeutic intervention. Indeed, other than lymphocyte homing, selective eosinophil accumulation is perhaps the best studied model of how different patterns of cell accumulation occur in various inflammatory diseases and, as a result, offers insights into how selective leukocyte trafficking may occur in other pathologic processes.

Early thoughts on eosinophil trafficking into tissues were dominated by the idea of a selective chemoattractant. An activity, termed eosinophil chemotactic factor of anaphylaxis (ECF-A) was detected in supernatants from anaphylactically challenged guinea pig lung that appeared to be selectively chemotactic for eosinophils.[2] This was subsequently found to consist of a mixture of leukotriene B_4, which is active on guinea pig eosinophils but less so on human eosinophils, and 18(s) 15(s) dihydroxyeicosatetraenoic acid.[3] ECF-A from human lung was later identified and characterized as 2 tetrapeptides, Val-Gly-Ser Glu and Ala-Gly-Ser Glu.[4] However, the later characterization of effective eosinophil chemotactic factors such as platelet-activating factor (PAF) revealed that the ECF-A tetrapeptides had negligible activity.[5] Indeed, there is limited evidence that anti-IgE challenged human lung mast cells are an important source of selective eosinophil chemoattractants, and it is probable that the effects of these cells on eosinophil migration are largely indirect, through the generation of cytokines such

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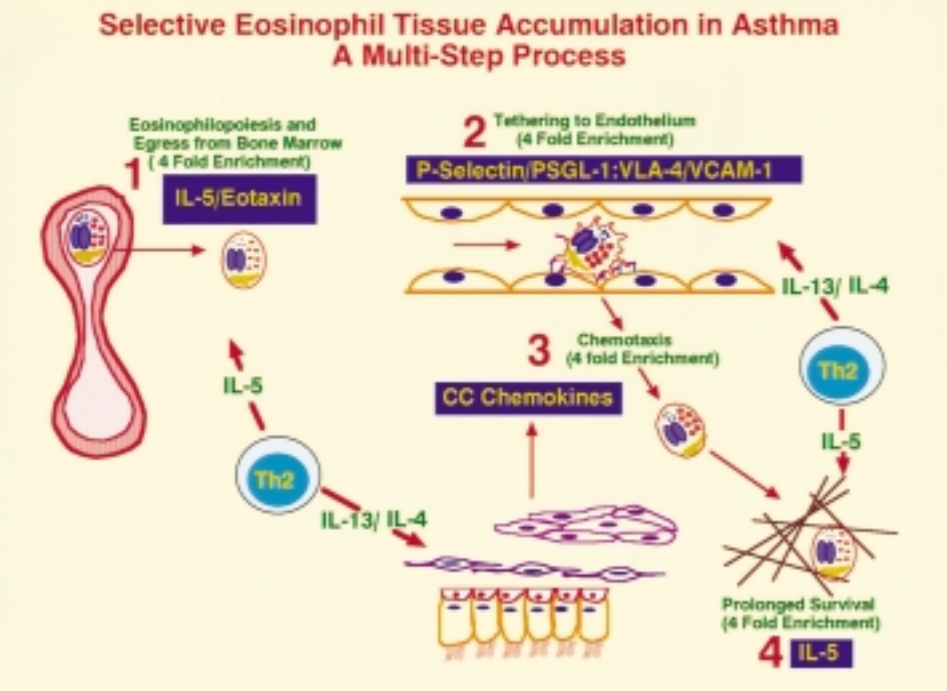

FIG 1. Schematic representation of multistep paradigm of eosinophil recruitment into tissue, illustrating that selective accumulation of eosinophils occurs as sequential and cumulative approximately 4-fold increases, in eosinophils compared with neutrophils, at several stages in life cycle of cell, with each step under separate molecular control, influenced either directly or indirectly by T_H2 cytokine production. The first step involves hematopoiesis and bone marrow egress mediated by IL-5 and chemotactic signals, the second step is through IL-4 and IL-13 up-regulation of P-selectin and vascular cell adhesion molecule-1 *(VCAM-1)* on vascular endothelium, the third step involves selective chemotaxis under influence of CC chemokines generated by IL-4– and IL-13–stimulated epithelial, fibroblast, and smooth muscle cells, and the fourth step is prolonged survival, again mediated by IL-5. *PSGL,* P-selectin glycoprotein ligand; *VLA,* very late activation antigen.

as TNF-α, IL-4, and IL-13.[6] In the late 1980s attention turned toward the importance of IL-5 and the possible role of adhesion pathways in controlling selective eosinophil accumulation.[7,8] More recently, the discovery of chemokines has revived interest in the central role of selective chemoattractants in directing eosinophil migration.

There has been a tendency to try to explain selective eosinophil accumulation in terms of a single molecular event. However, with many of the pieces of the eosinophil migration puzzle now in place, a complex picture has emerged, with major cytokine, chemokine, and adhesion receptor–mediated influences on selective eosinophil accumulation occurring at each stage in the life cycle of the cell, from hematopoiesis to apoptosis. The up to 200-fold increase in eosinophils compared with neutrophils that occurs in the airways in asthma appears to be the result of the cumulative effect of several 2- to 5-fold increases that occur from bone marrow to tissue. Borrowing from the events that control leukocyte adhesion to endothelium, I have called this the multistep paradigm of selective eosinophil accumulation.

The literature on eosinophils in general and eosinophil migration in particular is extensive and I apologize to those authors whose work I have not quoted. I have concentrated in this article on more recent studies and refer the reader, for more referencing of earlier work, to several detailed reviews on eosinophils that have appeared in the last decade.[1,9,10]

HOW SELECTIVE IS EOSINOPHIL MIGRATION IN ASTHMA?

Selective accumulation of eosinophils in the airways in asthma has become a central tenet of the pathology of the disease. This is based on a number of different types of studies, including postmortem analysis of the pathology of asthma deaths, studies of induced sputum, and the use of fiberoptic bronchoscopy to obtain endobronchial biopsy specimens and bronchoalveolar lavage fluid (BAL).[11] The majority of these studies have demonstrated a significant increase in the number of airway eosinophils compared with appropriate controls, without a corresponding increase in airway neutrophilia. The airway eosinophilia is very variable, but generally fairly modest, the difference from nonasthmatic subjects being the result of the paucity of eosinophils in normal airways. In asthmatic BAL eosinophils generally make up about 3% of the leukocytes, similar to neutrophils and lymphocytes. In bronchial biopsy specimens, which are probably

the most accurate reflection of the airway eosinophilia, when data are taken from a range of studies of clinical asthma, eosinophils are generally 2- to 4-fold more numerous than neutrophils, whereas in the normal airway neutrophils are 10- to 50-fold more numerous than eosinophils, giving a 20- to 200-fold increase in the eosinophil/neutrophil ratio in normal versus asthmatic airways, with a median of 60-fold. These studies actually tell us relatively little about the numbers of eosinophils and neutrophils that are migrating into the airways because the number present in the tissues is the result of balance between rate of migration and rate of removal. No kinetic studies have been reported in clinical asthma to fill this critical gap in the literature. However, it is likely that eosinophil accumulation is a combination of both increased migration and prolonged survival.

EOSINOPOIESIS, EOSINOPHIL EGRESS FROM THE BONE MARROW, AND THE ROLE OF IL-5 IN EOSINOPHIL TRAFFICKING

Eosinophils differentiate from bone marrow precursors under the influence of growth factors, including IL-3 and GM-CSF, which are active on early precursors, and IL-5, which acts as a late differentiation factor.[12] In humans IL-5 appears to be only active on eosinophils and basophils. There is plentiful evidence for an increase in IL-5 production in the airways in asthma,[13] and ectopic expression of IL-5 in airway epithelium in transgenic mice resulted in asthma-like changes in the airways.[14] IL-5 generated in the lung in asthma may therefore act hormonally on the bone marrow to increase eosinophilopoiesis. Alternatively, increased production of IL-5 locally in the bone marrow may be responsible.[15] There is also evidence for increased numbers of circulating eosinophil precursors in the peripheral blood of allergic patients, which may be able to migrate into the lung and differentiate in situ.[16]

There is considerable evidence that IL-5 is fundamentally required to mount an eosinophilic response in allergic disease. Antibodies against IL-5 in a number of animal models have prevented the peripheral blood and airway eosinophilia associated with antigen challenge and IL-5 gene deleted mice were unable to mount an eosinophilic response to allergic stimuli, although eosinophil production was not completely ablated.[17,18] IL-5, as well as regulating eosinophilopoiesis, is also able, as discussed below, to affect eosinophil tissue accumulation through its ability to prolong the survival of mature eosinophils. In addition, although not chemotactic in its own right, IL-5 is a potent enhancer in vitro of the chemotactic effects of established eosinophil chemoattractants.[19] Interestingly, this effect was only seen in eosinophils from normal donors, suggesting that in asthma they have already been primed in vivo. The importance of this synergism between IL-5 and chemoattractants has been demonstrated in animal models. The tissue eosinophilia induced by cutaneous injection of both leukotriene B_4 and eotaxin in guinea pigs was greatly enhanced by systemic administration of IL-5 and the cutaneous eosinophilia induced by eotaxin in wild-type mice was not seen in IL-5–deficient mice.[20,21] As well as a priming effect, the increase in the peripheral blood eosinophilia produced by systemic IL-5 may also have contributed to these observations because studies reintroducing the IL-5 gene in gene-deleted mice found that restoration of the peripheral blood eosinophilia was required to obtain a pulmonary eosinophilia after allergen challenge.[22]

The increased numbers of eosinophils seen in the blood of allergic individuals is a combination of increased hematopoiesis and rate of egress (Fig 1). The mechanisms involved in leukocyte migration from the marrow sinuses into the peripheral blood are still unclear. However, with use of an in vivo model in guinea pigs, Palframan et al[23,24] have demonstrated that eotaxin given intravenously caused a rapid peak of eosinophil egress from the bone marrow occurring over a few minutes, whereas intravenous IL-5 resulted in a delayed but more prolonged release. The 2 agents together resulted in a synergistic increase in migration, once again emphasizing the cooperative effects of IL-5 and a chemotactic stimulus on eosinophil locomotory behavior. Adhesion receptors were also involved in regulating eosinophil migration into the blood, with an anti-VLA-4 mAb accelerating egress and anti-Mac-1 preventing egress.

ADHESION

Whatever the percentage of circulating eosinophils, unless there are local signals on bronchial postcapillary endothelium leading to adhesion and transmigration, tissue accumulation of eosinophils will not occur. The pattern of expression of eosinophil adhesion receptors is in general similar to that of other leukocytes although eosinophils, unlike human neutrophils, express functional forms of very late activation antigen (VLA)-4, VLA-6, and $\alpha4\beta7$.[25] MAdCAM-1, the ligand for $\alpha4\beta7$ and almost exclusively expressed on gut endothelium, may be important in directing eosinophils to the intestinal wall where they normally reside. Eosinophils also express the newly described member of the $\beta2$ integrin family $\alpha d\beta2$, which like VLA-4 binds vascular cell adhesion molecule (VCAM)-1, although this receptor may be more important in modulating the function of tissue rather than peripheral blood eosinophils.[26] I will discuss selective signals for eosinophil endothelial adhesion in terms of each step of the established paradigm for leukocyte adhesion to endothelium: tethering, activation, and firm arrest (Fig 2).

Tethering

Because of its lack of expression on human neutrophils, VLA-4 has attracted considerable interest as a possible receptor mediating selective eosinophil adhesion. VLA-4 can promote both tethering and firm arrest. IL-4 and IL-13 up-regulate expression of VCAM-1, the ligand for VLA-4, on human umbilical vein endothelial cells (HUVEC) and transmigration through IL-

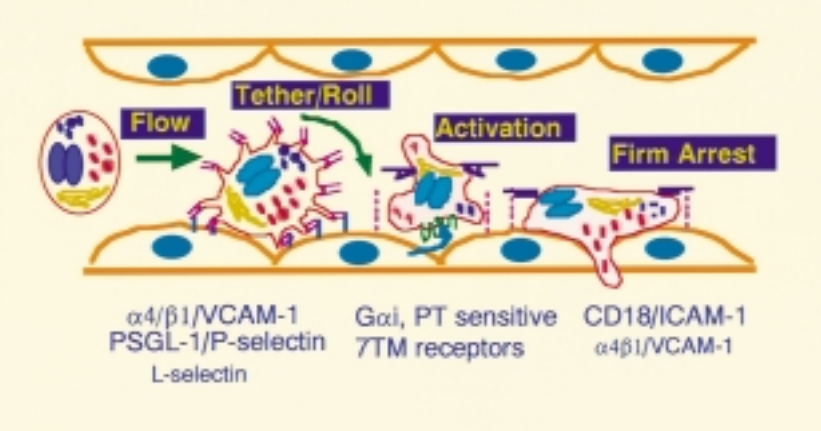

FIG 2. Schematic representation of steps mediating eosinophil adhesion to vascular endothelium. Eosinophils enter postcapillary endothelium under flow conditions and become tethered to endothelium through combined effects of VLA-4/VCAM-1, P-selecting glycoprotein ligand 1 *(PGSL-1)*/P-selectin and in some circumstances L-selectin. Activation, possibly through chemoattractant receptors, results in binding of CD18 integrins leukocyte function–associated antigen-1 *(LFA-1)* and Mac-1 to intercellular adhesion molecule-1 *(ICAM-1)*, which are the major receptors involved in transmigration, although VLA-4/VCAM-1 also make a contribution at this stage.

4/13–stimulated HUVECs was shown to be dependent on VLA-4.[8] IL-4/13-induced endothelial expression of VCAM-1 in the absence of a costimulus such as TNF-α is, however, weak, especially on cultured lung microvascular endothelium.[27,28] Consistent with this, we and others have found variable and often weak expression of VCAM-1 on airway endothelium in clinical asthma, with no increase over that of control subjects.[25] Some investigators have reported increased VCAM-1 expression, although the difference from controls has been modest.[29,30] A more consistent increase in VCAM-1 has been observed after antigen challenge with a correlation with eosinophil influx.[8,25] Peripheral blood eosinophils can constitutively bind VCAM-1 by VLA-4, although binding to VCAM-1 and fibronectin can be further enhanced by manganese and activating mAbs such as TS2/16 and 8A2 but not by physiologic stimulus such as IL-5 and RANTES, which, however, do up-regulate β2 function.[31] VLA-4 can tether eosinophils under flow. Thus the number of eosinophils rolling on IL-1–stimulated rabbit mesenteric vascular endothelium was partially decreased by anti-VLA-4 mAb[32] and eosinophils became tethered to purified VCAM-1 under flow conditions, although at a lower sheer stress than P-selectin.[33] VLA-4 was also important in mediating tethering of eosinophils to TNF-α–stimulated HUVECs.[34] A number of studies using a variety of animal models have demonstrated that blocking VLA-4 or VCAM-1 inhibited eosinophil migration into the lung and skin and prevented the development of bronchial hyperresponsiveness (BHR),[8,25,35] although these 2 effects have not always correlated.[36]

There is increasing evidence for a role for P-selectin in mediating eosinophil adhesion. Eosinophils bound with greater avidity to purified P-selectin than did neutrophils under shear conditions, especially at suboptimal concentrations of P-selectin.[33,37] The P-selectin gene promoter contains 2 STAT-6 binding sites[38] and chronic surface expression of P-selectin on HUVECs was induced by IL-4 and IL-13, although, unlike in the mouse, not by IL-1 or TNF-α.[39,40] Eosinophils, but not neutrophils, were able to adhere to IL-4– or IL-13–stimulated HUVECs under sheer stress, and binding was mediated both by antibodies against P-selectin/ P-selectin glycoprotein ligand 1 (PSGL-1) and VLA-4/VCAM-1, demonstrating a cooperative effect between these 2 pairs of receptors.[40,41] P-selectin was the only selectin involved in eosinophil adhesion to nasal polyp endothelium.[42] The reduced eosinophil infiltration seen in nasal polyps after treatment with fluticasone was associated with a reduction in expression of P-selectin but not VCAM-1.[43] The importance of P-selectin in eosinophil accumulation in allergic disease has been further underlined by studies in animal models. In a ragweed peritonitis model eosinophil accumulation was reduced by 75% in P-selectin–deficient mice with an additional contribution from VCAM-1 and intercellular adhesion molecule-1 (ICAM-1).[44] Eosinophil accumulation was reduced in the airways of P-selectin–deficient mice after antigen challenge[45,46] and anti-P-selectin, but not E-selectin, reduced eosinophil influx into the pleural cavity in a mouse pleuritis model.[47] In contrast to P-selectin, neutrophils bind with greater avidity to E-selectin than eosinophils,[8,33,48] although E-selectin did make a minor contribution toward eosinophil adherence to TNF-α–stimulated endothelium[34] and eosinophil influx into the skin in mice, consistent with the preferential expression of E-selectin in this organ.[49] There are no very clear-cut differences in L-selectin function between eosinophils and neutrophils, although anti-L-selectin did partially inhibit eosinophil binding to rabbit mesenteric endothelium.[32]

The reasons for the differences in the avidity of eosinophils and neutrophils for E and P selectin are not clear. PSGL-1 is a mucin-like homodimeric receptor,

although expression does not always correlate with function. For example, all lymphocytes express PSGL-1 but only about 15% can bind P-selectin. The majority of the P-selectin binding function is found in the N-terminal 19 amino acids of PSGL-1 and is crucially dependent on sulfation of at least 1 of 3 tyrosine residues as well as the appropriate glyocosylation of a single O-linked sugar residue in this region. In contrast, E-selectin, which can also bind PSGL-1 although with lower affinity than P-selectin, binds to the central mucin-like, O-linked, carbohydrate-rich region of the receptor. E-selectin binding correlates with expression of sialyl Lewis X–like carbohydrate moieties, expression of which is regulated by the glyocosyltransferase, fucosyltransferase V11. Although P-selectin binding function is also dependent on fucosyltransferase V11 as well as appropriate sulfytransferases, binding appears to be through sugars other than sialysl Lewis X.[50] Eosinophils, unlike neutrophils, express only low levels of sialyl-Lewis X, which probably explains their weak E-selectin binding. Eosinophils express a little more PSGL-l than neutrophils[37] which may explain increased binding affinity. Alternatively there may be, as yet to be defined, functionally important differences in glycosylation of PSGL-1 between the 2 cell types that influence binding.

In summary, the tethering step, which is an essential requirement of leukocyte transmigration into tissue, can play a major part in selective eosinophil migration through the combined effects of VLA-4/VCAM-1 and PSGL-1/P-selectin. Certainly, under conditions in which only IL-4 and IL-13 are expressed, as may be seen in mild to moderate asthma, only eosinophils would be expected to bind to endothelium, with P-selectin playing a major role in eosinophil capture. However, in more florid disease, for example, after allergen challenge or in exacerbations of asthma, in which larger amounts of TNF-α and IL-1 are likely to be generated, neutrophils would also be expected to be recruited by E-selectin and VLA-4/VCAM-1 would be the dominant receptor-mediating eosinophil capture.

Activation and firm arrest

After leukocytes become tethered to the endothelium, activation results in functional up-regulation of integrins that bind to ICAMs and VCAM expressed by the endothelium, resulting in the firm arrest that is a prerequisite of transmigration. The activation step is thought to be mediated by chemoattractants on the endothelium acting through G protein–linked pertussis toxin (PT)–sensitive serpentine receptors.[51] This process can be modeled in vitro by observing the behavior of leukocytes in flow chambers binding to purified adhesion proteins. Thus, when neutrophils flow across slides coated with P or E selectin and ICAM-1, they roll but do not stop unless an activating stimulus is exogenously added. Similarly, on HUVECs stimulated with optimal concentrations of TNF-α or IL-1 the majority of tethered neutrophils rapidly arrest. The activating stimulus expressed by the endothelium that stimulates neutrophil β2 integrin-mediated binding in these circumstances include PAF and IL-8.[52] Some chemoattractants such as PAF and eotaxin, but not RANTES, induced β2- and VLA-4–mediated eosinophil adhesion to HUVECs in static assays.[25,53] Eosinophil binding to purified adhesion proteins was up-regulated by RANTES, monocyte chemotactic protein (MCP)-3, and C5a in a VLA-4– and Mac-1–dependent fashion by PT-inhibitable receptors.[54] VLA-4–, unlike Mac-1–, dependent increases in adhesion, were transient and relied on actin polymerization. C3a and C5a were able to mediate firm arrest of eosinophils to rabbit mesenteric endothelium under flow conditions.[55] However, despite apparent eosinophil active chemokine production, eosinophil adhesion to TNF-α– and IFN-γ–stimulated HUVECs under flow was only slightly inhibited by either anti-CCR3, the major chemokine receptor on eosinophils, or PT. When HUVECs were stimulated with IL-4 alone, no eosinophil-active chemokine release was detected, suggesting that under conditions more relevant to allergic disease CC chemokines may not be playing a major role in the activation step.[56] Consistent with this, we observed only rolling behavior when eosinophils bound to IL-4– and IL-13–stimulated HUVECs,[40] although this was not the experience of Patel[41] who found that all the eosinophils arrested. A confounding factor is VCAM-1, which may be able to promote eosinophil arrest in the absence of an activating stimulus. In our studies of binding to nasal polyp endothelium, whereas neutrophil adhesion was inhibited by PT, an anti-IL-8R antibody and a PAF antagonist, eosinophil adhesion, although activation dependent, was not inhibited by PT or anti-CCR3.[57] This suggests that alternative endothelial-associated pathways may exist for activation of eosinophil β2 integrins.

A number of studies of eosinophil adhesion and transmigration through HUVECs have shown that these events are mediated by a combination of VLA-4/VCAM-1 and the β2 (CD18) integrins binding to ICAM-1, the relative importance of either pathway depending on the cytokines involved in stimulating the endothelium. Leukocyte function–associated antigen-1 and Mac-1 are both involved in CD18-mediated binding.[8,25,27,28] Eosinophil accumulation was reduced in ICAM-1 gene–deleted mice and by the use of anti-ICAM-1 mAbs.[44,45,58] The contribution from the CD18 integrins remains substantial even in the presence of good VCAM-1 expression, suggesting that this is not a site at which selection of eosinophils versus neutrophils occurs to any great extent. Consistent with this, in the frozen section assay (FSA) we found that most of the integrin contribution for both eosinophil and neutrophil migration was by β2 rather than by VLA-4/VCAM-1.[57] Interestingly, in the ICAM-2 gene–deleted mouse eosinophil accumulation was increased in the lung after antigen challenge through an unknown mechanism.[59]

In summary, the major contribution at the adhesion stage for selective eosinophil migration appears to be at the capture step, with P-selectin and VCAM-1 cooperating to tether eosinophils, but not neutrophils, to IL-4– and IL-13–stimulated endothelium. There are, however, potentially important differences in the activation step

between the 2 cell types that could result in further levels of specificity.

SELECTIVE CHEMOATTRACTANTS

Until this last decade most effective eosinophil chemoattractants that had been identified, such as PAF and C5a, were also active on neutrophils. Then, in 1992, RANTES (activating eosinophils through CCR-3 and CCR-1) and macrophage inflammatory protein (MIP)-1α (CCR-1) were shown to be effective eosinophil chemoattractants that were not active on neutrophils.[60,61] Characterization of a number of other eosinophil-active CC chemokines has since followed, including eotaxin (CCR-3)[62,63] eotaxin-2 (CCR-3),[64] MCP-2 (CCR-3),[65] MCP-3 (CCR-3),[66] MCP-4 (CCR-3),[67] and MIP-5 (CCR-1 and CCR-3).[68] Eotaxin and eotaxin-2 only signal through the CCR-3 receptor, whereas the other chemokines can bind to alternate noneosinophil-expressed chemokine receptors. The major chemokine receptor on eosinophils is CCR-3, which is also expressed by basophils and a small subset of peripheral blood T cells, but no other leukocytes, so that it is an attractive therapeutic target for the treatment of eosinophilic inflammation.[69,70] Expression of CCR-1 by eosinophils is generally low or absent, although higher levels have been detected on about 10% of both nonatopic and atopic donors.[71] There is some functional evidence that eosinophils may express other, as yet unidentified, chemokine receptors.[72] There are also some reports of IL-8 being a chemotactic factor for eosinophils[73] and IL-5 was reported to induce expression of IL-8 receptors,[74] although the literature is contradictory in this regard with some investigators being unable to detect IL-8 receptors on eosinophils.[75] In our hands IL-8 was only active on eosinophils from highly eosinophilic donors.[76]

There has been considerable interest in which chemokines may be involved in eosinophil recruitment in asthma. In asthma increased expression of a number of eosinophil-active chemokines, including eotaxin,[77,78] RANTES,[79] MCP-3[80] and MCP-4,[78] and MIP-1α,[79] has been demonstrated by a combination of reverse transcription–PCR, in situ hybridization, ELISA, and immunohistochemistry. In some cases expression has correlated with eosinophil counts. Low levels of constitutive expression have usually been detected in healthy control subjects.

A number of animal models have been used to study the role of chemokines (as well as growth factors and adhesion receptors) in asthma, in particular ova-sensitized and ova-challenged mice. The mouse model is particularly powerful because of the ability to study genetic modifications of the gene of interest, although substantial differences in the anatomy of the mouse and human lung caution against overinterpretation of the data, particularly in terms of the relationship between inflammatory changes and BHR. Evidence for a role for most of the CCR-3–binding chemokines, but particularly eotaxin, has been obtained with use of animal models.[21,81-87] However, as might be expected, complete abrogation of eosinophil migration has not been observed by negating any single chemokine.[88] Results with the CCR-3–gene deleted mouse, where a more profound effect might be expected, are awaited with interest. T cells, in particular T_H2 cells, do not appear to be a major source of CC chemokines, which are generated by structural cells such as the bronchial epithelium, endothelium, fibroblast, or smooth muscle. In this context it is particularly interesting that IL-4 and IL-13 stimulated the production of eotaxin from fibroblasts and epithelial cells, so linking eosinophil chemoattractant release with T_H2-related immunologic events.[89-91]

PROLONGED SURVIVAL

Eosinophils rapidly undergo apoptosis unless provided with support from eosinophil growth factors such as IL-5, GM-CSF, and IL-3, which have each been shown to be present in increased amounts in the airways of asthmatic patients. The signaling pathways involved in growth factor–induced eosinophil survival involve the Lyn, Jak 2, Raf 1, and mitogen-activated protein kinases.[92] Prolonged survival under the influence of locally generated growth factors has been considered to be an important mechanism for selective eosinophil accumulation in allergic disease. Direct evidence for prolonged survival was provided by a study where anti-IL-5 antibodies caused rapid loss of eosinophils from cultured explants of nasal polyps.[93] Triggering of eosinophil apoptosis in the airways of mice using an anti-Fas mAb resulted in decreased airway eosinophilia after allergen challenge.[94] The number of apoptotic eosinophils in the airways of asthmatic patients was increased in subjects treated with inhaled glucocorticoids (GC),[95] possibly as a result of either inhibition of growth factor production, or as a direct effect on eosinophil survival.[96]

THE MULTISTEP PARADIGM OF SELECTIVE EOSINOPHIL RECRUITMENT: IMPLICATIONS FOR DRUG DEVELOPMENT

I have estimated that there is a 50- to 100-fold increase in the accumulation of eosinophils over neutrophils in the airways in clinical asthma. Increased neutrophil migration may also occur in some individuals, so the total increase in eosinophils trafficking is likely to be even greater, but it is the mechanisms of selective trafficking that I am particularly focusing on here. The relative contribution of each stage in the life cycle of the eosinophil to selective trafficking can only be estimated and in any case probably varies both between and within individuals at different times in the disease process. The effect of increased hematopoiesis and release from the bone marrow on selective eosinophil migration can, however, be calculated from the peripheral blood eosinophil count, which in terms of both percentage and total numbers is about 4-fold greater in asthma compared with normal subjects, although there is considerable variability.[97]

Adhesion to endothelium is an absolute requirement for migration to occur. IL-4– and IL-13–stimulated endothelial cells support eosinophil but very little neutrophil binding, whereas TNF-α stimulation supports binding of both cell types equally.[98] The contribution of adhesion to selective eosinophil recruitment will therefore depend crucially on the relative amount of these cytokines that are generated. In our experiments with the FSA a striking finding was that up to 10-fold more eosinophils than neutrophils bound to nasal polyp endothelium (NPE),[37] giving support to the idea that the endothelium is indeed an important site for eosinophil enrichment. The combined effect of bone marrow and adhesion events therefore could easily result in the region of a 20-fold increase in eosinophils relative to neutrophils tethered to the endothelium.

Eosinophil chemoattractants, particularly chemokines, are clearly important in directing eosinophils into tissue. However, a number of studies have demonstrated increased expression of effective neutrophil chemoattractants, particularly IL-8,[99] in allergic disease so that any neutrophils that adhere to the vascular endothelium in allergic disease should be able to efficiently transmigrate into tissue. The effect of the chemotaxis step on selective trafficking of eosinophils versus neutrophils may therefore not be as great as has been suggested by the animal models discussed above, in which relatively few neutrophils are recruited. How much selectivity occurs at the chemotaxis stage is therefore guesswork, but for illustrative purpose let us estimate 4-fold. Similarly, although prolonged survival is undoubtedly a factor in maintaining eosinophil numbers in tissue, the magnitude of this effect on selective recruitment is difficult to gauge. Neutrophil survival factors, particularly GM-CSF, are also expressed in asthma, and these may have a comparable effect on neutrophil persistence. The exact numbers do not matter other than to illustrate that each stage can have a marked effect on selective recruitment and that it is unlikely that any single stage, let alone any single molecule, is likely to be wholly responsible. It can be seen that the cumulative effects of each stage are more than enough to result in very considerable enrichment of eosinophils seen in disease (Fig 1).

Although multiple molecular events direct recruitment, these events are integrated and controlled by the cytokines IL-5, IL-4, and IL-13.[100] In atopic disease at least, these cytokines are likely to be largely generated in a coordinate fashion by allergen-stimulated CD4 +ve T_H2 lymphocytes.

What does this multistep process mean for the development of drugs to inhibit eosinophil recruitment? An important feature of the paradigm that I have outlined above is that it should be possible to inhibit recruitment at each of the stages. IL-5 is crucial at a number of steps, but particularly in eosinophilopoiesis and in enhanced chemotactic responsiveness in the periphery. IL-5 antagonists are almost certainly going to have profound and specific effects on eosinophil trafficking in allergic disease, and the results of continuing trials with humanized mAbs against IL-5 are awaited with interest. P-selectin/PSGL-1 and VCAM-1/VLA-4 cooperate to tether eosinophils to endothelium and it is likely that antagonists of either receptor pair will inhibit eosinophil recruitment. The results of current clinical trials of low-molecular-weight potent VLA-4 antagonists in asthma are awaited with interest. There has been less progress in the development of potent selectin antagonists for asthma, although results in animal models of allergic disease suggest that sPSGL-1 could form the basis for a therapeutic strategy.[101] The plethora of eosinophil chemoattractants released in allergic disease, including lipid mediators,[102] may limit the effectiveness of individual chemokine antagonists, although CCR-3 antagonists are likely to be more effective. Clinical trials of CCR-3 antagonists may not be long coming because it appears considerably easier to develop low-molecular-weight, orally effective drugs against serpentine receptors than growth factor or adhesion receptors. However, the best strategy may be to suppress the production of T_H2-associated cytokines, for example, by altering the response to allergen sensitization toward a T_H1 response or through antagonizing transcription of T_H2 cytokines.[103]

In summary, the enormous effort that has gone into understanding the molecular basis for selective eosinophil recruitment in asthma and related diseases over the last 30 years has borne fruit, and in the coming 5 years we should have several drugs available that will more or less specifically inhibit their tissue accumulation. At the very least this will help us to ask the central question that has concerned eosinophil biologists for the last 3 decades. Do eosinophils really cause asthma?

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