

Eosinophil adhesion in allergic inflammation

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Eosinophils are primarily tissue dwelling cells, seen for example, in large numbers in the lamina propria of the normal gut. In normal tissue they appear as intact cells, but in disease they often undergo massive degranulation. A characteristic feature of allergic inflammation is the accumulation of eosinophils in the skin and airway, where they are not normally present. Preformed and newly formed, eosinophil-derived inflammatory mediators are thought to cause many of the symptoms of allergic disease.¹ Two linked questions central to our understanding of the role of the eosinophil in disease are therefore: (1) what are the mechanisms by which eosinophils migrate into tissue? and (2) how do eosinophils become activated to release their mediators? Migration of eosinophils into tissue involves both an adhesion process and directed locomotion under the influence of chemotactic mediators generated locally at the inflammatory focus. One of the key features of eosinophil-related diseases is that eosinophil accumulation often occurs with increased numbers of mononuclear cells but without an increase in neutrophils. Although this may be due in part to prolonged survival of eosinophils under the influence of locally generated cytokines such as interleukin (IL)-5 and IL-3, it nonetheless suggests a degree of selectivity in the migration process. Eosinophil adhesion in allergic inflammation involves a series of interactions as the cell migrates into the tissue. Initially, there is adhesion to the vascular endothelium followed by interaction with the extracellular matrix and resident cells, such as fibroblasts and epithelial cells (Fig. 1). Each of these steps involves a distinct set of eosinophil receptors. A summary of the adhesion receptors expressed by eosinophils and their ligands or counterreceptors is shown in Table I.

Abbreviations used

BHR:	Bronchial hyperresponsiveness
HUVECs:	Human umbilical vein endothelial cells
ICAM-1:	Intercellular adhesion molecule-1
IL:	Interleukin
LFA-1:	Leukocyte function associated antigen-1
mAb:	Monoclonal antibody
TNF- α :	Tumor necrosis factor- α
VCAM-1:	Vascular cell adhesion molecule-1
VLA-4:	Very late activation antigen-4

EOSINOPHIL ADHESION TO VASCULAR ENDOTHELIUM

Most of the early studies of eosinophil adhesion to endothelium used human umbilical vein endothelial cells (HUVECs) cultured in 96-well plates. HUVECs were cultured in medium or in the presence of various cytokines, particularly IL-1 and tumor necrosis factor- α (TNF- α). These studies were useful in establishing the receptors potentially involved in eosinophil adhesion to endothelium. Under standard assay conditions, eosinophils have a baseline adhesion to HUVECs that is resistant to antibodies against the known adhesion molecules. Adhesion is enhanced about two-fold by activation of eosinophils by chemotactic mediators such as platelet activating factor and, more selectively, by cytokines such as IL-5.²⁻⁴ This enhancement is mediated primarily by Mac-1 binding to an as yet unidentified ligand on HUVECs. Cytokine stimulation of HUVECs for several hours results in a marked enhancement of eosinophil adhesion compared with background. This is the result of increased expression of a number of adhesion receptors including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin.⁵⁻⁸ Expression is increased as a result of increased gene transcription with new protein synthesis. Eosinophils can also bind to P-selectin, which is rapidly and transiently expressed on the cultured endothelial cell membrane over a period of a few minutes, being recruited from intracellu-

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J ALLERGY CLIN IMMUNOL 1994;94:1163-71.

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0091-6749/94 \$3.00 + 0 1/0/59911

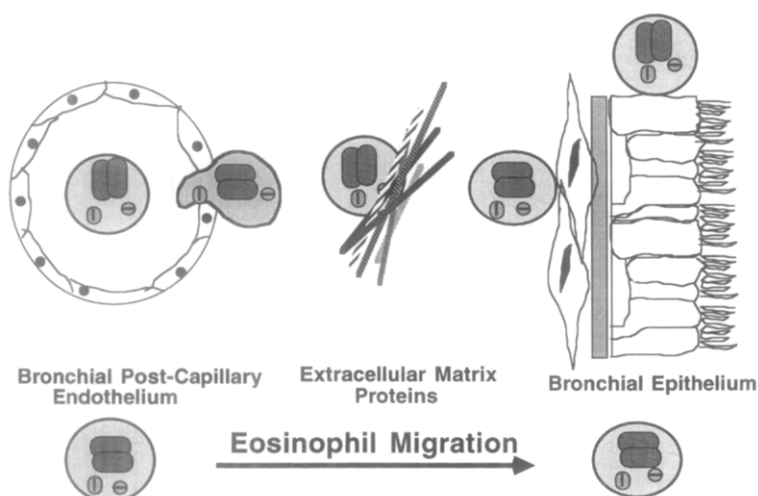


FIG. 1. Schematic representation of the potential adhesion interactions of the eosinophil as it migrates into tissue.

TABLE I. Eosinophil adhesion receptors and their counterstructures

Eosinophil 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)		
Selectins		
L-selectin	GlyCAM-1, CD34	
Carbohydrate		
PSGL-1	P-selectin	
E-selectin ligand	E-selectin	
Others		
CD44		Hyaluronate

MAdCAM-1, Mucosal addressin cell adhesion molecule-1; *PECAM*, platelet endothelial cell adhesion molecule; *GlyCAM*, glycosylated cell adhesion molecule-1; *PSGL-1*, P-selectin glycoprotein ligand-1.

lar stores as a result of stimulated with mediators such as thrombin and histamine.^{9, 10} Antibodies against L-selectin also inhibit eosinophil adhesion to cytokine-stimulated HUVECs under flow conditions.¹¹ Interestingly, in this study one anti-L-selectin antibody blocked eosinophil but not neutrophil adhesion, suggesting a difference in the nature of the L-selectin ligand recognized by these two cell types. ICAM-1 is a counterreceptor for the leukocyte integrins Mac-1 and leukocyte function associated antigen-1 (LFA-1) whereas VCAM-1 binds to the $\beta 1$ -integrin very late

antigen-4 (VLA-4) ($\alpha 4\beta 1$).^{12, 13} Because VLA-4 is expressed by eosinophils but not neutrophils, it was hypothesized that the VLA-4/VCAM-1 pathway may be partly responsible for selective migration of eosinophils through endothelium. This hypothesis was strengthened by the observation that IL-4 selectively induced expression of VCAM-1 on HUVECs¹⁴ and that eosinophil but not neutrophil adhesion to HUVECs was enhanced by IL-4 in a VLA-4-dependent manner.¹⁵ In addition, IL-4 transgenic mice had an eosinophilic inflammation of the conjunctiva.¹⁶ However, as discussed below,

expression of VCAM-1 in allergic inflammation is weak, which goes against this hypothesis.

The selectins bind, via their lectin domain, to O-linked sugars attached to membrane-expressed glycoproteins.¹⁷ The O-linked sugars involved in selectin binding are a family of sialylated, fucosylated glycans typified by sialyl Lewis X, with the strength of adhesion depending on the cell type, the protein backbone, and the exact structure of the sugar moiety involved. Two ligands for L-selectin have been described, expressed on high endothelial venules of peripheral lymph nodes. One is a 50 kd, sulfated, single-chain glycoprotein termed glycosylated cell adhesion molecule-1 (GlyCam-1), which is a heavily O-glycosylated mucin-like protein.¹⁸ An alternate ligand is CD34, which has a similar mucin-like structure. Unrelated in amino acid sequence, but also consisting of a heavily O-glycosylated glycoprotein (this time with a homodimeric structure), is the recently identified P-selectin ligand found on neutrophils and HL60 cells termed P-selectin glycoprotein ligand-1 (PSGL-1).¹⁹ The E-selectin ligand has not yet been fully characterized. Interestingly, there is evidence to suggest differences between the E-selectin ligand on eosinophils and neutrophils. Expression of sialyl-Lewis-X is much greater on neutrophils than on eosinophils and neutrophils binds more readily to purified E-selectin than eosinophils.²⁰ The two L-selectin ligands expressed on high endothelial venules in peripheral lymph nodes are not expressed on HUVECs, so that other ligands for L-selectin may yet be identified.

It is now clear that at least in the case of neutrophils, transmigration is a staged process with an initial tethering step mediated by selectins. This is followed by an activation stage mediated by chemotactic factors, which in turn is followed by a firmer adhesion step involving binding of integrin receptors to their immunoglobulin family counterreceptors, which results in transmigration through the endothelium.²¹ Simple adhesion assays with the 96-well plates in static systems therefore explore only part of the picture. Further information has been gained by investigating eosinophil transmigration through endothelial cells with the use of artificial models of the blood vessel wall. These studies have demonstrated that eosinophil transmigration through HUVECs was increased by cytokine stimulation and that eosinophils from allergic donors showed an increased migration capacity consistent with the idea that eosinophils from asthmatic donors

are activated.²² Similarly in vitro culture of resting eosinophils with granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5 increased the migration capacity of eosinophils from normal donors. The receptors that mediate transmigration of eosinophils through IL-1- or TNF- α -stimulated HUVECs appear to be primarily the leukocyte integrins LFA-1 and Mac-1 on the eosinophil binding to ICAM-1 and possibly other, as yet unidentified, ligands on the endothelium.²³ Antibodies against VLA-4 and VCAM-1 did not inhibit IL-1-stimulated transmigration. In contrast, eosinophil migration through IL-4-stimulated HUVECs was partly inhibited by anti-VLA-4 antibodies, as well as by antibodies against the leukocyte integrins.²⁴ Consistent with the premise that the selectins are not involved in transmigration, antibodies against E-selectin failed to block transmigration through IL-1-stimulated HUVECs.

MODULATION OF EXPRESSION OF EOSINOPHIL ADHESION RECEPTORS AND THEIR LIGANDS

For eosinophils to function, they must be able to both increase their adherence to surfaces during inflammatory responses and to "disadhere" to allow movement. Adhesion receptor function can be modulated either by changes in the number of expressed receptors or the conformation of already expressed receptors. Current evidence suggests that the main method of modulation of the selectins and immunoglobulin family adhesion receptors is through changing the number of expressed receptors, whereas integrin function is affected more by their conformational state. Short-term (less than 1 hour) stimulation of eosinophils with mediators such as platelet activating factor and IL-3 resulted in shedding of L-selectin and increased expression of Mac-1 as a result of recruitment from intracellular stores.²⁵ Interestingly, this pattern is also seen when eosinophils transmigrate through IL-1-stimulated HUVECs suggesting that the process of transmigration itself in some way results in eosinophil activation.²⁶ Twenty-four-hour culture of eosinophils with cytokines such as IL-3 and IL-5 results in a marked increase in Mac-1 expression as a result of new protein synthesis and induction of ICAM-1 expression.²⁷ This seems relevant to in vivo findings in that sputum, bronchoalveolar lavage eosinophils express ICAM-1 and have increased expression of Mac-1 and reduced expression of L-selectin.²⁸ VLA-4 appears to be consti-

tively active on eosinophils with little difference in the degree of VLA-4 expression between resting and activated eosinophils. However, one study did find evidence that β 1-integrin function on eosinophils was affected by the activation state of the receptor. Kuijpers et al.²⁹ observed that a monoclonal antibody (mAb) 8A2, which "activated" β 1-receptors inhibited eosinophil transmigration through fibronectin and HUVEC-coated polycarbonate filters, as if freezing the β 1-receptors in a high avidity state inhibited eosinophil migration.²⁹ The functional relevance of changes in adhesion receptor expression is not entirely clear. It has been postulated that shedding of L-selectin allows "disadhesion" and transmigration to occur, although there is no direct evidence of this. Mac-1 is clearly a crucial receptor in eosinophil migration, although activation of the receptor appears to be independent of changes in expression. ICAM-1 expression is associated with the ability of eosinophils to present antigen to T cells in vitro although whether this occurs in vivo is not known.³⁰

EXPRESSION OF ENDOTHELIAL ADHESION MOLECULES IN ALLERGIC INFLAMMATION

The influence of inflammatory mediators on the expression of adhesion receptors suggests that modulation of expression may direct leukocyte traffic into inflammatory sites. How consistent with this idea is the data on in vivo expression of adhesion receptors in allergic inflammation? To some extent, expression of endothelial adhesion receptors is organ-dependent. Normal skin endothelium has a low background expression of ICAM-1, E-selectin, and VCAM-1. Endothelial membrane expression of ICAM-1 and E-selectin in the skin is increased after allergen challenge, with the kinetics of upregulation broadly corresponding to the pattern observed in vitro with HUVECs.³¹ VCAM-1 expression is weak in both normal and allergen-challenged skin. 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 responsible for increased expression.³² TNF α from mast cells would be a likely candidate. Adhesion receptor expression in the skin of patients with atopic dermatitis has not been well documented. In both the upper and lower airways, a more complex pattern of expression is observed with good constitutive staining of ICAM-1 and E-selectin in the normal airway. This has made it more difficult to demon-

strate an increase in expression of these receptors after allergen challenge or in clinical disease. In the study by Bentley et al.³³ ICAM-1 and E-selectin expression were increased in the airways of patients with intrinsic asthma but not in those of patients with extrinsic asthma. Similar findings for ICAM-1 and E-selectin were reported by Montefort et al.³⁴ In a study by Bentley et al.,³³ no increases in adhesion molecule expression were seen after aerosol allergen challenge, although there was an almost significant trend toward an increase in VCAM-1 expression. In addition, there was a good correlation between numbers of airway eosinophils after allergen challenge and expression of both VCAM-1 and ICAM-1, suggesting that VCAM-1 may be involved in eosinophil recruitment after allergen challenge, if not in clinical disease. ICAM-1 and E-selectin expression were increased after segmental allergen challenge through a bronchoscope.³⁵ An increase in ICAM-1 and VCAM-1 expression was also observed in perennial rhinitis, although VCAM-1 was relatively poorly expressed.³⁶ P-selectin expression was not addressed by these studies, and because of the stored nature of P-selectin in endothelial cell granules, interpretation of P-selectin expression in immunohistochemical sections is difficult.³⁷ However, one study demonstrated the translocation of P-selectin to the endothelial cell membrane for up to 1 hour after the injection of the neuropeptides substance P, vasoactive intestinal peptide, and calcitonin gene-related peptide.³⁸

EOSINOPHIL ADHESION IN VIVO

Assessments of eosinophil adhesion in vivo have been restricted so far to studies in animal models with monoclonal antibodies. A number of studies have been performed on trapped wild cyanomolgous monkeys with a natural sensitivity to *Ascaris* antigen. Inhalation challenge with *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 or multiple. In the case of a single challenge, an early and sometimes dual (early and late) response was observed; in the case of a multiple challenge, a marked eosinophilia and increased BHR were seen. The pre-challenge state of the animal was also important in that those animals without baseline airway inflammation had a marked airway eosinophilia and increased BHR after multiple allergen chal-

TABLE II. Expression of adhesion proteins on nasal polyp endothelium

Adhesion protein	Total blood vessels stained positive (%)	Stain intensity (range)
P-selectin	44.7 ± 4.9	3 (2-4)
E-selectin	28.8 ± 6.9	2 (1-3)
ICAM-1	39.8 ± 4.8	2 (2-4)
VCAM-1	4.8 ± 1.7	1 (0-2)

Adhesion protein expression was determined by immunohistochemistry and expressed as the mean percent of total blood vessels (as determined by EN4 staining) that stained positive for each adhesion protein. Data are expressed as means ± SEM for nasal polyps from eight different patients.

allenges, whereas those animals with preexisting airway eosinophilia had markedly increased pre-challenge BHR and a late response after a single allergen challenge, characterized by increased numbers of airway neutrophils.³⁹ The airway eosinophilia and increased BHR after multiple allergen challenge was inhibited by an mAb against ICAM-1 but not E-selectin.⁴⁰ In contrast, the late response and airway neutrophilia after single challenge was inhibited by anti-E-selectin but not by anti-ICAM-1.⁴¹ 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. Anti-Mac-1 mAb, after repeated challenge, inhibited the development of BHR and reduced the levels of eosinophil cationic protein in the bronchoalveolar lavage fluid but did not inhibit the airway eosinophilia, suggesting that triggering through Mac-1 was involved in eosinophil activation and mediator release.⁴² In a sheep model of allergen challenge, anti-VLA-4 mAbs had a similar effect in that they inhibited the late response to allergen challenge and the release of eosinophil granule proteins into BAL fluid but not the airway eosinophilia seen in this model. Lastly, an anti-VLA-4 mAb inhibited the migration of eosinophils into the skin of guinea pigs both after injection of chemotactic factors and after passive cutaneous anaphylaxis.⁴³ These studies point to an important role for VLA-4, Mac-1 and ICAM-1 in eosinophil recruitment and activation in allergic inflammation and suggest that suitably designed adhesion receptor antagonists may be therapeutically effective.

EOSINOPHIL ADHESION TO NASAL POLYP ENDOTHELIUM

In an attempt to study eosinophil adhesion in a more physiologic setting than HUVEC-based sys-

tems, we undertook studies of adhesion of peripheral blood eosinophils to nasal polyp endothelium with the frozen-section Stamper-Woodruff assay.⁴⁴ Nasal polyps are readily available, large pieces of tissue in which the blood vessels can be readily identified. They are highly eosinophilic. The Stamper-Woodruff assay has been used extensively to characterize lymphocyte homing to high endothelial venules, but less used to study adhesion of other leukocytes. The assay involves layering the cells onto sections of tissue and allowing adhesion to occur on a rotating platform. The cells are therefore adhering under a degree of shear stress that favors binding through selectins. We initially investigated the expression of endothelial adhesion molecules in nasal polyps by using immunohistochemistry (Table II). We found good expression of ICAM-1, E-selectin, and P-selectin, on average, about 30% of blood vessels were stained. VCAM-1 expression was either very weak or absent. We then investigated eosinophil adhesion to nasal polyp endothelium. Eosinophils adhered in a specific manner to eosinophil cells with minimal adhesion to background structures. Adhesion was dose- and temperature-dependent, and room temperature was 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, whereas an antibody against Mac-1 had a significant though modest inhibitory effect. Most striking, however, was the almost total inhibition observed with an antibody against P-selectin (Fig. 2). Similar inhibition was seen with an IgG-P-selectin chimeric molecule that binds to the P-selectin ligand and blocks P-selectin interactions.⁴⁵ Because P-selectin is stored in Weibel-Palade granules within the endothelial cell, it is possible that eosinophils were binding to intracellular P-selectin. However, no eosinophil binding to normal skin endothelium was observed, in which P-selectin expression, as determined by immunohistochemistry, was similar

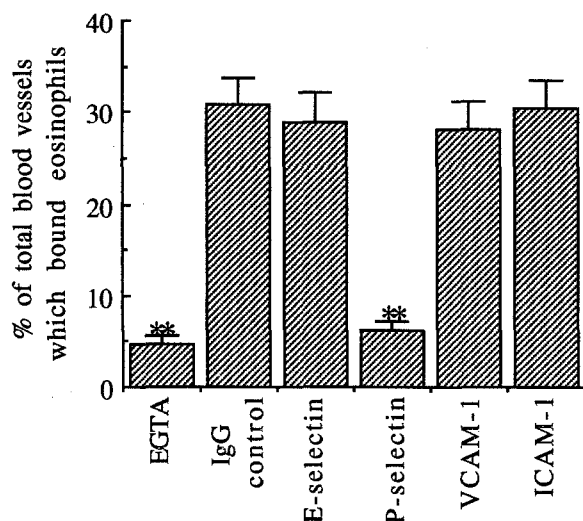


FIG. 2. Inhibition of eosinophil adhesion to nasal polyp endothelium by mAb against various endothelial adhesion molecules. Eosinophil adhesion was inhibited only by ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) and an antibody G1 against P-selectin.

to that observed in nasal polyps. In addition, immunofluorescence with confocal microscopy demonstrated membrane staining of P-selectin in nasal polyps compared with a granular pattern suggestive of intracellular staining in normal skin. These results suggest 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. The conditions of the assay, with quite vigorous rotation and the use of unstimulated eosinophils from normal donors, favor a selectin interaction. We are currently undertaking studies to determine whether eosinophils stimulated both *in vivo* and *in vitro* use a different pattern of adhesion receptors. It will also be of interest to determine whether there are differences in the pattern of adhesion between eosinophils and neutrophils because both *in vitro* and *in vivo* studies have suggested the dependence of neutrophil adhesion on all three selectins.⁴⁶

EOSINOPHIL ADHESION TO EXTRACELLULAR MATRIX

After migration through the endothelium, the eosinophil comes into contact with the proteins of the extracellular matrix. This is not simply a mesh in which the eosinophils are held, 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 the epithelium, and on leukocytes migrating from the peripheral blood. It is increasingly clear that tissue eosinophils have an activated phenotype. They express activation receptors such as CD69,⁴⁷ generate mRNA for a number of cytokines that do not appear to be generated by normal peripheral blood eosinophils,⁴⁸ and express the secreted form of eosinophil cationic protein as recognized by the mAb EG2.⁴⁹ However, it is far from clear what leads to this activated state. *In vitro* studies discussed above suggest that the process of migration through endothelium may result in eosinophil activation. In addition, locally generated chemotactic mediators such as platelet activating factor and RANTES may be involved. However, it is a reasonable hypothesis that interaction with the extracellular matrix, resulting in "outside in" signaling through integrin receptors, may be important in eosinophil priming and mediator release.⁵⁰ There have been relatively few studies of such interactions. Dri et al.⁵¹ studied the production of superoxide by eosinophils lying 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 with, for example, endothelial cells inhibiting superoxide production and fibrinogen priming eosinophils for enhanced superoxide generation after stimulation with N-formyl-methionyl-leucyl-phenylalanine. Anwar et al.⁵² demonstrated increased leukotriene generation by eosinophils adhering to fibronectin when compared with bovine serum albumin-coated surfaces. Perhaps a more profound effect was observed when eosinophils were cultured on plasma fibronectin, which resulted in prolonged survival of eosinophils as a result of autocrine generation of granulocyte-macrophage colony-stimulating factor and IL-3. Cytokine release and survival were inhibited by anti-VLA-4 mAb.⁵³ We have now extended these observations to show that tissue fibronectin is considerably more effective than plasma fibronectin in supporting eosinophil survival. This finding is consistent with the idea that survival is a result of triggering through VLA-4 because tissue fibronectin contains more of the alternatively spliced 111CS region that contains the binding site for VLA-4.⁵⁴ Survival was inhibited by antibodies against both VLA-4, $\alpha 4/\beta 7$, and Mac-1, suggesting that the effectiveness of fibronectin-

induced survival may be due to triggering through multiple integrin receptors. Consistent with this hypothesis were the observations that single antibodies, when bound to plates, did not support survival and that survival on purified VCAM-1 was considerably less than that on tissue fibronectin. It is likely that in addition to being important to eosinophil cytokine generation in disease, the interaction between eosinophils and fibronectin is a homeostatic mechanism by which eosinophils survive for prolonged periods in tissue before undergoing apoptosis and removal.

EPITHELIAL/EOSINOPHIL ADHESION INTERACTIONS

One of the most important features of asthma is thought to be the epithelial desquamation mediated by eosinophil-derived granule proteins. It is increasingly clear that the airway epithelium in asthma shows increased expression of ICAM-1,^{33, 55} and it has been suggested that the increased vulnerability of the epithelium to eosinophil-mediated damage is due to increased adhesiveness between eosinophils and epithelial cells mediated by ICAM-1 binding to LFA-1 and Mac-1. Several studies have reported increased ICAM-1 expression on epithelial cells.^{56, 57} This increase was associated with enhancement of neutrophil adhesion.^{58, 59} In addition, it has been observed that epithelial-derived cytokines can prolong eosinophil survival and that eosinophil-derived mediators can affect epithelial cilia beat frequency.^{60, 61} However, few studies have documented eosinophil adhesion to epithelial cells and the receptors involved. This area remains to be clarified.

SUMMARY AND CONCLUSIONS

Eosinophil adhesion has been studied in some detail in recent years, and a number of interesting observations have emerged. As with other aspects of eosinophil biology, there appears to be a greater similarity with basophils than with neutrophils in their pattern of adhesion interactions. A number of important differences with respect to neutrophils have emerged, which could be exploited for the treatment of eosinophil-mediated disease, including the observations that eosinophil adhesion can be modulated by selective cytokines such as IL-5 and that eosinophils, unlike neutrophils, express VLA-4 and $\alpha 4/\beta 7$. There is also tantalizing evidence emerging that eosinophils interact differentially with the selectins, with differing degrees of affinity of binding and possibly different counterreceptors. The extent to which

these observations will be useful in treating allergic disease remains to be seen.

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Distinguishing features of basophil and neutrophil activation by major basic protein

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Eosinophils play a prominent role in the pathogenesis triggered by the inflammatory late-phase reactions in allergic disease.^{1, 2} Although eosinophil release of mediators such as leukotriene C₄ and platelet activating factor clearly contributes to the pathogenesis,¹⁻³ a body of evidence has accumulated to demonstrate that the cationic proteins present within eosinophil granules are of at least comparable importance to the observed

pathogenesis.³ Most extensively studied among the various cationic proteins is major basic protein (MBP), a highly charged (isoelectric point = 10.9) 13.8 kd polypeptide that comprises a large portion of the eosinophil granule protein.^{3, 4} Results from a number of laboratories suggest that the severity of allergic late-phase reactions, and in particular airway hyperreactivity in asthma, is correlated with presence of MBP in sputum and at the reaction sites.^{2, 3} Although the pathogenic activity of MBP has been attributed most frequently to the well-described cytotoxic properties of the molecule,³ other findings have demonstrated that MBP activates various inflammatory cells in a noncytotoxic fashion. Specifically, micromolar concentrations of MBP stimulate basophil histamine release,⁵ neutrophil release of superoxide anion and specific granule enzymes,⁶ and

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J ALLERGY CLIN IMMUNOL 1994;94:1171-6.

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0091-6749/94 \$3.00 + 0 1/0/59913