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The role of adhesion molecules in human eosinophil and basophil recruitment

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Eosinophils and basophils accumulate in tissues during certain inflammatory processes, such as hypersensitivity diseases and experimental allergic late-phase responses in the nose, skin, or lungs.^{1, 2} However, the exact role of eosinophils and basophils in the pathophysiology of chronic allergic diseases remains incompletely defined. Eosinophil- and basophil-derived mediators can be identified during these inflammatory responses; many of these substances have been shown to induce or influence the pathophysiologic features of allergic disorders. The fact that levels of these cells and their mediators are reduced during resolution of allergic reactions further supports the hypothesis that eosinophils and basophils play a critical role in allergic diseases. Because these cells are preferentially attracted to extravascular inflammatory sites, mechanisms that favor their recruitment into the affected tissues must exist.²⁻⁴

In 1889, Julius Cohnheim, a former student of the pathologist Rudolph Virchow, published his landmark pathology textbook in which he carefully described all of the pathophysiologic features of inflammation, including leukocyte emigration into tissues.⁵ He noted that the vascular endothelium adjacent to the site of inflammation,

Abbreviations used

BAL:	Bronchoalveolar lavage
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
ICAM:	Intercellular adhesion molecule
IL:	Interleukin
MAdCAM:	Mucosal addressin cell adhesion molecule
MIP1 α :	Macrophage inflammatory protein 1 α
PECAM:	Platelet-endothelial cell adhesion molecule
sLe ^x :	Sialylated Lewis X antigen
TNF:	Tumor necrosis factor
VCAM:	Vascular cell adhesion molecule
VLA:	Very late activation

especially at the level of the postcapillary venule, was lined by margined leukocytes: "... the internal surface of the vein appears paved with a single but unbroken layer of colourless corpuscles without the interposition at any time of a single red one. It is the separation of the white from the red corpuscles that gives the venous stream in these cases that characteristic appearance, anything analogous to which you will look for in vain in the other vessels." His prediction, that there was "a molecular change in the vessel wall" resulting in the formation of some sort of "glue," was ultimately proven to be correct with the discovery, nearly a century later, of leukocyte and endothelial cell adhesion molecules. Within the past decade, an explosive growth in our knowledge of these molecules has occurred. Adhesion molecules are now known to be critical for virtually every step in cell recruitment, including margination, diapedesis, and chemotaxis. Several reviews have recently appeared in which various aspects of the function of adhesion molecules in allergic diseases are discussed.^{3, 6-8} Therefore this review briefly summarizes the known functions of endothelial and leukocyte adhesion molecules

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TABLE I. Biochemical and functional characteristics of several endothelial adhesion molecules

Endothelial cell adhesion molecule	CD designation	Molecular weight (kd)	Inducing stimuli*	Leukocyte ligands
ICAM-1	CD54	85-110	IL-1, TNF, IFN- γ	CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1)
ICAM-2	CD102	60	None	CD11a/CD18
PECAM-1	CD31	130	None	Unknown
VCAM-1	CD106	110	IL-1, TNF, IL-4	CD49d/CD29 (VLA-4, $\alpha 4\beta 1$) and Act-1 ($\alpha 4\beta 7$)
E-selectin	CD62E	115	IL-1, TNF	sLe ^x
P-selectin	CD62P	140	Histamine, thrombin	sLe ^x

*Surface expression of ICAM-1, ICAM-2, and PECAM-1 is constitutive.

TABLE II. Biochemical and functional characteristics of several eosinophil and basophil adhesion molecules

Adhesion molecule	CD designation	Molecular weight (kd)	Ligands
LFA-1 ($\alpha L\beta 2$)	CD11a/CD18	180/95	ICAM-1 (domain 1)
Mac-1 ($\alpha M\beta 2$)	CD11b/CD18	170/95	ICAM-1 (domain 3), C3bi
p150,95 ($\alpha X\beta 2$)	CD11c/CD18	150/95	Unknown
VLA-4 ($\alpha 4\beta 1$)	CD49d/CD29	150/130	VCAM-1, fibronectin
VLA-6 ($\alpha 6\beta 1$)*	CD49f/CD29	150/130	Laminin
Act-1 ($\alpha 4\beta 7$)	None	150/120	VCAM-1, MAdCAM-1, fibronectin
L-selectin	CD62L	90	GlyCAM-1, CD34, MAdCAM-1
sLe ^x	CD15s	Unknown	E-selectin, P-selectin

*Clearly expressed and functional on eosinophils; not detectable on basophils.

and examines the role that these molecules may play during eosinophil and basophil recruitment in vitro and in vivo, focusing in particular on work from our laboratories.

MOLECULAR MECHANISMS OF ENDOTHELIAL LEUKOCYTE ATTACHMENT

Many cell surface glycoproteins on endothelial cells are capable of mediating adhesion. These molecules can be subdivided on the basis of shared structural characteristics. For example, many adhesion molecules have globular domains resembling those found in immunoglobulins. On endothelial cells, those belonging to the immunoglobulin gene superfamily, which consists of more than a dozen structures, include intercellular adhesion molecule-1 (ICAM-1), ICAM-2, platelet-endothelial cell adhesion molecule-1 (PECAM-1), and vascular cell adhesion molecule-1 (VCAM-1)⁹ (Table I). Another family of adhesion molecules, of which there are only three known members, is the selectin gene superfamily.¹⁰ Two members of this family, E-selectin (originally called ELAM-1 or endothelial-leukocyte adhesion molecule-1)

and P-selectin (formerly GMP-140) can be expressed on the surface of endothelial cells.

Although ICAM-1, ICAM-2, and PECAM-1 are normally expressed on the endothelial surface, E-selectin and VCAM-1 are not, and various cytokines are capable of inducing or enhancing their expression (Table I).¹¹ P-selectin is also absent from the surface of resting endothelium, but in contrast to the other endothelial adhesion molecules, it exists preformed within granules (the so-called Weibel-Palade bodies) and is rapidly expressed (i.e., within minutes) after stimulation with agents such as histamine and thrombin.¹⁰ The N-terminal lectin domains of E-selectin and P-selectin bind to carbohydrate structures, such as those containing sialylated Lewis X antigen (sLe^x) or sialic acid $\alpha 2$ -3 galactose $\beta 1$ -4 (fucose $\alpha 1$ -3)N-acetylglucosamine).¹⁰

On leukocytes, additional families of adhesion molecules have been recognized; many of these are expressed by eosinophils and basophils (Table II). One of these families, the integrins, consists of more than a dozen heterodimers with distinct α chains and a common β chain,¹² and are responsi-

ble for adhesion to endothelial cells, complement protein fragments, extracellular matrix proteins, and other ligands.^{12, 13} For example, the very late activation antigen-4 (VLA-4) heterodimer (CD49d/CD29, $\alpha 4\beta 1$) can bind to both VCAM-1 and the CS-1 region of fibronectin.¹⁴ Another β subunit, $\beta 7$, can also pair with $\alpha 4$ ($\alpha 4\beta 7$ or Act-1). This heterodimer, like VLA-4, is capable of binding to fibronectin and VCAM-1,^{15, 16} but unlike VLA-4, $\alpha 4\beta 7$ binds to another adhesion molecule, mucosal addressin cell adhesion molecule-1 (MAdCAM-1) that has structural homology to both VCAM-1 and ICAM-1 and appears to be involved in lymphocyte homing to the gut mucosa.^{17, 18} The adhesive functions of integrins require divalent cations and can be augmented after cell activation as a result of movement of additional preformed molecules from within the cytoplasm to the cell surface or as a result of rapid conformational changes in the molecule.

The third member of the selectin family, L-selectin (formerly LECAM-1), is found on leukocytes, including eosinophils and basophils, and mediates localization of circulating neutrophils to inflammatory sites independent of integrins.¹⁰ This adhesion protein appears to function as an anchor, which is rapidly shed (rather than upregulated like integrins) during leukocyte activation and extravasation.¹⁰ L-selectin also binds to carbohydrates, and recently identified ligands include the extensively glycosylated structures GlyCAM-1¹⁹ and CD34.²⁰

GENERATION OF A SELECTIVE CELLULAR INFILTRATE

It has been proposed that a series of steps is necessary for the emigration of leukocytes into tissue sites.²¹ Under the influence of shear stress caused by blood flow, circulating leukocytes must first undergo a reversible process of margination, during which cells "roll" or reversibly attach to the endothelium. Both *in vitro* and *in vivo* studies suggest that these "tethering" adhesive interactions are mediated via carbohydrate-selectin interactions (probably involving both L- and P-selectin).¹⁰ The next step requires leukocyte activation, or "triggering," perhaps as a result of their exposure to chemoattractants released by endothelial cells or nearby tissue-dwelling cells.^{22, 23} Immobilization of activating stimuli on the endothelial cell surface may serve to facilitate these triggering events.²⁴ Alternatively, contact of leukocytes with endothelial adhesion molecules may activate the cells directly,²⁵ a process referred to

as "inside-out signaling." Associated with these events are increases in both avidity and expression of integrins on the leukocyte surface. Leukocyte rolling and activation is followed by firm leukocyte-endothelial adhesion and subsequent trans-endothelial migration (diapedesis), during which the leukocytes emigrate between endothelial cells and enter the extravascular space. These final events are mediated by $\beta 1$ and $\beta 2$ integrins and adhesion molecules belonging to the immunoglobulin family (e.g., ICAM-1, VCAM-1, and PECAM-1; see below), although the array of adhesion molecules available for these interactions varies among the different leukocyte types. According to this model, preferential recruitment of a given cell type is the net result of many separate events, rather than the effect of a unique cell-specific chemoattractant or adhesion molecule pathway for each cell type. This paradigm would suggest that a particular pattern of cellular influx would result from a sequence of relatively selective recruitment events in which overlapping cell adhesion mechanisms and chemotactic factors function in concert. For many cell types, evidence is rapidly accumulating in support of this hypothesis. With a variety of assays *in vitro* and *in vivo*, several laboratories have now examined eosinophil, basophil, and neutrophil adhesion responses and have identified a number of differences among these cell types.

COMPARING THE FUNCTION OF ADHESION MOLECULES DURING EOSINOPHIL, BASOPHIL, AND NEUTROPHIL ATTACHMENT TO ENDOTHELIAL CELLS

Methods have been established to study adhesion *in vitro* under conditions that simulate blood flow *in vivo*.^{26, 27} These assays are particularly useful in studying carbohydrate-selectin adhesion events, because integrins play a minimal role, at least in initiating adhesion, under conditions of shear stress. In comparing adhesion of eosinophils and neutrophils, it was demonstrated that both cell types can bind to cytokine-activated endothelium in a calcium-dependent manner.²⁸ Adhesion was mediated in part by leukocyte L-selectin, since monoclonal antibodies recognizing L-selectin significantly inhibited adhesion. One L-selectin antibody, LAM 1-11, had an unexpected activity in that it inhibited eosinophil but not neutrophil adhesion under conditions of shear stress *in vitro*, raising the possibility that eosinophils use an epitope on the L-selectin molecule that is not used by neutrophils.²⁸ Eosinophils that have shed

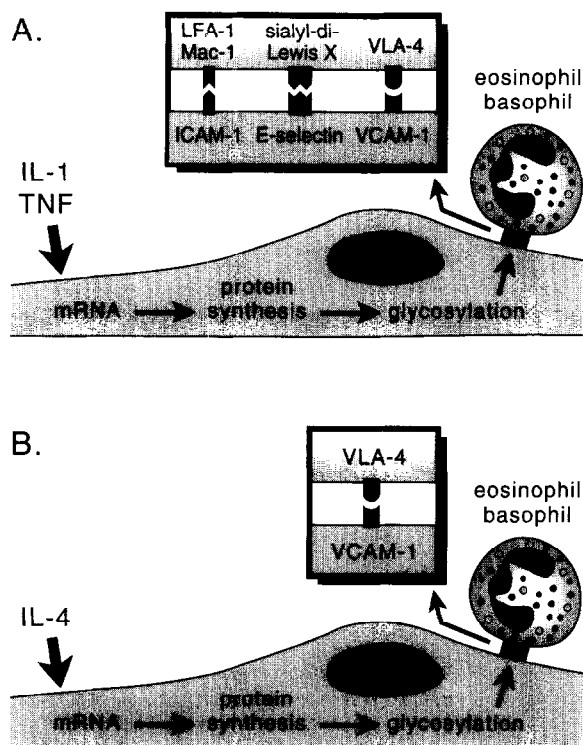


FIG. 1. Schematic representation of the effects of cytokine activation of endothelium on eosinophil and basophil adhesion. Note that a different pattern of adhesion molecules is expressed by endothelial cells after treatment with IL-1 or TNF (**A**) compared with treatment with IL-4 (**B**). Because neutrophils do not express $\alpha 4\beta 1$ (VLA-4), they cannot bind to VCAM-1. Copyright © 1993 by Jacqueline A. Schaffer. Reprinted with permission.

their L-selectin, either as a result of activation in vivo (e.g., as seen with eosinophils isolated from late-phase bronchoalveolar lavage (BAL) fluids²⁹) or in vitro (e.g., as seen after exposure to certain cytokines or chemoattractants³⁰) adhered poorly in this assay.²⁸ Basophils were also shown to express L-selectin and shed this molecule on activation in vitro or in vivo, although the shedding, compared with other granulocytes, is less complete.^{29, 31} No information is available about the function of L-selectin on basophils.

Recent data suggest that P-selectin can mediate adhesion and rolling of neutrophils under flow conditions. Unlike L-selectin, P-selectin will support adhesion under static conditions as well.^{10, 32} Like neutrophils, eosinophils have been shown to bind to P-selectin immobilized on plastic surfaces or when expressed on the surface of P-selectin-transfected Chinese hamster ovary (CHO) cells; antibodies to the lectin domain of P-selectin block adhesion.³²⁻³⁴ Based on a variety of pieces of in-

formation, including the effects of treatment with neuraminidase or various proteases, adhesion to selectins appears to be mediated by sialylated glycoprotein ligands on the surface of the eosinophil and neutrophil.³⁴⁻³⁶ Indeed, a 120 kd neutrophil ligand for P-selectin has been recently identified.³⁷ However, the ability of several of these enzymes to reduce neutrophil binding was greater than that seen for eosinophils, suggesting some differences in the level of expression or biochemical composition of their P-selectin ligands.³⁴ Unlike eosinophil adhesion to other ligands, eosinophils failed to spread on P-selectin and exhibited a reduced capacity to degranulate or produce superoxide anion, suggesting that attachment to P-selectin inhibited eosinophil function.³³ Whether similar functional interactions occur between basophils and P-selectin is not known.

In the mid 1980s, it was discovered that exposure of endothelial cells to interleukin-1 (IL-1), tumor necrosis factor (TNF), phorbol diesters, or bacterial lipopolysaccharide causes an enhanced adhesiveness for neutrophils.³⁸⁻⁴⁰ Subsequent studies revealed that the same basic results occur with eosinophils⁴¹ and basophils.⁴² Activation of endothelium by these stimuli induces expression of endothelial adhesion molecules including ICAM-1, E-selectin, and VCAM-1 (Fig. 1, A). To determine whether there was any selectivity among these three adhesion molecules for eosinophils or basophils, antibodies against ICAM-1, E-selectin, and VCAM-1 were compared for their ability to inhibit adherence of eosinophils, basophils, and neutrophils to IL-1-stimulated endothelial cell monolayers.⁴³ Specific antibodies to ICAM-1 and E-selectin inhibited adherence of all three leukocyte types, in agreement with results of another published report.⁴⁴ In contrast, anti-VCAM-1 antibody was extremely effective in inhibiting eosinophil adherence but had no effect on neutrophil adherence, suggesting that eosinophils, unlike neutrophils, recognize VCAM-1.⁴³ In these studies basophil adherence was also demonstrated to be partly mediated through VCAM-1, although the inhibitory effect seen with anti-VCAM-1 antibody was less impressive.

Since it has been established that a counterligand for VCAM-1 on lymphocytes was VLA-4,¹⁴ immunofluorescence and flow cytometry were used to examine VLA-4 expression on these cells. As expected, neutrophils did not express VLA-4, whereas eosinophils and basophils did.^{43, 45-47} Furthermore, antibodies to VLA-4 inhibited eosinophil and basophil but not neutrophil adhesion to

IL-1-stimulated endothelium.^{45, 47} To independently confirm that eosinophils and basophils could adhere to VCAM-1, the ability of these cells to adhere to a truncated recombinant form of VCAM-1, which had been immobilized to plastic plates, was tested. Eosinophils and basophils, unlike neutrophils, adhered more avidly to this surface compared with control surfaces, and the adhesion was inhibited with specific antibodies against VCAM-1 and VLA-4.^{3, 46, 48}

The finding that VCAM-1/VLA-4-mediated adhesion was different among eosinophils, basophils, and neutrophils raised the possibility that specific induction of VCAM-1 on endothelial cells might promote eosinophil and basophil adherence, but not neutrophil adherence. Previous studies suggested that the cytokine IL-4 was capable of selectively inducing VCAM-1 expression in endothelial cells (Fig. 1, B).⁴⁹ Incubation of endothelial cells with IL-4 did not influence neutrophil adherence but did induce eosinophil and basophil adherence, which was inhibited by more than 70% with either anti-VCAM-1 or anti-VLA-4 antibodies.⁴⁸ These findings were consistent with the observations that mice bearing an IL-4 transgene develop tissue eosinophilia and an allergic syndrome and that mice inoculated with a tumor cell line, which has been transfected with the IL-4 gene, develop local eosinophilia at the tumor site.⁵⁰ Therefore IL-4, by selectively promoting VCAM-1 expression, might contribute to the preferential recruitment of eosinophils (and basophils) compared with neutrophils seen during certain inflammatory responses. However, because VLA-4 is expressed on other cell types, including lymphocytes and monocytes, it seems unlikely that the VCAM-1/VLA-4 adhesion pathway is solely responsible for selective eosinophil and basophil recruitment. Finally, the discovery that eosinophils and basophils, but not neutrophils, express Act-1 ($\alpha 4\beta 7$)⁵¹ (and unpublished observations), a molecule that recognizes both VCAM-1 and MAdCAM-1, suggests that this integrin may also play a role in preferential recruitment responses.

Studies in which a truncated recombinant form of E-selectin immobilized on plastic plates was used confirmed that eosinophils, basophils, and neutrophils are capable of binding to E-selectin.^{52, 53} All three cell types adhered to E-selectin, and their adhesion was dependent on leukocyte surface expression of sialic acid, since neuraminidase treatment and removal of sialic acid abolished essentially all adhesive activity.^{52, 53} Interest-

ingly, basophils bound best to E-selectin, followed by neutrophils and eosinophils. This relative rank order of binding efficiency was not directly related to the quantity of sLe^x on the cell surface. Flow cytometric studies with antibodies specific for sLe^x, as well as an extended chain form of sLe^x (sialyl-dimeric Le^x), revealed that neutrophils had the greatest amount of sLe^x, whereas all three cells had comparable levels of sialyl-dimeric Le^x.⁵² (and unpublished observations). That the extended chain form of sLe^x may be responsible for adhesion to E-selectin was suggested by experiments in which endo- β -galactosidase was used. This enzyme removes the sialyl-dimeric Le^x (representing $\approx 10\%$ of the total pool of sLe^x on neutrophils but essentially all of the sLe^x on eosinophils) and almost completely inhibits binding of both eosinophils and neutrophils.⁵² Thus although the bulk of sLe^x remained on the surface of the neutrophils after treatment with endo- β -galactosidase, adhesion was dramatically impaired. These same experiments revealed that sialyl-dimeric Le^x is the important and predominant form of sLe^x on the eosinophil surface.

FUNCTION OF ADHESION MOLECULES DURING TRANSENDOTHELIAL MIGRATION

Transendothelial migration is a process by which cells entering a local inflammatory site traverse the vascular endothelium. Migration of neutrophils across endothelial monolayers *in vitro* has been shown to be enhanced by prior activation of the endothelium with IL-1 or TNF.⁵⁴ Several *in vitro* studies have now analyzed the molecular mechanisms regulating eosinophil transendothelial migration. As is the case with other leukocyte types, treatment of endothelial monolayers with IL-1 or TNF increased eosinophil transendothelial migration.^{55, 56} Eosinophil transendothelial migration through IL-1-treated endothelium was almost completely inhibited by antibodies to CD18 (β_2 integrin) on the leukocyte; CD29 (β_1 integrin) antibody had little or no effect.⁵⁶ Antibodies directed against the CD11a/CD18-1 binding site on ICAM-1 were moderately effective in inhibiting transmigration; a combination of anti-VCAM-1, ICAM-1, and E-selectin was more effective than anti-ICAM-1 antibody alone.⁵⁶ An additional study used an antibody that activates, rather than inhibits, $\beta 1$ integrin function to study its effect on eosinophil migration. Treatment with this antibody dramatically inhibited eosinophil chemotaxis and transendothelial migration, presumably by enhancing ad-

hesion and thereby preventing migration by tightly immobilizing these cells.⁵⁷ These data support the hypothesis that transendothelial migration of eosinophils involves the function and expression of adhesion molecules on both the leukocyte and the endothelium and suggest that the mechanisms regulating leukocyte adhesion may differ from those mediating transmigration. Recently, studies have also implicated PECAM-1 in leukocyte transendothelial migration responses.⁵⁸ Thus far, the role of PECAM-1 in eosinophil transmigration remains unknown, and mechanisms of basophil transendothelial migration have not been carefully studied.

FUNCTION OF ADHESION MOLECULES DURING MIGRATION THROUGH TISSUES

Once leukocytes enter the extravascular space, migration through the tissue is dependent on their ability to bind to extracellular matrix proteins. Because most of the leukocyte receptors for matrix proteins belong to the $\beta 1$ integrin family, eosinophils were recently examined for their expression of these surface structures.^{59, 60} Eosinophils were found to express both $\alpha 4$ and $\alpha 6$, the former functioning as a ligand for the CS-1 region of fibronectin, and the latter functioning as a laminin receptor.^{13, 59} Interestingly, adhesion to fibronectin was shown to activate production of IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which augmented eosinophil survival in an autocrine fashion.⁶¹ Studies examining expression of $\beta 1$ integrins in human basophils have documented expression of VLA-3, VLA-4, and VLA-5.^{61a} With the exception of VLA-4, the functions of these integrins have not been investigated.

EFFECT OF CYTOKINES ON EOSINOPHIL AND BASOPHIL ADHESION AND CHEMOTAXIS

Several cytokines have the ability to selectively enhance eosinophil or basophil adhesion responses. For example, exposure of eosinophils to IL-3, IL-5, or GM-CSF will promote their adhesion to various surfaces and induce or augment chemoattractant-induced adhesion responses.⁶²⁻⁶⁵ For basophils, IgE-dependent degranulation or treatment with IL-3 will enhance adhesion to endothelial cells,^{66, 67} and IL-3 and IL-5 are potent basophil chemoattractants.⁶⁸ In each instance, these treatments have little or no effect on neutrophils, and adhesion is mediated primarily through $\beta 2$ integrins.

In addition to the active role of the vascular endothelium in expressing adhesion counterligands for various leukocytes, mediators synthesized and released by activated endothelial cells or other resident tissue cells may influence cell adhesion. For example, it has been shown that neutrophil transendothelial migration across IL-1-activated endothelial cells requires, or is facilitated by, endothelial derived IL-8, a chemoattractant that appears to be necessary for penetration of the endothelial barrier.²³ IL-8 is a member of the C-X-C or α -subfamily of the chemokines, which consists of more than 15 structurally related 8 to 10 kd proteins that have secretagogue and chemotactic activities.^{69, 70}

Although IL-8 has been reported to have some chemotactic effect on primed eosinophils, this activity is quite modest.^{71, 72} It was reasoned therefore that another member of the chemokine family may subserve for eosinophils (and basophils) the role that IL-8 plays for neutrophils. To determine whether other members of the chemokine family might have preferential eosinophil-activating properties, a panel of chemokines was tested for their ability to promote eosinophil and neutrophil chemotaxis and transendothelial migration.⁷³ Among the nine chemokines examined, RANTES was by far the most effective in inducing eosinophil chemotaxis and transmigration, whereas MIP1 α had much less activity, findings consistent with other recent reports.^{74, 75} For neutrophils, IL-8 and GRO were active in chemotaxis and transmigration assays, whereas RANTES and MIP1 α had no effect. In the transendothelial migration assay, the effects of RANTES on eosinophils were synergistic with IL-5, and both anti-CD18 and anti-VLA-4 antibodies were needed to effectively inhibit RANTES-induced transmigration across IL-1-activated endothelium.^{3, 76} Although the ability of endothelial cells to release RANTES is currently unknown, these data suggest that RANTES and perhaps other chemoattractants promote VLA-4/VCAM-1 adhesive interactions during transmigration *in vitro*. Transendothelial migration of eosinophils is potentiated up to 10-fold by exposure to the eosinophil-activating cytokines IL-3, IL-5, or GM-CSF.⁷⁶ Because activated endothelial cells can produce GM-CSF, experiments were performed in which specific antibodies to GM-CSF were present during the transmigration assay. Antibodies to GM-CSF inhibited eosinophil transendothelial migration across IL-1-activated endothelial monolayers by almost 50%, whereas antibodies to IL-8 had

little effect.⁷⁶ Interestingly, eosinophils purified from late-phase reaction BAL fluids display a similarly potentiated transendothelial migration response.⁷⁶ These data suggest that endothelial-derived GM-CSF may preferentially stimulate eosinophil transendothelial migration, whereas endothelial-derived IL-8 may play a similar role for neutrophils. Several chemokines can affect basophil mediator release⁷⁷; and among these, IL-8, MCP-1, MIP1 α , and RANTES have been reported to have chemotactic activity for basophils *in vitro*.^{78, 79}

EXPRESSION AND FUNCTION OF ADHESION MOLECULES *IN VIVO* DURING ALLERGIC INFLAMMATION

Among the initial evidence suggesting the existence of different adhesion-related mechanisms for cell recruitment among granulocyte subtypes were findings in patients with leukocyte adhesion deficiency disease, a rare congenital immunodeficiency caused by diminished or absent β 2 integrin expression on all leukocytes.⁸⁰ Children with this disease experience severe and often fatal bacterial soft tissue infections, despite a persistent peripheral blood neutrophilia, because without β 2 integrins, their neutrophils cannot leave the circulation to enter infected sites. An intriguing observation was made when sites of infection were examined histologically. Eosinophils (and mononuclear leukocytes) were found to be present in these lesions, even though neutrophils were virtually absent. These findings suggested that eosinophils, unlike neutrophils, possessed β 2-integrin-independent mechanisms for recruitment into tissues. This is likely to be mediated, at least in part, by VLA-4 and Act-1, which are found on eosinophils and not neutrophils (see above). Further support for the potential role of VLA-4/VCAM-1-mediated eosinophil recruitment is provided by the demonstration of VCAM-1 staining of blood vessels, without E-selectin staining, in skin biopsy specimens from patients with eosinophilic vasculitis.⁸¹

Studies in which immunohistochemical techniques are used have demonstrated that intradermal injection of allergic individuals with allergen activates the vascular endothelium to express E-selectin and VCAM-1 and to increase its expression of ICAM-1 in an IgE-dependent manner.^{44, 82-84} Allergen challenge of the eye induces ICAM-1 expression on conjunctival epithelium.⁸⁵ There is indirect evidence that endothelial activation also occurs within the human airway after

endoscopic intrabronchial allergen challenge, because increased levels of a soluble form of E-selectin are observed in BAL fluids.²⁹ In non-human primates, allergen inhalation results in E-selectin expression on the airway vascular endothelium within 6 hours.⁸⁶ The pattern of endothelial activation seen during allergic inflammation *in vivo* suggests that endothelial cells are being exposed to cytokines such as IL-1, TNF, and/or IL-4. Several recent studies have identified conditions under which these cytokines may be generated in allergic reactions. For example, IL-1 is released *in vivo* during experimental cutaneous allergic reactions,⁸⁷ mast cells can release TNF and perhaps IL-4,^{88, 89} and IL-4 mRNA has been detected in allergic inflammatory sites by *in situ* hybridization.⁹⁰ Especially convincing is the observation that E-selectin expression induced by allergen injection in human skin can be inhibited if the site is immediately biopsied and placed into culture with a mixture of antibodies that neutralize IL-1 and TNF.⁸³

Experiments directly implicating endothelial cell adhesion molecules in the pathophysiology of allergic rhinitis and asthma are limited. When bronchial mucosal biopsy specimens were obtained from normal subjects and subjects with mild, stable allergic asthma, immunohistochemical analyses revealed similar levels of expression of ICAM-1 and E-selectin, despite an increased number of eosinophils in the mucosa of the subjects with asthma.⁹¹ After 6 weeks of treatment with inhaled budesonide, tissue eosinophilia in the subjects with asthma was reduced, yet no significant change in the pattern of ICAM-1 and E-selectin expression was observed. In contrast, when nasal airway tissue was obtained from subjects with perennial allergic rhinitis, an increased expression of ICAM-1 and VCAM-1, but not E-selectin, was seen compared with tissues from nonallergic control subjects.⁹² To examine changes in the expression of adhesion molecules in the nasal mucosa after allergen provocation, biopsy specimens of the inferior turbinates of subjects with allergic rhinitis and nonallergic control subjects were obtained 24 hours after localized challenge with antigen.⁹³ In this study constitutive expression of ICAM-1 and modest levels of VCAM-1, but no E-selectin expression were found in the nasal mucosa of nonallergic subjects. A comparison of endothelial cell adhesion molecule expression 24 hours after allergen challenge in allergic and nonallergic subjects revealed a significant increase in VCAM-1 expression, a

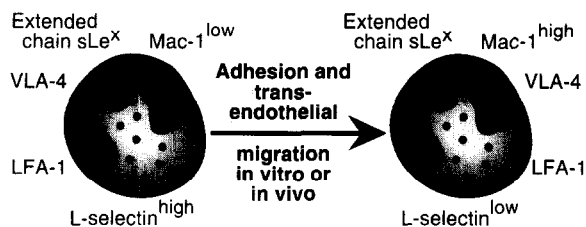


FIG. 2. Changes in adhesion molecule expression that occur during eosinophil migration. Levels of Mac-1 and L-selectin are altered, whereas expression of LFA-1, VLA-4, and the extended chain form of sLe^x (i.e., sialyl-dimeric Le^x) remain essentially unchanged. Copyright © 1993 by Jacqueline A. Schaffer. Reprinted with permission.

modest but significant increase in E-selectin expression, and no change in ICAM-1 expression in allergic subjects. Associated with evidence of endothelial activation was an increase in eosinophils, seen only in the allergic subjects, along with a weak correlation between the percentage of vessels expressing VCAM-1 and the number of eosinophils in the tissues.

Changes in the expression of cell surface adhesion molecules occur on eosinophils and basophils during their movement from the circulation into tissues, implying an involvement during allergic inflammation. A comparison of levels of adhesion molecules on granulocytes recovered from blood and either sputum, BAL, or nasal lavage after antigen challenge revealed increased expression of CD11b,^{8, 29, 94-96} diminished levels of L-selectin,^{8, 29, 97} and little or no change in expression of CD11a, VLA-4, or sialyl-dimeric Le^x^{29, 95, 96} (and unpublished observations). Similar phenotypic changes have been observed after eosinophil trans-endothelial migration *in vitro*^{56, 98} (Fig. 2). Although these data demonstrate dynamic changes in eosinophil and basophil adhesion molecule expression during inflammation, these changes also occur on neutrophils and have been observed on cells obtained from subjects with other inflammatory reactions.^{99, 100} Therefore these events cannot by themselves account for selectivity in cell recruitment but instead probably represent a common consequence of cell recruitment.

Despite evidence for endothelial activation during allergic inflammation, it is clear that several conditions exist under which eosinophil and basophil recruitment can occur in the apparent absence of cytokine-induced endothelial activation. First, injection of platelet activating factor into the skin of normal subjects causes a local tissue neutrophilia, whereas in allergic subjects, a tissue

eosinophilia arises.¹⁰¹ Second, intradermal injection of the neuropeptides substance P, vasoactive intestinal polypeptide, and calcitonin gene-related peptide induced P-selectin and E-selectin expression on the vascular endothelium in association with leukocyte accumulation.¹⁰² However, in four of six subjects injected with substance P, the infiltrate seen 4 to 8 hours later was primarily eosinophilic; this was not observed in biopsy specimens from subjects injected with the other neuropeptides. Regardless of the stimulus used and the subsequent inflammatory infiltrate, investigators were unable to detect any VCAM-1 expression or changes in ICAM-1 expression. Third, intradermal injection of dogs with the chemokine RANTES resulted in a striking local eosinophilic and monocytic infiltrate that was detectable within 4 hours and increased for at least 24 hours.¹⁰³

Direct proof of adhesion molecule involvement in cell recruitment would require studies in which specific antagonists are used. Although such data have yet to be reported for allergic rhinitis or asthma in human beings, blocking monoclonal antibodies have been infused *in vivo* in animals to study their effects on experimental models of asthma. In one study airway hyperreactivity and bronchoalveolar eosinophilia that accompanied repeated allergen challenge were effectively reduced by intravenous infusion or inhalation of anti-ICAM-1 antibody, but not by an antibody recognizing E-selectin.^{104, 105} In this same model when antibodies against leukocyte CD11b were given instead, the antigen-induced rise in airway responsiveness was again blunted, but this time the number of eosinophils recovered by BAL was unaffected.¹⁰⁶ Interestingly, eosinophil peroxidase activity in the BAL fluids was reduced by antibody treatment, suggesting an effect of the antibody on eosinophil activation and degranulation rather than on recruitment *per se*. In another study in which a single allergen challenge protocol that induces airway neutrophilia rather than eosinophilia was used, infusion of the anti-E-selectin antibody, but not the anti-ICAM-1 antibody, reduced airway inflammation.⁸⁶ Similar studies with anti-VCAM-1 antibodies *in vivo* have not yet been reported. However, in guinea pigs pretreatment with a species cross-reactive mouse anti-human $\alpha 4$ antibody inhibited chemotactic factor-induced accumulation of eosinophils in the skin, although it is unclear whether VCAM-1 or fibronectin is involved in this response.¹⁰⁷ With a different approach, investigators have begun to use antago-

nists of eosinophil-activating or endothelium-activating cytokines in an attempt to reduce allergic inflammatory responses. For example, neutralization of IL-5 by antibody treatment dramatically reduced lung eosinophilia in guinea pigs,¹⁰⁸ and a recent report demonstrated that treatment with a soluble form of the IL-1 receptor impressively blocks the cutaneous late-phase response in human volunteers.¹⁰⁹ On the basis of these and other studies, many novel pharmaceutical approaches are being tried in an attempt to prevent cell recruitment responses, and results from additional studies in human beings should be forthcoming.¹¹⁰ Only then will we truly be able to functionally define the role of adhesion molecules and cytokines in human allergic diseases, including rhinitis and asthma.

CONCLUSIONS

Largely on the basis of *in vitro* studies, several distinct but interrelated hypothetical mechanisms have been developed, which provide a basis for the selective recruitment and accumulation of eosinophils and basophils observed during allergic reactions. Such selectivity may be explained in part by: (1) endothelial expression of VCAM-1 induced by IL-1, TNF, or IL-4, which induces adherence of VLA-4-positive cells (i.e., eosinophils, basophils, monocytes, lymphocytes) but not VLA-4-negative cells (neutrophils); (2) priming of circulating leukocytes by IL-3, IL-5, or GM-CSF, which promotes adhesion and transendothelial migration of eosinophils and basophils but not neutrophils; (3) generation of chemotactic peptides such as RANTES, MIP1 α , and others, which stimulate migration of eosinophils and basophils but not neutrophils; (4) interactions of recruited eosinophils and basophils, but not neutrophils, with specific extracellular matrix proteins found within tissues; and (5) prolongation of the survival of eosinophils (and perhaps basophils), but not neutrophils, by IL-3 or IL-5. Although the relative importance of these processes remains unclear, *in vivo* data are now available to document the existence of some of these mechanisms. It therefore seems likely that a complex combination of these and additional, as yet undescribed, mechanisms is responsible for the selective accumulation of eosinophils and basophils in allergic diseases.

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REFERENCES

1. Sur S, Adolphson CR, Gleich GJ. Eosinophils: biochemical and cellular aspects. In: Middleton E Jr, Reed CE, Ellis NF, Adkinson NF Jr, Yunginger JW, Busse W, eds. *Allergy: principles and practice*. 4th ed. St. Louis: Mosby, 1993:169-200.
2. Bochner BS. Basophils. In: Frank MM, Austen KF, Claman HN, Unanue ER, eds. *Samter's immunological diseases*. 5th ed. Boston: Little, Brown, and Company, 1994 (in press).
3. Schleimer RP, Ebisawa M, Georas SN, Bochner BS. The role of adhesion molecules and cytokines in eosinophil recruitment. In: Gleich GJ, Kay AB, eds. *Eosinophils in allergy and inflammation*. New York: Marcel Dekker, Inc, 1993:99-114.
4. Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. *Am J Respir Cell Mol Biol* 1993;8:349-55.
5. Cohnheim J. *Inflammation Lectures in General Pathology*. London: New Sydenham Society, 1889.
6. Haskard DO, Lee TH. The role of leukocyte-endothelial interactions in the accumulation of leukocytes in allergic inflammation. *Am Rev Respir Dis* 1992;145:S10-3.
7. Montefort S, Holgate ST, Howarth PH. Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. *Eur Respir J* 1993;6:1044-54.
8. Baroody FM, Lee B-J, Lim MC, Bochner BS. Implicating adhesion molecules in nasal allergic inflammation. *Eur Arch Otorhinolaryngol* 1994 (in press).
9. Springer TA. Adhesion receptors of the immune system. *Nature* 1990;346:425-34.
10. Bevilacqua MP, Nelson RM. Selectins. *J Clin Invest* 1993;91:379-87.
11. Bevilacqua MP. Endothelial-leukocyte adhesion molecules. *Annu Rev Immunol* 1993;11:767-804.
12. Albelda SM, Buck CA. Integrins and other cell adhesion molecules. *FASEB J* 1990;4:2868-80.
13. Hemler ME. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu Rev Immunol* 1990;8:365-400.
14. Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990;60:577-84.
15. Postigo AA, Sanchez-Mateos P, Lazarovits AI, Sanchez-Madrid F, de Landazuri MO. $\alpha 4\beta 7$ Integrin mediates B-cell binding to fibronectin and vascular cell adhesion molecule-1: expression and function of $\alpha 4$ integrins on human B-lymphocytes. *J Immunol* 1993;151:2471-83.
16. Ruegg C, Postigo AA, Sikorski EE, Butcher EC, Pytela R, Erle DJ. Role of integrin $\alpha 4\beta 7/\alpha 4\beta P$ in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J Cell Biol* 1992;117:179-89.
17. Berlin C, Berg EL, Briskin MJ, et al. $\alpha 4\beta 7$ Integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 1993;74:185-95.
18. Briskin MJ, McEvoy LM, Butcher EC. MAdCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgA₁. *Nature* 1993;363:461-4.
19. Lasky LA, Singer MS, Dowbenko D, et al. An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell* 1992;69:927-38.
20. Baumhueter S, Singer MS, Henzel W, et al. Binding of L-selectin to the vascular sialomucin CD34. *Science* 1993; 262:436-8.
21. Butcher EC. Leukocyte-endothelial cell recognition:

- three (or more) steps to specificity and diversity. *Cell* 1991;67:1033-6.
22. Zimmerman GA, Prescott SM, McIntyre TM. Endothelial cell interactions with granulocytes: tethering and signaling molecules. *Immunol Today* 1992;13:93-100.
23. Huber AR, Kunkel SL, Todd RF III, Weiss SJ. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science* 1991;254:99-102.
24. Tanaka Y, Adams DH, Hubscher S, Hirano H, Siebenlist U, Shaw S. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1 β . *Nature* 1993;361:79-82.
25. Lo SK, Lee S, Ramos RA. Endothelial-leukocyte adhesion molecule-1 stimulates the adhesive activity of leukocyte integrin CR3 (CD11b/CD18, Mac-1, $\alpha_m\beta_2$) on human neutrophils. *J Exp Med* 1991;173:1493-1500.
26. Spertini O, Luscinskas FW, Kansas GS, et al. Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. *J Immunol* 1991;147:2565-73.
27. Smith CW, Kishimoto TK, Abbass O, et al. Chemotactic factors regulate lectin adhesion molecule 1 (LECAM-1) dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. *J Clin Invest* 1991;87:609-18.
28. Knol EF, Kansas GS, Tedder TF, Schleimer RP, Bochner BS. Human eosinophils use L-selectin to bind to endothelial cells under non-static conditions [Abstract]. *J ALLERGY CLIN IMMUNOL* 1993;91:334.
29. Georas SN, Liu MC, Newman W, Beall WD, Stealey BA, Bochner BS. Altered adhesion molecule expression and endothelial activation accompanies the recruitment of human granulocytes to the lung following segmental antigen challenge. *Am J Respir Cell Mol Biol* 1992;7:261-9.
30. Neeley SP, Hamann KJ, White SR, Baranowski SL, Burch RA, Leff AR. Selective regulation of expression of surface adhesion molecules Mac-1, L-selectin, and VLA-4 on human eosinophils and neutrophils. *Am Rev Respir Cell Mol Biol* 1993;8:633-9.
31. Bochner BS, Sterbinsky SA. Altered surface expression of CD11 and Leu-8 during human basophil degranulation. *J Immunol* 1991;146:2367-73.
32. Geng JG, Bevilacqua MP, Moore KL, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* 1990;343:757-9.
33. Vadas MA, Lucas CM, Gamble JR, Lopez AF, Skinner MP, Berndt MC. Regulation of eosinophil function by P-selectin. In: Gleich GJ, Kay AB, eds. *Eosinophils in allergy and inflammation*. New York: Marcel Dekker, Inc, 1993:69-80.
34. Wein M, Bochner BS. Comparison of neutrophil and eosinophil ligands for P-selectin [Abstract]. *J ALLERGY CLIN IMMUNOL* 1994;93:268.
35. Zhou Q, Moore KL, Smith DF, Varki A, McEver RP, Cummings RD. The selectin GMP-140 binds to sialylated, fucosylated lactosaminoglycans on both myeloid and nonmyeloid cells. *J Cell Biol* 1991;115:557-64.
36. Larsen GR, Sako D, Ahern TJ, et al. P-selectin and E-selectin. Distinct but overlapping leukocyte ligand specificities. *J Biol Chem* 1992;267:11104-10.
37. Moore KL, Stults NL, Diaz S, et al. Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. *J Cell Biol* 1992;118:445-56.
38. Dunn CJ, Fleming WE. Increased adhesion of polymorphonuclear leukocytes to vascular endothelium by specific interaction of endogenous (interleukin-1) and exogenous (lipopolysaccharide) substances with endothelial cells "in vitro". *Eur J Rheumatol Inflamm* 1984;7:80-6.
39. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA Jr. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocytic cell lines. *J Clin Invest* 1985;76:2003-11.
40. Schleimer RP, Rutledge BK. Cultured human vascular endothelial cells acquire adhesiveness for leukocytes following stimulation with interleukin-1, endotoxin, and tumor-promoting phorbol esters. *J Immunol* 1986;136:649-54.
41. Lamas AM, Mulroney CR, Schleimer RP. Studies on the adhesive interaction between human eosinophils and cultured vascular endothelial cells. *J Immunol* 1988;140:1500-5.
42. Bochner BS, Peachell PT, Brown KE, Schleimer RP. Adherence of human basophils to cultured umbilical vein vascular endothelial cells. *J Clin Invest* 1988;81:1355-60.
43. Bochner BS, Luscinskas FW, Gimbrone MA Jr, et al. Adhesion of human basophils, eosinophils, and neutrophils to IL-1 activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991;173:1553-7.
44. Kyan-Aung U, Haskard DO, Poston RN, Thornhill MH, Lee TH. Endothelial leukocyte adhesion molecule-1 and intercellular adhesion molecule-1 mediate the adhesion of eosinophils to endothelial cells in vitro and are expressed by endothelium in allergic cutaneous inflammation in vivo. *J Immunol* 1991;146:521-8.
45. Walsh GM, Mermoud J, Hartnell A, Kay AB, Wardlaw AJ. Human eosinophil, but not neutrophil, adherence to IL-1-stimulated human umbilical vascular endothelial cells is $\alpha 4\beta 1$ (very late antigen-4) dependent. *J Immunol* 1991;146:3419-23.
46. Weller PF, Rand TH, Goelz SE, Chi-Rosso G, Lobb RR. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci U S A* 1991;88:7430-3.
47. Dobrina A, Menegazzi R, Carlos TM, et al. Mechanisms of eosinophil adherence to cultured vascular endothelial cells: eosinophils bind to the cytokine-induced endothelial ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. *J Clin Invest* 1991;88:20-6.
48. Schleimer RP, Sterbinsky SA, Kaiser J, et al. Interleukin-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J Immunol* 1992;148:1086-92.
49. Thornhill MH, Kyan-Aung U, Haskard DO. IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. *J Immunol* 1990;144:3060-5.
50. Tepper RI, Pattengale PK, Leder P. Murine interleukin-4 displays potent anti-tumor activity in vivo. *Cell* 1989;57:503-12.
51. Tidswell M, Brown T, Erle D. Characterization of the lymphocyte and eosinophil cell adhesion molecule integrin of $\alpha 4\beta 7$ [Abstract]. *Am Rev Respir Dis* 1993;147:A542.
52. Bochner BS, Sterbinsky SA, Bickel CA, Werfel S, Wein M, Newman W. Differences between human eosinophils and neutrophils in the function and expression of sialic

- acid-containing counterligands for E-selectin. *J Immunol* 1994;152:774-82.
53. Bochner BS, Newman W, McIntyre BW, Yamada T, MacGlashan DW Jr, Schleimer RP. Counter-receptors for endothelial cell adhesion molecules on human eosinophils and basophils [Abstract]. *FASEB J* 1992;6:A1435.
54. Moser R, Schleiffenbaum B, Groscurth P, Fehr J. Interleukin 1 and tumor necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. *J Clin Invest* 1989;83:444-55.
55. Moser R, Fehr J, Olgiate L, Bruijnzeel PL. Migration of primed human eosinophils across cytokine-activated endothelial cell monolayers. *Blood* 1992;79:2937-45.
56. Ebisawa M, Bochner BS, Georas SN, Schleimer RP. Eosinophil transendothelial migration induced by cytokines. I. Role of endothelial and eosinophil adhesion molecules in IL-1 β -induced transendothelial migration. *J Immunol* 1992;149:4021-8.
57. Kuijpers TW, Mul EPJ, Blom M, et al. Freezing adhesion molecules in a state of high-avidity binding blocks eosinophil migration. *J Exp Med* 1993;178:279-84.
58. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 1993;178:449-60.
59. Georas SN, McIntyre BW, Ebisawa M, Bednarczyk J, Schleimer RP, Bochner BS. Expression of a functional laminin receptor (α 6 β 1, VLA-6) on human eosinophils. *Blood* 1993;82:2872-9.
60. Ebisawa M, Schleimer RP, Bickel C, Bochner BS. Phenotyping of purified human peripheral blood eosinophils using the blind panel of monoclonal antibodies. In: Schlossman S, Boumsell L, Gilks W, eds. *Leukocyte typing V: white cell differentiation antigens*. Oxford: Oxford University Press, 1994 (in press).
61. Anwar ARF, Moqbel R, Walsh GM, Kay AB, Wardlaw AJ. Adhesion to fibronectin prolongs eosinophil survival. *J Exp Med* 1993;177:839-43.
- 61a. Bochner BS, Sterbinsky SA, Knol EF, et al. Function and expression of adhesion molecules on human basophils. *J ALLERGY CLIN IMMUNOL* 1994 (in press).
62. Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A. Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701-5.
63. Warringa RAJ, Koenderman L, Kok PTM, Kreukniet J, Bruijnzeel PLB. Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. *Blood* 1991;77:2694-2700.
64. Warringa RAJ, Mengelers HJJ, Kuijper PHM, Raaijmakers JAM, Bruijnzeel PLB, Koenderman L. In vivo priming of platelet-activating factor-induced eosinophil chemotaxis in allergic asthmatic individuals. *Blood* 1992;79:1836-41.
65. Tomioka K, MacGlashan DW Jr, Lichtenstein LM, Bochner BS, Schleimer RP. GM-CSF regulates human eosinophil responses to F-Met peptide and platelet activating factor. *J Immunol* 1993;151:4989-97.
66. Bochner BS, MacGlashan DW Jr, Marcotte GV, Schleimer RP. IgE-dependent regulation of human basophil adherence to vascular endothelium. *J Immunol* 1989;142:3180-6.
67. Bochner BS, McKelvey AA, Sterbinsky SA, et al. Interleukin-3 augments adhesiveness for endothelium and CD11b expression in human basophils but not neutrophils. *J Immunol* 1990;145:1832-7.
68. Tanimoto Y, Takahashi K, Kimura I. Effects of cytokines on human basophil chemotaxis. *Clin Exp Allergy* 1992;22:1020-5.
69. Oppenheim JJ, Zachariae COC, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol* 1991;9:617-48.
70. Schall TJ. Biology of the RANTES/SIS cytokine family. *Cytokine* 1991;3:165-83.
71. Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Walmann P, Kay AB. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* 1992;79:2952-9.
72. Warringa RAJ, Mengelers HJJ, Raaijmakers JAM, Bruijnzeel PLB, Koenderman L. Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J ALLERGY CLIN IMMUNOL* 1993;91:1198-1205.
73. Yamada T, Ebisawa M, MacGlashan DW Jr, et al. RANTES is a chemoattractant for human eosinophils [Abstract]. *J ALLERGY CLIN IMMUNOL* 1993;91:314.
74. Kameyoshi Y, Dorschner A, Mallet AI, Christophers E, Schroder JM. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992;176:587-92.
75. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;176:1489-95.
76. Ebisawa M, Yamada T, Klunk D, et al. Regulation of eosinophil and neutrophil transendothelial migration by cytokines and chemokines [Abstract]. *J ALLERGY CLIN IMMUNOL* 1993;91:313.
77. MacDonald SM, Bochner BS, Lichtenstein LM. The role of basophil-cytokine interactions in asthma. In: Holgate ST, Austen KF, Lichtenstein LM, Kay AB, eds. *Asthma: Physiology, Immunopharmacology, and Treatment*. Fourth International Symposium. London: Academic Press, 1993:99-108.
78. Leonard EJ, Skeel A, Yoshimura T, Noer K, Kutvirt S, Van Epps D. Leukocyte specificity and binding of human neutrophil attractant/activation protein-1. *J Immunol* 1990;144:1323-30.
79. Bischoff SC, Krieger M, Brunner T, et al. RANTES and related chemokines activate human basophil granulocytes through different G-protein-coupled receptors. *Eur J Immunol* 1993;23:761-7.
80. Anderson DC, Schmalstieg FC, Finegold MJ, et al. The severe and moderate phenotypes of heritable MAC-1, LFA-1, p150,95 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features. *J Infect Dis* 1985;152:668-89.
81. Chen K-R, Pittelkow MR, Su WPD, Gleich GJ, Newman W, Leiferman KM. Recurrent cutaneous necrotizing eosinophilic vasculitis: a novel eosinophil mediated syndrome. *Arch Dermatol* 1994 (in press).
82. Bochner BS, Lamas AM, Benenati SV, Schleimer RP. On the central role of vascular endothelium in allergic reactions. In: Dorsch R, ed. *Late phase allergic reactions*. Boca Raton, Florida: CRC Press, 1990:221-35.
83. Leung DYM, Pober JS, Cotran RS. Expression of endo-

- thelial-leukocyte adhesion molecule-1 in elicited late phase allergic reactions. *J Clin Invest* 1991;87:1805-9.
84. Schleimer RP, Bochner BS. Letter to the editor. *J Immunol* 1991;147:380-1.
85. Ciprandi G, Buscaglia S, Pesce G, Villaggio B, Bagnasco M, Canonica G. Allergic subjects express ICAM-1 on epithelial cells of conjunctiva after antigen challenge. *J ALLERGY CLIN IMMUNOL* 1993;91:3783-92.
86. Gundel RH, Wegner CD, Torcellini CA, et al. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *J Clin Invest* 1991;88:1407-11.
87. Bochner BS, Charlesworth EN, Lichtenstein LM, et al. Interleukin-1 is released at sites of human cutaneous allergic reactions. *J ALLERGY CLIN IMMUNOL* 1990;86:830-9.
88. Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF. Human dermal mast cells contain and release tumor necrosis factor α , which induces endothelial leukocyte adhesion molecule. 1. *Proc Natl Acad Sci U S A* 1991;88:4220-4.
89. Bradding P, Feather IH, Howarth PH, et al. Interleukin-4 is localized to and released by human mast cells. *J Exp Med* 1992;176:1381-6.
90. Robinson DS, Hamid Q, Ying S, et al. T_H2 -like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
91. Montefort S, Roche WR, Howarth PH, et al. Intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1) expression in the bronchial mucosa of normal and asthmatic subjects. *Eur Respir J* 1992;5:815-23.
92. Montefort S, Feather IH, Wilson SJ, et al. The expression of leukocyte-endothelial adhesion molecules is increased in perennial allergic rhinitis. *Am J Respir Cell Mol Biol* 1992;7:393-8.
93. Lee B-J, Naclerio RM, Bochner BS, Taylor RM, Lim MC, Baroody FM. Nasal challenge with allergic upregulates the local expression of vascular endothelial adhesion molecules [Abstract]. *J ALLERGY CLIN IMMUNOL* 1994; 93:183.
94. Hansel TT, Walker C. The migration of eosinophils into the sputum of asthmatics: the role of adhesion molecules. *Clin Exp Allergy* 1992;22:345-56.
95. Sedgwick JB, Calhoun WJ, Vrtis RF, Bates ME, McAllister PK, Busse WW. Comparison of airway and blood eosinophil function after in vivo antigen challenge. *J Immunol* 1992;149:3710-8.
96. Kroegel C, Liu MC, Hubbard WC, Lichtenstein LM, Bochner BS. Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoid production. *J ALLERGY CLIN IMMUNOL* 1994;93:725-34.
97. Mengelers HJJ, Maikoe T, Hooibrink B, et al. Down modulation of L-selectin expression on eosinophils recovered from bronchoalveolar lavage fluid after allergen provocation. *Clin Exp Allergy* 1993;23:196-204.
98. Walker C, Rihs S, Braun RK, Betz S, Bruijnzeel PLB. Increased expression of CD11b and functional changes in eosinophils after migration across endothelial cell monolayers. *J Immunol* 1993;150:4061-71.
99. Kishimoto TK, Jutila MA, Berg EL, Butcher EC. Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science* 1989;245: 1238-41.
100. Jutila MA, Rott L, Berg EL, Butcher EC. Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and MAC-1. *J Immunol* 1989; 143:3318-24.
101. Henocq E, Vargaftig BB. Accumulation of eosinophils in response to intracutaneous PAF-acether and allergen in man. *Lancet* 1986;2:1378-9.
102. Smith CH, Barker JNWN, Morris RW, MacDonald DM, Lee TH. Neuropeptides induce rapid expression of endothelial cell adhesion molecules and elicit granulocytic infiltration in human skin. *J Immunol* 1993;151:3274-82.
103. Meurer R, Van Riper G, Feeney W, et al. Formation of eosinophilic and monocytic intradermal inflammatory sites in the dog by injection of human RANTES but not human monocyte chemoattractant protein 1, human macrophage inflammatory protein 1 α , or human interleukin 8. *J Exp Med* 1993;178:1913-21.
104. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
105. Wegner CD, Rothlein R, Clarke CC, et al. Inhaled ICAM-1 reduces antigen-induced airway hyperresponsiveness in monkeys [Abstract]. *Am Rev Respir Dis* 1991;143:A418.
106. Wegner CD, Gundel RH, Letts LG. Expression and probable roles of cell adhesion molecules in lung inflammation. *Chest* 1992;101:34S-39S.
107. Weg VB, Williams TJ, Lobb RR, Nourshargh S. A monoclonal antibody recognizing very late activation antigen-4 inhibits eosinophil accumulation in vivo. *J Exp Med* 1993;177:561-6.
108. Gulbenkian AR, Egan RW, Fernandez X. Interleukin-5 modulates eosinophil accumulation in allergic guinea pig lung. *Am Rev Respir Dis* 1992;146:263-5.
109. Mullarkey MF, Leiferman KM, Peters MS, et al. Human cutaneous allergic late-phase response is inhibited by soluble human IL-1 receptor (IL-1R). *J Immunol* 1994; 152:2033-41.
110. Wein M, Bochner BS. Adhesion molecule antagonists: future therapies for allergic diseases? *Eur Respir J* 1993; 6:1239-42.