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Updates on cells and cytokines

Cellular adhesion and its antagonism

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Inflammation, defined more than a century ago as the development of localized swelling, pain, redness, and often loss of function, is the net effect of substances released by selectively recruited and activated tissue-resident and circulating cells. The microscopic appearances of these inflammatory infiltrates vary widely among disorders yet are remarkably consistent within a given disorder. It is the consistent pattern of development and resolution of inflammatory reactions, elicited in response to such diverse agents as tissue injury, infection, or allergen exposure, that suggests the existence of a series of overlapping but distinct mechanisms for cell recruitment. For example, eosinophils, basophils, and subsets of T lymphocytes accumulate at sites of chronic allergic disease after the instillation of allergen to the skin and airways. Recently, an appreciation has grown for the direct role that these cells and their products play in producing allergic inflammation. In addition, the concept that these cells play central roles in the development of allergic disease is supported by the detection of their mediators at sites of allergic inflammatory reactions and the reduction of these cells and their mediators during the resolution of these responses. This review summarizes the currently accepted paradigms by which cell adhesion events contribute to inflammation and outlines progress toward the development of adhesion molecule-directed therapeutics.

Abbreviations used

ICAM:	Intercellular adhesion molecule
VCAM:	Vascular cell adhesion molecule
VLA:	Very late antigen

ROLES OF ADHESION MOLECULES IN INFLAMMATION

Among the many molecules involved in the cellular inflammatory response are adhesion molecules. These structures, exceeding 35 in number, participate in virtually every step of cell migration. They are subdivided into families (integrins, immunoglobulin superfamily members, selectins, and sialylated carbohydrate counterligands for selectins, including the sialomucins) on the basis of shared structural characteristics and functions (Fig. 1). Furthermore, they are now known to be necessary for margination (leukocyte-endothelial interactions), diapedesis (transendothelial migration), directed cellular locomotion (chemotaxis and haptotaxis), and transepithelial migration in the airways.

LEUKOCYTE TETHERING AND ROLLING DURING MARGINATION

Leukocyte recruitment results from a complex process that involves the interaction of adhesion molecules on the leukocyte surface with molecular counterligands on vascular endothelial cells, extracellular matrix proteins, and other tissue structures (Fig. 2). During the earliest stages of this process, circulating leukocytes undergo tethering and rolling on the endothelial surface. These events involve the formation of numerous weak reversible bonds primarily between selectins and their carbohydrate-containing counterligands. The selectin family has only three known members, E-selectin, P-selectin, and L-selectin. E-selectin is found exclusively on the postcapillary endothelium and is gradually and tran-

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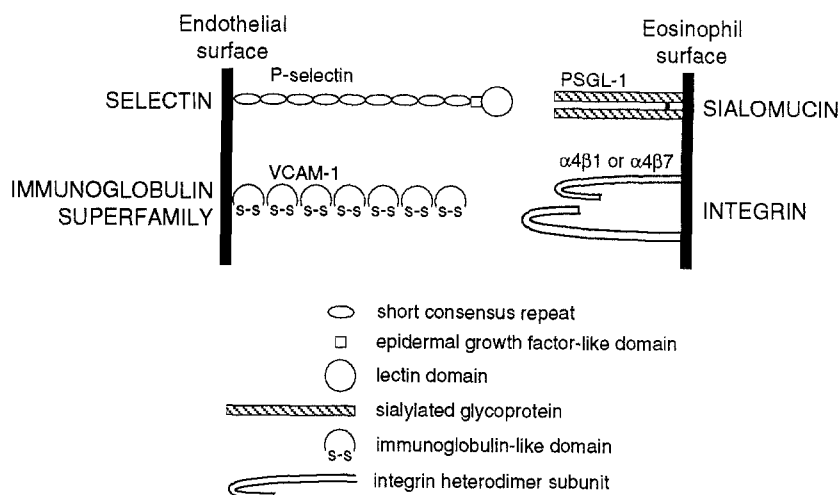


FIG. 1. Adhesion molecule counterligands for leukocyte-endothelial interactions. Shown are a few examples to illustrate how these structures differ and how members of each adhesion molecule family (selectins and sialylated selectin ligands, immunoglobulin superfamily members, and integrins) interact during cell recruitment. Note that transmembrane and intracytoplasmic domains for each of these structures are not shown. PSGL-1, P-selectin glycoprotein-1.

siently expressed in response to cytokines (e.g., IL-1, tumor necrosis factor) and other agents, whereas P-selectin can appear within minutes of activation from preformed stores on platelets and endothelium. In contrast, L-selectin expression is restricted to leukocytes, and in response to chemotactic agents it is rapidly and irreversibly shed by enzymatic cleavage through as yet unidentified proteases, probably belonging to the matrix metalloprotease family. Although selectins can mediate adhesion under static conditions, their major function in vivo is to mediate leukocyte-endothelial tethering (through L- and P-selectin) and rolling (through all selectins) under forces of shear stress and during lymphocyte trafficking to peripheral and mesenteric lymph nodes.¹ Selectins are exclusively responsible for leukocyte tethering, but they are not the only type of adhesion molecules capable of mediating rolling adhesion under conditions of shear stress caused by blood flow. Indeed, the integrin very late antigen-4 (VLA-4 [$\alpha 4\beta 1$, CD49d/CD29]), by interacting with vascular cell adhesion molecule-1 (VCAM-1), appears unique in that it has also been shown to mediate rolling adhesion.²

Many sialylated, fucosylated mucin-like ligands for selectins have been identified.^{3,4} For example, the tetrasaccharide sialyl Lewis^x, containing $\alpha 2,3$ -linked terminal sialic acid residues and $\alpha 1,3$ -linked fucose, can bind to all three selectins. However, it appears that the most prominent ligand for P-selectin is P-selectin glycoprotein ligand-1, a glycoprotein homodimer that is widely expressed on leukocytes and other cells, whereas E-selectin ligands on normal human granulocytes appear to be extended-chain sialyl dimeric Lewis^x-containing glycolipids termed *myeloglobins*. For skin-homing lymphocytes of the memory phenotype (CD45RO⁺), a distinct sialylated molecule termed the *cutaneous lymphocyte antigen* mediates binding to E-selectin. For L-selectin,

recently identified fucosylated, sialylated, sulfated ligands on human endothelium include CD34, mucosal addressin cell adhesion molecule-1, and an as yet unidentified cytokine-inducible structure.

Recently, tremendous advances have been made in identifying the enzymes ($\alpha 2,3$ sialyl-transferases and $\alpha 1,3$ fucosyl-transferases) involved in the synthesis of the carbohydrate portions of selectin ligands such as sialyl Lewis^x. For example, at least five $\alpha 1,3$ fucosyltransferases have been discovered, and on the basis of studies done with knockout mice, $\alpha 1,3$ fucosyltransferase VII appears especially important for leukocyte synthesis of selectin ligands.⁵ In addition, a rare genetic disease in which fucosylation is abnormal results in leukocyte adhesion deficiency disease type II, in which sialyl Lewis^x synthesis and leukocyte rolling and recruitment responses are impaired.³

FIRM ADHESION DURING MARGINATION, DIAPEDESIS, AND TISSUE MIGRATION

The initial reversible tethering and rolling of leukocytes on the endothelium may be followed by the induction of firm adhesion (Fig. 2), which is influenced by chemokines, platelet-activating factor, leukotrienes, and other chemoattractants that act through seven transmembrane receptors on the leukocyte. These adhesive interactions are mediated by leukocyte integrins interacting with endothelial adhesion molecules belonging to the immunoglobulin superfamily such as intercellular adhesion molecule-1 (ICAM-1) and VCAM-1. Leukocytes subsequently transmigrate from the vascular endothelium to the tissue, a process influenced by integrins and platelet-endothelial cell adhesion molecule-1.³

For all leukocytes, the processes of firm adhesion, locomotion, and transendothelial migration in response to stimulation with chemotactic factors are either par-

TABLE I. Therapeutic strategies for antagonizing adhesion molecules

Block cell surface expression
Cytokine and cytokine-receptor antagonists
Antisense nucleotides
Fucosyl- or Sialyl-transferase inhibitors
Metalloprotease inhibitors
Block function
Low molecular weight competitive antagonists
Oligosaccharides and glycomimetics
Linear and cyclic peptides
Monoclonal antibodies
Soluble adhesion molecule constructs
Integrin "activation" inhibitors

tially or completely dependent on a subset of integrins, the β_2 -integrins.³ Ligands for β_2 -integrins include ICAM-1, ICAM-2, and ICAM-3, as well as fibrinogen, the complement fragment C3bi, and other structures. Until recently, only three β_2 -integrins (CD18) had been identified: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), and CD11c/CD18 (p150,95). A fourth β_2 -integrin, $\alpha\delta\beta_2$, has now been identified, and it too can interact with members of the immunoglobulin superfamily such as ICAM-3. Further support for the critical role of β_2 -integrins in cell accumulation is provided by studies of patients with rare genetic mutations in β_2 -integrins (leukocyte adhesion deficiency type I), in which leukocyte recruitment responses are impaired.

Results of additional studies indicate that on the cell surface, both the number and functional state of integrins can be regulated. Changes in the affinity for counterligand binding can thus occur. In the latter case, affinity can change rapidly with little or no change in the level of cell-surface expression, a process that occurs as a result of inside-out signaling.⁶ For example, the affinity of $\alpha 4\beta 1$ -integrins on eosinophils for its ligands can be quickly and reversibly upregulated or downregulated depending on the stimulus, allowing the cell to adhere and detach as it migrates.^{7,8} Certain cytokines such as IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor are believed to contribute to allergic inflammation. They do this in part by increasing the expression and function of β_2 -integrins on eosinophils and basophils and by potentiating (or "priming") transendothelial migration and other responses to chemoattractants. Thus cytokines and chemokines that promote eosinophil and basophil accumulation can contribute to cell migration by altering integrin function.

Besides their role in adhesion, integrins have important functions as signal-transducing molecules, mediating so-called "outside-in" signaling to influence adherent cell function.⁹ This does not appear to occur exclusively through the integrins themselves, however, because intracytoplasmic domains of integrins lack kinase or phosphatase activity of their own, as well as sequence homology with known signaling proteins. Instead, outside-in signaling may occur as a result of interactions between

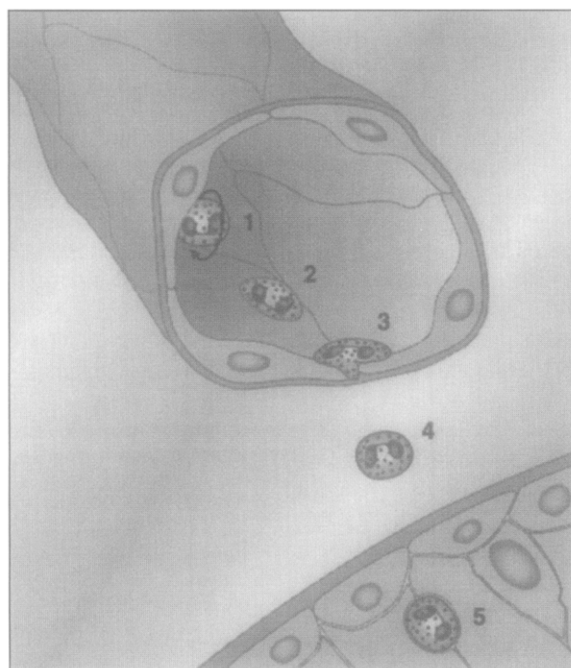


FIG. 2. Proposed sequence of adhesion-dependent events by which circulating granulocytes are recruited to extravascular sites during inflammation. Local production of endothelial-activating substances permits tethering and rolling, mediated predominantly by selectins and their counterligands, although $\alpha 4$ -integrins can also contribute (1). Firm adhesion and cell spreading, occurring as a result of cytoskeletal alterations and mediated by leukocyte-integrin interactions with immunoglobulin superfamily members on the endothelium, may follow as a result of their exposure to chemotactic factors or other leukocyte activators (2). These or similar stimuli facilitate transendothelial migration through intercellular spaces between adjacent endothelial cells, events dependent on integrins and immunoglobulin superfamily members (3). Transendothelial migration may also require the function of matrix metalloproteases that induce L-selectin shedding and permit leukocyte penetration by digesting small holes in the basement membrane. Once in the extravascular compartment, cells may remain tissue-resident, a process maintained by integrin interactions with matrix proteins, or undergo apoptosis (4). In some cases, such as in the airway, recruited cells migrate to the epithelial compartment in response to epithelial-derived cytokines and chemokines (5). Like transendothelial migration, these events are at least partially dependent on integrins and immunoglobulin superfamily members.

integrins and other associated molecules on or near the cell surface, such as cytoskeletal proteins. Furthermore, integrins associate with nonintegrin cell-surface molecules such as members of the transmembrane 4 family of proteins (e.g., CD53, CD63, CD81, and CD82),¹⁰ which may also be involved in the regulation of integrin function or outside-in signaling through integrins.

Among the integrins, the $\alpha 4\beta 1$ heterodimer (VLA-4) is of particular interest in allergic inflammation. This is due in large part to its prominent expression on eosinophils and basophils and its lack of expression on neutrophils. VLA-4 binds to fibronectin and VCAM-1 on activated endothelial cells. Expression of VCAM-1 on endothelium is induced within hours of exposure to

bacterial endotoxin or certain cytokines (e.g., IL-1, tumor necrosis factor, IL-4, IL-13) and reaches maximal levels in 1 to 2 days. Among the endothelial-activating cytokines, only IL-4 and IL-13 are known to selectively induce VCAM-1 expression. Another β -subunit, β_7 , can pair with α_4 ($\alpha_4\beta_7$) and, like VLA-4, is capable of binding to fibronectin and VCAM-1. Unlike VLA-4, however, $\alpha_4\beta_7$ binds to another cytokine-inducible adhesion molecule, mucosal addressin cell adhesion molecule-1, which is important in the homing of lymphocyte subsets (and perhaps eosinophils and basophils, which also express $\alpha_4\beta_7$) to the gut mucosa.¹¹ However, its role in allergic inflammatory diseases of the gastrointestinal tract is unknown.

Once leukocytes enter the extravascular space, migration through the matrix protein-rich basement membrane and tissue parenchyma is influenced primarily by receptors belonging to the β_1 -integrin family (Fig. 2). This is also true for mast cells that ultimately reside in perivascular tissue spaces. On the basis of *in vitro* studies with eosinophils, basophils, and mast cells, it appears that these adhesive interactions alter many functional responses (e.g., degranulation, survival), presumably through outside-in signaling mentioned above.¹²⁻¹⁴ Finally, many leukocytes trafficking through the lung ultimately undergo transepithelial migration before appearing in the airway lumen. Cytokines such as tumor necrosis factor and viral infections of the epithelial cells increase leukocyte-epithelial cell adhesion. The adhesion pathways involved are not entirely clear because this is only partially inhibited by antibodies to ICAM-1 or β_2 -integrins.¹⁵ What determines whether a recruited cell remains in the tissue parenchyma or migrates into the airways is not known but may be influenced by epithelial-derived factors such as chemokines.

FUNCTIONAL ROLES OF ADHESION MOLECULES IN VIVO IN ALLERGIC INFLAMMATION

The expression and potential role of endothelial adhesion molecules in allergic diseases *in vivo* has recently been reviewed.^{16, 17} Numerous studies have detected enhanced expression of E-selectin, ICAM-1, and/or VCAM-1 at sites of allergic or eosinophilic inflammation of the skin, nose, and lower airways. In addition to endothelial changes, altered expression of adhesion molecules occurs on eosinophils and basophils during their allergen-induced movement from the circulation into tissues. More recently, several adhesion molecule knock-out mice that display uniquely altered inflammatory responses have been created, some of which are now being studied in models of allergic inflammation.¹⁸

One of the challenges we now face is to determine the relative importance of each of these adhesion molecules for allergic airway disease in human beings. Direct proof of adhesion-molecule involvement in human allergic diseases will, by necessity, require the use of adhesion-molecule antagonists. Although no data yet exist on their role in allergic diseases in human beings, efforts towards the generation of such agents have been motivated in

large part by the success with blocking antibodies in animal studies.^{19, 20}

In addition to antibodies, many novel pharmaceutical approaches are being tested for their ability to prevent cell recruitment responses by blocking the expression and/or the function of adhesion molecules (Table I). Efforts to block expression of adhesion molecules have focused on the stimuli that induce adhesion-molecule expression (e.g., cytokines), the intracellular molecular pathways controlling adhesion-molecule expression, or the posttranslational modification of the adhesion structures themselves (e.g., glycosyltransferases for selectin ligands or proteases involved in L-selectin shedding). Efforts that instead attempt to block the function of adhesion molecules have searched for low molecular weight competitive antagonists, carbohydrate-based structures (especially for selectins), peptides, monoclonal antibodies, soluble forms of the adhesion molecules themselves, or agents that alter outside-in or inside-out integrin signaling. Although a great deal of progress has been made in this area, and many such antagonists have become available for *in vitro* and animal use,³ information on the role of each adhesion molecule in allergic disease *in vivo* must await studies with these antagonists in human beings.

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