Leukocyte-Endothelial Adhesion Molecules

By Timothy M. Carlos and John M. Harlan

In 1985, a review article appeared in this journal that examined the interactions of leukocytes with vascular endothelium during an immune or inflammatory response. The basic physiology of leukocyte emigration as well as disease states in which alterations of leukocyte-endothelial interactions may contribute to pathology were discussed. Since then there has been an explosion of interest in this topic. This explosion has been fueled by remarkable advances in the elucidation of the molecular basis of leukocyte adherence to endothelium and the potential for new therapies directed at these adhesion molecules.

Before 1985 only a single protein involved in leukocyte adherence to endothelium had clearly been identified immunologically—the murine lymphocyte homing receptor gp90DIE. In the subsequent years nine endothelial and nine leukocyte surface proteins involved in this heterotypic adhesion have been molecularly cloned (Fig 1, A and B). In addition, several other distinct leukocyte and endothelial molecules have been identified functionally or immunologically (Fig 1C).

In this review, we focus on the expression, regulation, and function of the endothelial proteins known to be involved in the adhesion of leukocytes. We also consider the leukocyte counter-receptors for these proteins, and examine the current view of the role of these adhesion proteins in leukocyte-endothelial interactions.

To provide a detailed review we have focused solely on the role of these adhesion proteins in leukocyte-endothelial interactions. We do not address the many additional functions subserved by these molecules such as binding of parasites (eg, malaria), viruses (eg, rhinovirus), tumor cells, and hematopoietic precursors, or their participation in signal transduction.

Finally, although we have attempted to be comprehensive with respect to several aspects of leukocyte-endothelial adhesive interactions, we have undoubtedly overlooked some important contributions in these areas, and we apologize for these oversights. The interested reader may wish to consider other recent reviews on this topic, particularly those relating to altered expression of adhesion molecules in human disease because this important aspect is not addressed in this review.

HOMING RECEPTORS AND VASCULAR ADDRESSINS

Lymphocytes recirculate between blood and lymphatics, gaining entrance into the latter at specialized endothelium on postcapillary venules in lymphoid tissue. These high endothelial venules (HEVs) express specific surface proteins that have been designated as vascular addressins. Vascular addressins selectively bind subsets of circulating lymphocytes that express complementary (homing) receptors.

The specificity of this interaction has been shown in vivo with binding studies examining lymphocyte or leukemic cell line adhesion to HEV in frozen sections of lymphoid tissue. Currently, four vascular addressins have been identified functionally: peripheral lymph node (PNA), mucosal lymph node (ie, Peyer’s patches or appendix) (MAd), y-synovial, and skin. Peripheral node addressins include GlyCAM-1 and CD34 (see “Counter-structures for selectins”); an MAd is designated MAdCAM-1 (see “Endothelial Immunoglobulin-like Proteins”). The complementary leukocyte homing receptors for these vascular addressins are L-selectin for GlyCAM-1, CD34, and MAdCAM-1 (see “Selectins”) and αβ, for MAdCAM-1 (see “Leukocyte Integrins”).

The tissue distribution and function of some of these vascular addressins have been characterized by monoclonal antibodies (MoAbs). The MoAb MECA-79 identifies 50-kD and 90-kD sulfated glycoproteins expressed by HEV in murine and human peripheral lymph nodes and blocks lymphocyte binding to peripheral, but not mucosal, lymph nodes in vitro and in vivo. MoAb MECA-79 also reacts with venules at cutaneous sites of chronic, but not acute, inflammation in humans, suggesting that PNA is expressed in other, non-HEV sites. The MoAb MECA-367 identifies the MAd expressed by HEV in mucosa-associated lymphoid tissues. MoAb MECA-367 immunoprecipitates an ~60–65-kD protein from labeled mesenteric lymph node stroma and inhibits lymphocyte homing to mucosal, but not peripheral, lymph nodes in vitro and in vivo.

MoAbs directed against the lymphocyte homing receptors for PNA and MAd have also been shown to inhibit binding of lymphoid cells to HEV. Antibodies that react with L-selectin prevent the binding of lymphocytes to PNA, whereas MoAbs directed against an integrin (LPAM-1, αβ) inhibit the adhesion of lymphocytes to MAd.

SELECTINS

The selectin family is comprised of three proteins designated by the prefixes E (endothelial), P (platelet), and L (leukocyte). E-selectin (CD62E) and P-selectin (CD62P) are expressed by endothelial cells, and L-selectin (CD62L) is expressed only on leukocytes (Fig 1A). Structural features common to the selectins are the presence of an NH2-terminal C-type (Ca2+-dependent) lectinlike binding domain, an epitopes.

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Fig 1. Leukocyte and endothelial adhesion molecules. (A) Selectin receptors recognize carbohydrate counter-structures expressed on glycoproteins (or glycolipids). E-selectin (CD62E; ELAM-1) and P(platelet)-selectin (CD62P; GMP-140, PADGEM) recognize sialylated, fucosylated carbohydrate moieties on leukocytes including sialyl Lewis" (SLe"; CD15s). E-selectin also binds the cutaneous lymphocyte antigen (CLA) expressed on a subset of memory T cells. P-selectin has been reported to bind to SLe" (or related sialylated, fucosylated structures) expressed on P-selectin–glycoprotein ligand-1 (PSGL-1) and L(lymphocyte)-selectin (CD62L). E-selectin has been reported to bind to SLe" (or related sialylated, fucosylated structures) expressed on P-selectin–glycoprotein ligand-1 (PSGL-1), L-selectin, CD66, CD11/CD18, and a 150-kD glycoprotein on murine leukocytes. L-selectin binds to an as yet unidentified cytokine-induced counter-structure on nonlymphoid endothelium; to sialylated, fucosylated, and sulfated carbohydrate structures expressed on murine glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1); to CD34 on high endothelial venules in murine peripheral lymphoid tissue; and to the murine mucosal lymphoid addressin, MadCAM-1. (B) Interactions between leukocyte integrin receptors and ligands on the endothelial cells that are members of the Ig-like superfamily include: aβ2 (CD11a/CD18; LFA-1) with the first Ig domain of intercellular adhesion molecule-1 (ICAM-1; CD54) or the first domain of ICAM-2 (CD102); aβ2 (CD11b/CD18; Mac-1, MO-1, CR3) with the third Ig domain of ICAM-1 and with another as yet unidentified structure(s); aβ2 (CD11c/CD18; p150/95) with an as yet unidentified ligand(s); aβ1 (CD49d/CD29; VLA-4) with the first or fourth Ig domain of vascular cell adhesion molecule-1 (VCAM-1, INCAM-110; CD106); and aβ1 with mucosal addressin cell adhesion molecule-1 (MadCAM-1) expressed on high endothelial venules in murine mucosal lymphoid tissue. (C) Other adhesion pathways, apart from integrin–Ig-like and selectin–carbohydrate interactions, have been proposed. Fibrinogen may promote adhesion by bridging between αβ2 (CD11b/CD18) and ICAM-1 in vitro. The integrin receptor αβ2 as the type 3 complement receptor (CR3) binds to iC3b fixed to endothelial cells in vitro and in vivo. The integrin receptor αβ1 (CD49d/CD29; VLA-4) recognizes the CS-l region of fibronectin expressed on the lumenal surface of endothelium in vivo. Platelet-endothelial adhesion molecule-1 (PECAM-1; CD31), a member of the Ig-like superfamily, is expressed on both leukocytes and endothelial cells and mediates transendothelial migration in vitro and in vivo. Vascular adhesion protein-1 (VAP-1) expressed on human synovial endothelium promotes binding of lymphocytes ex vivo.
dermal growth factor (EGF)-like region, a variable number of consensus repeats of sequences similar to those appearing in complement-regulatory proteins, a membrane-spanning region, and a short cytoplasmic region. The selectins share an overall identity of 40% to 60% at the nucleotide and protein levels whereas the lectin and EGF domains are 60% to 70% homologous. The genes for the selectin family are closely linked on chromosome 1 (q21-24). Genes for other complement-binding proteins (C4 binding protein and decay accelerating factor) and factor V are also located in this region in both humans and mice, suggesting that the selectin family may have arisen by gene duplication.

**E-selectin**

E-selectin (CD62E) was initially described as a 115-kDa antigen that was induced on cultured human umbilical vein endothelium after stimulation by interleukin-1 (IL-1) and that was involved in the adhesion of neutrophils and several leukemic cell lines. The subsequent molecular cloning of E-selectin showed the C-type lectinlike binding domain, the EGF-like domain, and six complement-regulatory protein regions. Translation of E-selectin yields a core protein of 64 kDa with 11 potential N-glycosylation sites. The 32- amino acid cytoplasmic domain contains tyrosine residues that have been suggested to mediate the internalization of other transmembrane proteins, and may account for the short half-life of E-selectin at the cell surface. Murine and rabbit E-selectin have been molecularly cloned, and show a high degree (>70%) of homology with human E-selectin.

Mapping of E-selectin domains by MoAbs has shown that the NH2-terminal nine amino acids of the lectin domain and an epitope within the EGF-like region are important for ligand binding. Computer modeling and generation of site-specific mutants of the lectin domain of human E-selectin showed that three positively charged amino acids (arginine 97, lysine 111, and lysine 113) are critical for ligand binding and two residues (tyrosine 48 and lysine 111) have been shown to be important for function. Two variant forms of E-selectin that arise by alternative splicing of mRNA have been described. In one variant there is deletion of the seventh consensus repeat, whereas in the other variant the transmembrane region is deleted. Hydrophobicity plots of the latter suggest that this variant would be a soluble protein. Consistent with this possibility, soluble E-selectin has been detected in the plasma of normal individuals (0.15 to 0.30 µg/mL). However, it has not been shown that this is the secreted form rather than protein shed from platelets or endothelial cells. At this concentration in plasma, 20% to 40% of the binding sites for P-selectin on neutrophils would be saturated, raising the possibility that soluble plasma E-selectin may modulate leukocyte adhesion to E-selectin expressed on endothelium.

**Murine** and bovine P-selectin have been cloned. There is greater than 80% homology between these species and human P-selectin lectin, EGF, transmembrane, and cytoplasmic domains. Murine P-selectin contains eight whereas bovine P-selectin contains six consensus repeats that are ~70% homologous to human P-selectin.

Computer modeling and generation of site-specific mutants of the lectin domain of human P-selectin have indicated that two residues (tyrosine 48 and lysine 111) are critical for ligand binding. Two other amino acids (tyrosine 94 and lysine 113) may also be important for function.

**L-selectin**

Although both E- and P-selectin are expressed by endothelial cells, L-selectin (CD62L) is found only on leukocytes. L-selectin is the human homologue of the murine peripheral lymph node homing receptor that was originally identified by the MoAb MEL-14. Although originally described as a lymphocyte homing receptor, it was subsequently shown to be expressed on most other peripheral blood leukocytes, and is involved in leukocyte traffic in the systemic microcirculation.

The cloning of human L-selectin demonstrated that it shares the organization of extracellular domains found in P- and E-selectins. L-selectin has two consensus repeats of the complement-regulatory protein domains. The sequence of human L-selectin encodes a core protein of 37 kDa that has eight possible sites for N-linked glycosylation. There are no serine- or threonine-rich regions, which agrees with the lack of O-linked sugars. The molecular weight of L-selectin differs among lymphocytes (~75 kDa), neutrophils (~95 to 105 kDa), and monocytes (~110 kDa). This variability is thought to result from differences in posttranslational glycosylation among these subsets of leukocytes. L-selectin has 22 cysteine residues, 19 of which are in the consensus repeat and EGF domains, suggesting a role for disulfide bond for-
mation in providing proper spatial conformation for the lectin region that is the proposed site of ligand binding.

Murine,24,75 rat,76 and bovine77 L-selectins have also been molecularly cloned. There is approximately 80% homology between human L-selectin and these other species with the highest degree of conservation in the lectin, EGF, transmembrane, and cytoplasmic regions.

Mapping of L-selectin domains by MoAbs has determined that the NH2-terminal nine amino acids are critical for ligand binding.68,79 Maintenance of spatial conformation by the EGF and consensus repeat units of L-selectin is also important for ligand binding because MoAbs that interact with these domains affected binding in the lectin domain.52,80,81

One group has reported that two forms of human L-selectin are generated by alternative splicing.71 One form retains a transmembrane region, whereas a second form is attached to the cellular membrane by a phosphotyrosinol linkage.

There is abundant evidence that L-selectin is shed with activation of leukocytes82-85 with the loss of L-selectin because of proteolytic cleavage near the membrane insertion.82,85 Soluble circulating L-selectin was measured in the plasma of normal individuals (normal plasma level = 1.6 μg/ml), and this concentration of soluble L-selectin was able to partially inhibit leukocyte adhesion to cytokine-stimulated endothelium.86 Thus, circulating L-selectin, like soluble circulating P-selectin, may modulate leukocyte adhesion to endothelium during inflammation.25

COUNTER-STRUCTURES FOR SELECTINS

As noted previously, L-selectin was initially characterized as the murine homing receptor that mediated lymphocyte binding to HEV in peripheral lymph nodes.3 Using the Stumper-Woodruff assay of leukocyte binding to HEVs of frozen sections of lymphoid tissue, it was shown that lymphocyte binding was observed at 4°C, was sensitive to staurosporine treatment of the HEV, and was inhibited by phosphorylated oligosaccharides.87 These characteristics suggested that the counter-structure in HEV recognized by this homing receptor was a carbohydrate moiety.87 This proposal was validated by the molecular cloning of L-selectin that showed an NH2-terminal C-type lectin domain.70,77 The presence of lectinlike domains in E- and P-selectin suggested that leukocyte binding to these receptors would also involve recognition of carbohydrates. In the past several years multiple studies have examined the characteristics of carbohydrate ligands for the three selectin receptors. Additionally, several proteins have been shown to participate in selectin binding (Fig 1A).

Carbohydrate Ligands

The carbohydrate determinants recognized by the selectins have recently been reviewed in detail36,37,88 and will be considered here only briefly.

E-selectin. In 1990 and 1991 several groups identified the fucosylated tetrasaccharide, sialyl Lewis X (SLex: NeuNAcα2,3Galβ1,4(Fucα1,3)GlcNAc) (CD15) or closely related structures, as a ligand for E-selectin.59-61 Both the sialic acid and the fucose linkages were shown to be critical for efficient binding.63,64 Sialyl Lewis X and other fucosylated lactos-amines are heavily expressed on neutrophils and monocytes91,97 and are also found on natural killer (NK) cells.98,99 Peripheral blood T and B lymphocytes do not normally express SLex, but do when activated ex vivo.100 A subset of skin-homing lymphocytes expresses the cutaneous lymphocyte antigen (CLA) recognized by the HECA-452 MoAb.101,102 and these cells bind to E-selectin.103,104 The CLA-positive cells do not express SLex, but exhibit the Lewis X antigen (Le') after treatment with neuraminidase.101 Thus, the CLA antigen appears to be a sialylated, fucosylated structure closely related to SLex.

E-selectin also recognizes an isomer of SLex, sialyl Lewis A (SLexα: NeuNAcα2,3Galβ1,3(Fucα1,4GlcNAc).94,95,101 The HECA 452 MoAb recognizing CLA binds to SLex as well as SLexα.102 Because SLexα is expressed on some tumor cells but is not usually found on leukocytes, this interaction is more relevant to tumor metastases than to leukocyte trafficking.20

P-selectin. Initial studies showed that P-selectin recognized the Lewis X (Le') trisaccharide (CD15), Galβ1,4(Fucα1,3)GlcNAc.103 although subsequently the sialylated tetrasaccharide, SLex, was shown to be a higher affinity ligand.104-107 Like E-selectin, P-selectin also binds to SLex.104 However, both SLex and SLexα fail to compete for binding to activated platelets, suggesting that P-selectin may use other structural modifications.105 In this regard, P-selectin was also demonstrated to bind to sulfated glycolipids106,108-110 and certain sulfated polysaccharides such as heparin.111

The physiologic importance of fucose in carbohydrate ligands for E- and P-selectin was shown recently by studies in two patients with an inherited defect in neutrophil adhesion resulting from a generalized abnormality in fucose metabolism.112 Because the patients exhibited neutrophilia and recurrent infections such as the classic leukocyte adhesion deficiency (LAD) syndrome,113 but had normal levels of β2 integrins, the syndrome was designated LAD type II. In the initial report it was shown that neutrophils from both LAD type II patients did not express SLex and failed to bind to E-selectin or cytokine-activated endothelium. Subsequent studies showed that neutrophils from an LAD type II patient did not bind to recombinant E-selectin, or to P-selectin expressed on histamine-activated human umbilical vein endothelial cells (M.L. Phillips, B. Schwartz, A. Etzioni, R. Bryer, H. Ochs, J.C. Paulson, J.M. Harlan, manuscript submitted). From these investigations it is clear that SLex or other fucose-containing carbohydrate structures are critical for neutrophil binding to E- and P-selectin. Consequently, selectin interactions with potential protein, sulfated glycolipid, or polysaccharide ligands alone are not sufficient for neutrophil adherence under static assay conditions.

L-selectin. L-selectin is involved in leukocyte adherence to nonlymphoid microvasculature as well as to PNAD HEV.49 Like E- and P-selectin, murine L-selectin has been shown to bind to SLex (and SLexα).94,107,114 Although MoAbs to SLex react minimally with nonlymphoid endothelium in vivo or in vitro, an SLex antigen was recently found to be expressed on human HEV, suggesting that it may serve as a ligand for L-selectin.97,115 In the murine system, L-selectin ligands in HEV are sialylated and fucosylated like SLex, but are also sulfated.116
L-selectin and P-selectin (but not E-selectin) also recognize sulfatides and sulfated-polysaccharides such as fucoidan and heparin. Recently, heparinlike ligands for L-selectin were identified in cultured nonlymphoid endothelial cells, and these structures are candidates for L-selectin ligands in the systemic microvasculature.

### Protein Ligands

Although lectin-carbohydrate binding is critical to selectin-mediated adhesion, certain proteins may also participate in the adhesive interaction. Many lectin-carbohydrate interactions, probably including selectin binding to various ligands in vitro, are of low affinity. Appropriate presentation of specific carbohydrate moieties to lectin domains by membrane protein components may contribute to higher affinity binding of selectins to cellular ligands.

**E-selectin.** Several leukocyte surface structures modified by SLζ, Leζ, or related structures have been reported to bind to E-selectin, including L-selectin, CD66, and β3 integrins. Other studies have characterized the human myeloid ligand of E-selectin to be resistant to a variety of proteases and to O-glycoprotease.

Using an E-selectin–IgG chimeric protein as a probe, a protein ligand with a molecular weight of 150 kD reduced and 130 kD nonreduced was recently identified on murine neutrophils and the HL60 leukemic cell line. E-selectin binding to this glycoprotein was calcium-dependent and sensitive to neuraminidase pretreatment of the cell lysate, characteristics of E-selectin–dependent leukocyte adhesion. Interestingly, adhesion to E-selectin also was reduced by pretreatment of leukocytes with N-glycosidase, but not O-sialoglycosidase.

**P-selectin.** In contrast to E-selectin, P-selectin–dependent adhesion was shown to be abolished by pretreatment of human myeloid cells with various proteases. Pretreatment of neutrophils with an O-glycoprotease that selectively removes proteins that are heavily glycosylated also inhibited neutrophil binding to P-selectin. Interestingly, the O-glycoprotease did not dramatically alter surface SLζ expression. Furthermore, only a small portion of the SLζ-positive material bound to a P-selectin affinity column. These observations suggest that the high-affinity ligand for P-selectin is only a minor component of SLζ-modified glycoproteins on the neutrophil surface.

These results strongly implicate a specific glycoprotein ligand for P-selectin. Neutrophil L-selectin–bearing SLζ has been reported to bind to P-selectin as well as to E-selectin. However, candidate high-affinity glycoprotein ligands for P-selectin, distinct from L-selectin, have been described. Affinity purification of neutrophil membrane extracts with P-selectin showed a glycoprotein of molecular weight 240 kD nonreduced and 120 kD reduced, suggesting that it may be a disulfide-linked heterodimer. Binding of P-selectin to this glycoprotein was calcium-dependent, and was specifically inhibited by a blocking anti–P-selectin MoAb.

One P-selectin ligand, a mucinlike protein designated P-selectin glycoprotein ligand (PSGL-1), was recently molecularly cloned. After cotransfection of both a specific fucosyltransferase and a cDNA library from HL60 cells, a 220-kD glycoprotein was expressed in COS cells that conferred adhesion to P-selectin (and, interestingly, E-selectin as well) that was abolished by EDTA or by inhibitory selectin-specific MoAbs. Analysis of cell lysates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that this protein migrated as a single band of 110 kD after reduction, suggesting that it is a disulfide-linked dimer. Whether PSGL-1 is the dimeric glycoprotein identified in earlier studies awaits further studies.

Another P-selectin–specific leukocyte ligand was recently identified. A glycoprotein (160 kD nonreduced, 80 kD reduced) was found on mouse neutrophils and HL60 cells that mediated adhesion to P-selectin but not E-selectin. This binding was sensitive to EDTA and sialidase pretreatment of leukocytes. Adhesion to P-selectin was abolished by preincubation of leukocyte with either N-glycosidase and O-sialoglycosidase. In contrast, the 150-kD E-selectin ligand was resistant to the latter enzyme.

Additional glycoprotein ligands (230 kD and 130 kD) were identified that mediate adhesion to both E- and P-selectin and that are sensitive to O-sialoglycosidase but not N-glycosidase.

**L-selectin.** Using an L-selectin–IgG chimeric protein to precipitate Sζ-labeled proteins from murine peripheral lymph node organ culture, two glycoproteins, a predominant one of 50 kD and a minor one of 90 kD, were identified. Sequence data derived from the lower molecular weight sulfated glycoprotein (sgp) were used to develop probes to screen a murine lymph node cDNA library. A cDNA was cloned predicting a mature protein of 132 amino acids with a core protein molecular weight of 14 kD. This protein was shown to contain 29% of its amino acids as either serine or threonine that are clustered in two regions in which ~50% of the core protein structure is composed of these amino acids. Because there is only one possible site for N-linked glycosylation and because the molecular weight of the mature protein is ~50 kD, 70% of the mass is apparently caused by O-linked glycosylation. The sequence of this L-selectin ligand is similar to other heavily glycosylated proteins such as CD34 and MUC-1, and these sialoglycans have been proposed as a new family of adhesion proteins (vide infra). The regions of O-linked glycosylation are thought to provide a scaffold for the presentation of the polyanalyl carbohydrate that have been demonstrated to be involved in ligand binding. Because of its dependence on glycosylation, the 50 kD L-selectin ligand was designated GlyCAM-1 (glycosylation-dependent cell adhesion molecule-1). Interestingly, the structure of GlyCAM-1 does not contain a transmembrane region; hence, the mechanism by which GlyCAM-1 attaches to the cell surface is unclear. Indeed, it may function primarily as a circulating soluble protein.

Recently, the cloning of rat GlyCAM-1 was reported. The rat protein was highly homologous to murine GlyCAM-1 with ~70% of the residues being identical. Rat GlyCAM-1 contains the serine/threonine-rich clusters that are ~68% and ~48% homologous to the murine sequence.

Additional studies have identified sgp as the sialomucin, CD34, that is expressed on hematopoietic progenitors and endothelium. Partial amino acid sequencing of sgp revealed...
showed a protein core identical to murine CD34. Moreover, a rabbit polyclonal antibody to recombinant murine CD34 was shown to react with HEV of peripheral lymph nodes and with sgp\textsuperscript{\textregistered} on analysis by immunoprecipitation. Thus, CD34 is a PNA\textsuperscript{\textregistered}.

**ENDOTHELIAL Ig-LIKE PROTEINS**

The Ig gene superfamily consists of cell-surface proteins that are involved in antigen recognition (C1-type) or complement-binding or cellular adhesion (C2-type). Common features of C2-type proteins include a variable number of extracellular Ig-like domains with conserved cysteine sequences that form disulfide bonds to stabilize \( \beta \)-sheets of the tertiary structure. Members of the C2-type Ig gene superfamily include CD2, CD58 (LFA-3), and CD56 (NACAM). Five members of this family expressed by endothelial cells are involved in leukocyte adhesion: intercellular adhesion molecule-1 (ICAM-1; CD54), ICAM-2 (CD102), vascular cell adhesion molecule-1 (VCAM-1; CD106), platelet-endothelial cell adhesion molecule-1 (PECAM-1; CD31), and the mucosal adressin (MAdCAM-1) (Fig 1B).

**ICAM-1**

Human ICAM-1 is a single-copy gene located on chromosome 19. Molecular cloning showed that ICAM-1 has a core protein of 55 kD with five extracellular Ig-like domains. Amino acid substitutions in the extracellular domains have indicated that the primary binding site for leukocyte CD11a/CD18 (LFA-1) is located in the NH\textsubscript{2}-terminal first domain of ICAM-1. Initial electron microscopy of soluble ICAM-1 suggested a hinge between the second and third domains of the extracellular region; however, a recent report has shown that the hinge in ICAM-1 occurs between the third and fourth Ig-like domains. The location of this hinge may be germane for leukocyte adhesion to endothelium, because a second ligand-binding site for a leukocyte integrin (CD11b/CD18, Mac-1) was localized to the third Ig-like domain.

Leukocyte adhesion to the second ligand-binding site of ICAM-1 also is affected by the degree of glycosylation of ICAM-1. There are eight possible sites for N-linked glycosylation in the five Ig-like extracellular domains of ICAM-1. ICAM-1 is expressed on leukocytes, fibroblasts, epithelial cells, as well as endothelial cells. The molecular weights of ICAM-1 extracted from these tissues varied between 76 and 114 kD, suggesting that there is variable posttranslational modification of ICAM-1. Affinity of leukocytes for the binding site within the third Ig-like region of ICAM-1 was found to increase as the degree of glycosylation decreased. This observation suggested that selectivity of leukocyte adhesion via CD11b/CD18 may be dictated in part by the posttranslational glycosylation of ICAM-1 at the tissue level.

The cytoplasmic domain of ICAM-1 consists of a 28-residue, highly charged sequence rich in lysine and arginine residues. The cytoplasmic domain, or an 8-amino acid portion of the cytoplasmic region of ICAM-1 (RQRKIKKR), has been shown to bind to the cytoskeleton of COS cells transfected with the cDNA of human ICAM-1 and to the cytoskeleton of Epstein-Barr virus (EBV)-transformed B cells. The binding of ICAM-1 to the cytoskeleton was found to occur through linkage with \( \alpha \)-actinin, a cytoskeleton protein that may serve to anchor actin filaments to the cell membrane. When expressed as a glycosphosphatidylinositol-linked membrane protein ICAM-1 was expressed diffusely on the cell surface. Therefore, linkage with the cytoskeleton may localize ICAM-1 within regions of the endothelial cell membrane to facilitate leukocyte adherence and transmigration.

Murine and rat ICAM-1 have been molecularly cloned, and both have five extracellular Ig-like domains like human ICAM-1. However, in comparison with human ICAM-1, there is limited homology at the nucleotide (mouse = 65%, rat = 56%) or at the protein levels (mouse = 50%, rat = 51%) among these species. However, a partial cDNA sequence of canine ICAM-1 prepared by polymerase chain reaction (PCR) amplification of conserved sequences in domains 2 through 5 of human and murine ICAM-1 was slightly more homologous to human ICAM-1 (nucleotide = 74%, protein = 61%).

Mice deficient in ICAM-1 have recently been produced by targeted-gene disruption, and studies in this model should provide important information on the specific contribution of this adhesion molecule to leukocyte recruitment during inflammatory and immune reactions.

**ICAM-2**

Intercellular adhesion molecule-2 is another member of the Ig gene superfamily that is expressed on endothelium and is involved in leukocyte adherence. Human ICAM-2 is a single-copy gene located on chromosome 17. Molecular cloning of ICAM-2 showed a core protein of 29 kD with six residues for possible N-linked glycosylation which, if fully used, would yield a mature protein of 46 kD. ICAM-2 has only two extracellular Ig-like domains. However, these domains are 34% homologous to the two NH\textsubscript{2}-terminal Ig-like domains of ICAM-1. The ligand-binding site for CD11a/CD18 (LFA-1) is located in these domains of ICAM-1; hence, ICAM-2 is a second endothelial ligand for this leukocyte integrin. However, the observation that CD11b/CD18 (Mac-1) binds to the third Ig-like domain of ICAM-1 suggests that ICAM-2 does not serve as an endothelial ligand for this leukocyte integrin.

Murine ICAM-2 has been molecularly cloned and shares \(~60%\) homology to human ICAM-2 at the protein level. However, the transmembrane and cytoplasmic regions of murine ICAM-2 are more highly conserved (75% protein homology). In contrast to human ICAM-2, murine ICAM-2 has five potential residues for N-linked glycosylation.

**VCAM-1**

The third member of the Ig gene superfamily that serves as an endothelial adhesion molecule for leukocytes is VCAM-1. The initial molecular cloning of VCAM-1 reported six extracellular Ig-like domains (6D VCAM-1). However, 6D VCAM-1 arises due to alternative splicing of a seven-domain form of VCAM-1 (7D VCAM-1) that is the dominant form.
expressed by cultured human endothelial cells. The VCAM-1 cDNA initially cloned lacked a domain (designated domain 4) that is homologous to the NH₂-terminal domain of VCAM-1. Homologous, domains 1 through 3 are highly homologous to domains 4 through 6, suggesting that 7D VCAM-1 arose by gene duplication. The cloning of 7D VCAM-1 predicted a core protein of ~81 kD with seven potential sites of N-linked glycosylation in 7D VCAM-1 which, if fully used, would yield a mature protein of ~102 kD. This observation is in general agreement with immunoprecipitation studies that showed a surface protein of ~110 kD on cytokine-activated endothelium.

The additional domain in 7D VCAM-1 may have a functional role in leukocyte adhesion to endothelium. One ligand-binding site for leukocytes on both 6D and 7D VCAM-1 was shown to be located within the NH₂-terminal first domain. Similar to the presence of two ligand-binding sites for leukocytes on ICAM-1 (CD11a/CD18/1 domain 1-2 and CD11b/CD18/domain 3), leukocytes have also been shown to bind to domain 4 of 7D VCAM-1.

Murine and rat VCAM-1 have been molecularly cloned. In contrast to ICAM-1, VCAM-1 appears to have been highly conserved through evolution. Both rat and mouse VCAM-1 are highly homologous at the protein level to the 7D form of human VCAM-1 (77% and 76%, respectively).

A glycosylphosphatidylinositol-linked form of murine VCAM-1, generated by alternative splicing of VCAM-1 mRNA and able to support VLA-4-dependent leukocyte adhesion after cleavage by phosphatidylinositol-specific phospholipase C, has been reported. Whether alternative splicing of human VCAM-1 occurs remains to be determined.

**MAdCAM-1**

The most recent member of endothelial adhesion proteins in the Ig gene superfamily to be molecularly cloned is the mucosal addressin, MAdCAM-1. MAdCAM-1 was initially characterized by MoAb MECA-367 as a 58- to 66-kD antigen that was present on high endothelial venules in murine mucosal lymph nodes (eg, Peyers patches) and that was involved in lymphocyte emigration. Monoclonal and polyclonal antibodies against murine mucosal addressin were used to screen a cDNA library prepared from a tumor necrosis factor α (TNFα)-stimulated murine endotheloma cell line. A novel protein was cloned that has structural homology to two other endothelial Ig-like proteins involved in leukocyte adhesion, ICAM-1 and VCAM-1. The NH₂-terminal Ig domain of MAdCAM-1 is 30% homologous to the first Ig domain of rat ICAM-1 and human VCAM-1, whereas the second Ig domain has an equal degree of homology to the fifth Ig domain of VCAM-1. However, there are two novel aspects of MAdCAM-1. First, the third Ig-like domain of MAdCAM-1 is 30% homologous with another Ig involved in mucosal immunity, the Ca2 constant region of the Ig loop of human IgA. Second, between the second and third Ig domains is a 37-amino acid region rich in serine and threonine. These two amino acids constitute 41% of the structure of this region and are possible sites for O-linked glycosylation. A subset of MAdCAM-1 isolated from mesenteric lymph nodes was found to express the MEGA-79 carbohydrate antigen and to support L-selectin-dependent rolling. Thus, MAdCAM-1 is unique in being able to bind both αβ integrin and L-selectin.

**PECAM-1**

Recent reports have suggested that another member of the endothelial Ig-superfamily, PECAM-1 (CD31), may have a role in leukocyte adhesion and, particularly, in transmigration. CD31 is a widely distributed ~130 kD glycoprotein found on endothelial cells, platelets and some leukocytes. Greater than 95% of monocytes and neutrophils, but only ~50% of peripheral blood lymphocytes, express CD31. Among T lymphocyte subsets 100% of naive and 50% of memory CD8+ T lymphocytes are CD31+, but only 20% of CD4+ T cells and few memory CD4+ lymphocytes express CD31+. The cloning of CD31 showed six, C2-like extracellular Ig-like domains. The core protein has a molecular weight of ~80 kD with nine possible residues for N-linked glycosylation. With a molecular weight of ~130 kD for the mature protein, glycosylation may account for 40% of the mass of CD31.

Several observations suggested that CD31 might be involved in leukocyte adhesion. The molecular structure of CD31 is homologous to both carcinoembryonic antigen and ICAM-1, proteins that have been shown to be involved in homotypic and heterotypic cell adhesion, respectively. In contrast to ICAM-1 that is expressed over the entire surface of resting endothelial cells, CD31 was found to be localized at intercellular junctions. CD31 was shown to play a key role in homotypic adhesion of endothelial cells as well as in the binding of platelets to myeloid cells.

Because of the molecular similarity to FcγR, a role for CD31 in leukocyte adhesion was proposed. The binding of a CD31 MoAb increased CD8+ T-cell adhesion to fibronectin and VCAM-1, but not fibrinogen or collagen, consistent with activation of integrins containing α4 subunits (eg, α4β1 or α4β7). The binding of CD31 MoAbs to monocytes (but not neutrophils) induced a respiratory burst. Thus, in addition to a role for CD31 in adhesion, binding to CD31 may also transduce activating signals to leukocytes.

Finally, treatment of leukocytes or endothelial cells with either soluble CD31 or blocking CD31 MoAbs was recently shown to prevent monocyte or neutrophil transmigration in vitro (see “Transmigration’’). Treatment of either the leukocytes or the endothelium separately was effective, suggesting that a homophilic adhesive interaction, ie, leukocyte CD31 binding to endothelial CD31, was involved in neutrophil and monocyte transmigration. Similarly, in vivo administration of blocking CD31 polyclonal antibodies prevented the recruitment of neutrophils during acute peritoneal inflammation or skin allograft rejection.
LEUKOCYTE INTEGRINS

Integrins are transmembrane cell surface proteins that bind to cytoskeletal proteins and communicate extracellular signals. Each integrin consists of a noncovalently linked, heterodimeric α and β chains. To date, 8 known β chain subunits and 12 of 15 reported α subunits have been molecularly cloned. Integrins have been arranged in subfamilies according to the β subunits and each β subunit may have from one to eight different α subunits associated with it. However, in recent years it has also become apparent that individual α subunits may be associated with several different β subunits (e.g., the αc, chain of the classic vitronectin receptor (αcβ3) can also associate with β1, β2, β4, and β8 chains with changes in its ligand-binding capability. Thus, as many as 21 different integrin combinations have been reported.

Integrin α chains have several common structural characteristics. First, there are ~7 tandem repeats of ~60 amino acids that share homology with EF-hand structures of the calcium-binding proteins calmodulin and troponin. Three or four of these regions are thought to contribute to a divalent cation-binding domain. However, divalent cation specificity differs among the various α chains with α2β1 (CD11a/CD18) and α5β1 (very late activation antigen-2 [VLA-2]) requiring magnesium, whereas calcium is necessary for α5β1 (VLA-5) function. Second, a region of ~180 amino acids similar to domains found in cartilage matrix protein, von Willebrand factor, and complement factor B is inserted between the divergent cation-binding tandem repeats in several integrins (α2β1 and α5β1 [VLA-1 and -2, respectively] and α2β1, α2β3, and α2β2 [CD11a or LFA-1, CD11b or Mac-1 and CD11c or p150.95, respectively). There are few cysteine-rich regions or sites for N-linked glycosylation in this area, suggesting that it may be important for ligand binding. Several MoAbs that inhibited or activated β2 leukocyte integrin function were shown to bind to this I (inserted) domain of their respective α chains.

Recently, a novel, divergent cation-binding site that is required for metal-dependent ligand binding was identified within the A (I) domain of CD11b. Third, several α chains (α5β1, α6β1, α6β1 [VLA-3, -5, and -6 respectively], α3β5, and α2β1) have a site in the extracellular region near the membrane insertion domain that is proteolytically cleaved yet remains attached to the α chain by disulfide linkage. The cytoplasmic domains of several α chains are constitutively phosphorylated. A conserved sequence—KXGFFK— in the cytoplasmic domain of the α-subunits has been shown to bind to calreticulin and to be critical for the modulation of integrin avidity.

Integrin β chains also have characteristic features. Tandem repeats of four cysteine-rich regions that are thought to be essential for tertiary structure are conserved among the various β chains. Approximately 100 amino acids from the NH2-terminus are additional conserved units that are critical for maintenance of the α/β heterodimer. Disruption by point mutations in either of these regions leads to absence of expression of the β2 leukocyte integrins (leukocyte adhesion deficiency type I). The cytoplasmic domain of the β-subunit, in concert with the α-subunit, is also necessary for avidity modulation.

Within the integrin family of adhesion receptors only five members have so far been shown to be involved in leukocyte adhesion to endothelium: the β2 leukocyte integrins (CD11a/CD18, CD11b/CD18 and CD11c/CD18), the β1 integrin VLA-4 (α4β1, CD49d/CD29), and α4β1 (Fig 1B).

β2 Integrins

The β2 leukocyte integrins share a common β chain (CD18) that has the typical structure described above. Three α subunits are noncovalently associated with CD18: CD11a (lymphocyte function-associated-1 or LFA-1), CD11b (Mac-1, Mo-1) and CD11c (p150.95). The CD11 subunits contain an I domain but are not proteolytically cleaved. The expression of the β2 integrins is restricted to leukocytes, but among subtypes of leukocytes the distribution of CD11/CD18 differs. Peripheral blood lymphocytes express primarily CD11a/CD18 whereas neutrophils, monocytes, and NK cells express all three β2 integrins. Intracellular storage pools of CD11a/CD18 and CD11c/CD18 are present in neutrophils and monocytes whereas there is no storage pool of CD11a/CD18. Surface expression of CD11b/CD18 and CD11c/CD18 is increased by a variety of agonists: calcium ionophore, phorbol esters, FMLP, GM-CSF, CSF5, TNF-α, and LTB4. Increased expression of CD11b/CD18 also has been noted after neutrophil adhesion to E-selectin.

Ligands for the β2 leukocyte integrins include proteins expressed by cells (ICAM-1 for CD11a/CD18, CD11b/CD18; ICAM-2 for CD11a/CD18; and ICAM-3 for CD11a/CD18) as well as soluble proteins (fibrinogen and factor X for CD11b/CD18 and complement fragments for CD11b/CD18 and CD11c/CD18). Neutrophil and monocyte adhesion to endothelium relies primarily on the CD11a/CD18 and CD11b/CD18 leukocyte integrins with only a minor role for CD11c/CD18. Lymphocyte adhesion involves the interaction of CD11a/CD18 with the endothelial ligands ICAM-1 and ICAM-2.

The significance of the β2 leukocyte integrins has been demonstrated by the LAD Type I syndrome. In this autosomal recessive disorder there is partial or total absence of expression of the β2 leukocyte integrins on all leukocytes leading to a defect in the recruitment of neutrophils to sites of inflammation. Although neutrophils are absent in inflamed tissues, other leukocytes (mononuclear leukocytes and eosinophils) are able to emigrate to sites of inflammation, presumably via VLA-4. Absent or deficient β2 integrin expression results from heterogeneous mutations in the β subunit that impairs synthesis or prevents association with the α chain. A CD18-mutant mouse with 2% to 16% normal CD18 expression was recently produced by gene targeting, thus providing a model of partial deficiency for the study of inflammation.

β1 Integrins

The β1 integrins share CD29 as their common β subunit. This widely distributed family of integrins contains a series of cellular receptors for extracellular matrix proteins including fibronectin, collagen, laminin, and vitronectin. One member of the β1 integrins, α4β1 (VLA-4, CD49d/CD29), has
been shown to be involved in lymphocyte,\textsuperscript{195,212-216} monocyte,\textsuperscript{217,218} eosinophil,\textsuperscript{219-221} basophil,\textsuperscript{220,221} and NK cell\textsuperscript{222} adhesion to cytokine-activated endothelial cells. Because neutrophils do not express VLA-4,\textsuperscript{211} they cannot use this pathway to adhere to stimulated endothelium. The unique absence of VLA-4 on neutrophils likely accounts for the absence of VLA-4 on neutrophils, whereas pretreatment with blocking CD11a or CD11b MoAbs or by pretreatment of endothelial cells with ICAM-1 MoAbs that inhibited CD11a:ICAM-1-dependent adhesion.\textsuperscript{202} The combination of the CD11b with either the CD11a or the ICAM-1 MoAbs totally inhibited the binding of activated neutrophils to resting endothelial cell, whereas pretreatment with CD11a and ICAM-1 MoAbs was no better than each MoAb alone. These results suggested that a separate endothelial ligand for CD11b/CD18 existed.

A second study reported that activated endothelial cells adhered to plastic coated with purified CD11b and that this binding was totally inhibited by blocking CD11b MoAbs.\textsuperscript{241} A combination of blocking ICAM-1 MoAbs (RR1/1 and R6.5) partially inhibited the adhesion of activated endothelial cells to purified CD11b. Pretreatment of FMLP-activated neutrophils with the CD11b MoAb alone prevented 73\% of hetertopic conjugates formed with lipopolysaccharide (LPS)-stimulated endothelial cells whereas the combination of CD11b and CD11a MoAbs inhibited conjugate formation by 98\%. The combination of ICAM-1 MoAbs again only partially inhibited aggregation (48\%). The observation that the ICAM-1 MoAb R6.5, which was shown to prevent CD11b-dependent binding to the alternate binding domain (D3) on ICAM-1, was ineffective in totally preventing endothelial adhesion to purified CD11b or in blocking the binding of activated neutrophils to endothelium stimulated for 24 hours with LPS (a time chosen to avoid E-selectin-dependent neutrophil adhesion), again indicated that there was another ligand for CD11b on endothelium. Most importantly, the observation that neutrophils still emigrate into inflamed peritoneum in ICAM-1-null mice also indicates the existence of a second ligand.\textsuperscript{214}

EVIDENCE FOR OTHER ADHESION PATHWAYS

In addition to the defined integrin/Ig-like and selectin/carbohydrate interactions described above, a number of other adhesion pathways have been described (Fig 1, A through C).

A Second CD11b/CD18 Ligand on Vascular Endothelium

Two reports have suggested that leukocyte CD11b/CD18 (Mac-1) binds to a second ligand on endothelial cells distinct from ICAM-1.\textsuperscript{202,241} One study showed that the adhesion of phorbol dibutyrate-activated neutrophils to unstimulated endothelium was partially inhibited by pretreatment of neutrophils with blocking CD11a or CD11b MoAbs or by pretreatment of endothelial cells with ICAM-1 MoAbs that inhibited CD11a:ICAM-1-dependent adhesion.\textsuperscript{202} The combination of the CD11b with either the CD11a or the ICAM-1 MoAbs totally inhibited the binding of activated neutrophils to resting endothelial cell, whereas pretreatment with CD11a and ICAM-1 MoAbs was no better than each MoAb alone. These results suggested that a separate endothelial ligand for CD11b/CD18 existed.

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SIALOMUCINS

With the recent molecular cloning of GlyCAM-1,\textsuperscript{133} MAdCAM-1,\textsuperscript{171} and PSGL-1,\textsuperscript{132} and the identification of CD34 as a ligand for L-selectin,\textsuperscript{138} a fourth family of adhesion molecules, the sialomucins, has been proposed.\textsuperscript{135,138} The common structural elements of this family of adhesion molecules are regions rich in O-linked sugars that provide an extended structure for the exposure of multiple terminal sugars. In addition, one of these sialomucins—MAdCAM-1—also contains Ig-like regions, thus providing a binding site for anintegrin receptor.\textsuperscript{25,179} Therefore, the characterization of additional members of this family may identify molecules that subserve multiple adhesive functions.

An Induced Endothelial Ligand for CD11c

Endothelial cells stimulated for 24 hours with IL-1 or LPS, but not unactivated endothelial cells, were shown to

\[\text{CD34 as a ligand for L-selectin, a fourth family of adhesion molecules, the sialomucins, has been proposed.}\]
bind to purified CD11c/CD18. This adhesion was inhibited specifically by a blocking CD11c MoAb or by EDTA-induced chelation of divalent cations. Binding of activated endothelial cells to purified CD11c/CD18 required a higher density of soluble protein, suggesting that the affinity of this β2 leukocyte integrin for its endothelial ligand may be low, or that leukocyte capping of CD11c/CD18 may be required for binding.

An Alternate Endothelial Ligand for VLA-4 (CD49d/CD29)

Two studies have reported that the binding of lymphoid cell lines or peripheral blood lymphocytes to TNF-α-stimulated endothelial cells was more potently inhibited by CD49d than by VCA1 blocking MoAbs. The CD49d-dependent, VCA1-independent lymphocyte adhesion was reported to increase with time after TNF-α treatment of endothelium and the majority of adhesion 7 hours poststimulation was VCA1-independent. The CS-1 fragment of fibronectin has been shown to function as an alternate pathway of VLA-4 binding to stimulate endothelium in vivo (vide infra); however, coinubcation of lymphocytes with a polyclonal, antifibronectin antiserum did not affect binding to TNF-α-activated endothelium in vitro.

A Ligand on Nonlymphoid Endothelium for L-Selectin

Although the PNAd is the original ligand described for L-selectin, the observation that a blocking L-selectin MoAb inhibited phagocyte emigration into nonlymphoid tissue showed that there are also counter-receptors on nonlymphoid endothelium for L-selectin. L-selectin was shown to be expressed on unstimulated or LPS- or cytokine-treated human umbilical vein endothelium. Partial amino acid sequence of VAP-1 also indicated that it is a novel adhesion molecule. MoAb 1B2 inhibited the adhesion of lymphocytes to tonsil, peripheral lymph node, and synovial HEV as well as the binding of peripheral blood lymphocytes to immobilized VAP-1, thereby confirming VAP-1 as an endothelial adhesion molecule for lymphocytes. Whether VAP-1 is the proposed synovial vascular addressin has yet to be established.

L-VAP-2

An additional putative endothelial adhesion molecule for lymphocytes that is expressed on venules in lymphoid and nonlymphoid tissues has been identified. This 70-kDa protein, designated lymphocyte-vascular adhesion protein-2 (L-VAP-2), is constitutively expressed on human umbilical vein endothelial cells, and its expression is not upregulated by cytokines. L-VAP-2 is also expressed on B lymphocytes and CD8+ T cells. A MoAb to L-VAP-2 partially reduced lymphocyte adhesion to cultured endothelial cells. By molecular weight and tissue distribution, it is proposed that L-VAP-2 is a novel adhesion molecule involved in lymphocyte adherence to endothelium.

CD14: A Monocyte-Specific Adhesion Pathway?

Several MoAbs directed to CD14 were reported to inhibit binding of monocytes, but not neutrophils, to cytokine-activated human endothelial cells in vitro. CD14 is a glycosyl-phosphatidylinositol lipid-anchored surface protein that is highly expressed on monocytes, and, to a much lesser extent, on neutrophils. It functions as a receptor for LPS complexed with LPS-binding protein, an acute-phase protein. The nature of the induced endothelial counter-structure for CD14 is unknown. Interestingly, cross-linking of monocyte CD14 by MoAbs has been shown to activate CD11/CD18-dependent adherence, raising the possibility that binding of monocyte CD14 by its endothelial ligand may trigger subsequent β2 integrin-mediated adherence.

Finally, the sialomucin CD34 has recently been identified as a ligand for L-selectin in HEV. CD34 is expressed on endothelium in diverse vascular beds, and, when appropriately glycosylated by endothelial activation, it could potentially serve as a ligand for L-selectin on nonlymphoid vasculature.

VAP-1: A Novel, Synovial Adhesion Protein That Binds Lymphocytes

Vascular adhesion protein-1 (VAP-1) is a recently described 90-kDa endothelial adhesion protein that was identified by an MoAb (1B2) generated by immunizing mice with human synovium. Immunochemistry studies using MoAb 1B2 showed that VAP-1 was expressed on mucosal, peripheral lymph node, and synovial HEV, but was absent on endothelium from large vessels. In contrast to E- and P-selectin, ICAM-1, ICAM-2, and VCAM-1, VAP-1 was not expressed on unstimulated or LPS- or cytokine-treated human umbilical vein endothelium. Partial amino acid sequence of VAP-1 also indicated that it is a novel adhesion molecule. MoAb 1B2 inhibited the adhesion of lymphocytes to tonsil, peripheral lymph node, and synovial HEV as well as the binding of peripheral blood lymphocytes to immobilized VAP-1, thereby confirming VAP-1 as an endothelial adhesion molecule for lymphocytes. Whether VAP-1 is the proposed synovial vascular addressin has yet to be established.
CD18-Independent Neutrophil Emigration

In studies in a rabbit model, a CD18 MoAb was observed to inhibit completely neutrophil emigration in response to multiple inflammatory stimuli in the abdominal wall of rabbits, but failed to inhibit emigration after instillation of certain stimuli in the lung. These studies demonstrating a CD18-independent pathway of neutrophil emigration in the rabbit lung were supported by the finding of extravasated neutrophils at foci of bronchopneumonia in a patient with severe LAD type I syndrome, although no neutrophils were present at sites of infection elsewhere in the body. Subsequently, it was shown that the CD18-independent neutrophil emigration could also be induced in rabbit peritoneum in response to certain stimuli, if mononuclear phagocytes had previously been recruited to the peritoneum. These results suggest that a product of mononuclear phagocytes may be responsible for the induction of the CD18-independent pathway of neutrophil emigration. The adhesion molecules involved in β2 integrin-independent neutrophil emigration have not been identified.

iC3b

CD11b/CD18 was initially identified as a receptor for iC3b and was defined as CR3. CD11b/CD18-dependent neutrophil adhesion to endothelium was shown to be rapidly induced by fixation of complement on the endothelial surface in vivo. Similarly, neutrophil adhesion to xenogenic endothelium in vitro was shown to be mediated by CD11b/CD18 binding to iC3b deposited on endothelium after activation of complement by naturally occurring antibodies against endothelial cells. These pathways of adhesion may be particularly relevant to phagocyte-mediated vascular injury in vasculitides as well as in acute xenograft rejection.

Fibrinogen

Fibrinogen is a soluble ligand for CD11b/CD18. Recently, fibrinogen was shown to promote leukocyte adherence to endothelium by binding both leukocytes and endothelial cells. Purified fibrinogen or plasma fibrinogen increased leukocyte adherence by binding to CD11b/CD18 on the leukocyte and ICAM-1 on the endothelial cell. The physiologic relevance of this novel "bridging" mechanism remains to be determined.

CS-1

As discussed previously, the β2 integrin VLA-4 recognizes the extracellular matrix protein fibronectin as well as VCAM-1. A major binding site for VLA-4 in fibronectin is found within the CS-1 segment, a 25-amino acid sequence present within the alternatively spliced III CS region. Analysis of rheumatoid synovium showed that CS-1-containing fibronectin isoforms were expressed exclusively on endothelium and in extracellular matrix. Lumenal expression of CS-1 was confirmed by electron microscopy, and binding of T-lymphoblastoid cells to frozen sections of synovial tissues was inhibited by a blocking VLA-4 MoAb and CS-1 peptides. These studies show that CS-1-containing fibronectin may serve as an endothelial ligand for VLA-4-expressing leukocytes.

REGULATION OF ENDOTHELIAL ADHESION PROTEINS

The regulation of endothelial and leukocyte adhesion molecules involves both quantitative changes in surface expression and qualitative changes in avidity (Fig 2). For the endothelial adhesion molecules quantitative alterations in surface expression predominate, although qualitative changes affecting adhesion have been reported. Among the endothelial adhesion proteins there are both similarities and differences regarding the agents that induce them and the kinetics of their expression. For example, a triad of agents—IL-1, TNF-α, and LPS—stimulate the expression of ICAM-1, VCAM-1, and E-selectin, but the kinetics of the induced surface expression in vitro differ with E-selectin having a shorter half-life than ICAM-1 or VCAM-1. Stimulated surface expression of ICAM-1, E-selectin, and VCAM-1 appears to result in large part from increased transcriptional regulation. However, surface expression of P-selectin may also involve a rapid mobilization of cytoplasmic granules induced by noncytokine agents, and surface expression of E-selectin is in part regulated by rapid internalization. Therefore, important differences in regulatory mechanisms exist among these proteins that may help to explain the recruitment of subsets of leukocytes to specific sites of endothelium during an inflammatory or immune response.

A detailed consideration of the signaling pathways, promoter elements, and transcriptional factors involved in regulation of endothelial adhesion protein gene expression is beyond the scope of this review. Instead we will focus on the modulation of surface expression by various inflammatory stimuli.

ICAM-2

ICAM-2 was found to be expressed constitutively on vascular endothelium both in vivo and in vitro, and was not subject to upregulation by cytokines (TNF-α, IL-1, or IFN-
has also been reported to induce E-selectin, ICAM-1, and VCAM-1. In other studies phorbol esters induced E-selectin and upregulated ICAM-1, but were only weak inducers of VCAM-1. Hypoxia/reoxygenation, the generation of oxygen radicals, and TNF-α have been shown to induce the endothelial expression of ICAM-1 and E-selectin. Lysophosphatidylcholine treatment of endothelium derived from rabbit aorta or human iliac artery stimulated the expression of ICAM-1 and VCAM-1, but not E-selectin. IFNγ stimulated the upregulation of endothelial expression of ICAM-1, but not E-selectin or VCAM-1, whereas neither IFNα nor IFNβ were active in inducing any of the three endothelial adhesion proteins in vitro. Several reports have shown that IL-4 stimulated the expression of VCAM-1 but not E-selectin or ICAM-1 whereas IL-3 induced the expression of E-selectin.

From these observations there appear to be both common and specific pathways of induction of the endothelial adhesion proteins. Gram-negative sepsis, for example, may be associated with circulating endotoxin leading to the generation of monokines (IL-1 and TNF-α) that could induce the expression of E-selectin, ICAM-1, VCAM-1, and P-selectin on vascular endothelium. However, certain chronic inflammatory disorders may be associated with the generation of lymphokines (IFNγ or IL-4) that would favor recruitment of mononuclear leukocytes because they do not induce major neutrophil ligands, E- or P-selectin. Similarly, the generation of lysophosphatidylcholine during hyperlipidemia may induce endothelial ligands ICAM-1 and VCAM-1 that would preferentially recruit mononuclear leukocytes to sites of athrogenesis. Thus, the various patterns of cytokines or inflammatory mediators may lead to the differential induction of endothelial adhesion proteins.

Differences in the kinetics of endothelial expression of E-selectin, VCAM-1, and ICAM-1 may also contribute to the selective recruitment of leukocyte subtypes to sites of inflammation. In vitro the surface expression of E-selectin has been reported to peak 4 hours poststimulation with a return to basal levels of expression within 24 hours. However, studies in vivo have shown that E-selectin persists beyond 24 hours, indicating that additional factors may determine the duration of E-selectin expression. Endothelial expression of VCAM-1 and ICAM-1 in vitro peaks by ~6 hours and 12 hours, respectively, and both proteins persist for at least 72 hours after induction by TNF-α.

The induced expression of E-selectin, VCAM-1, and ICAM-1 is largely dependent on synthesis of new mRNA and protein because, in contrast to P-selectin, there are apparently no storage forms of these endothelial adhesion proteins. The de novo endothelial surface expression of E-selectin on cultured human endothelial cells requires the synthesis on new protein and mRNA because cycloheximide and actinomycin D were shown to inhibit the generation of E-selectin. In pulse-chase experiments it was found that treatment of endothelial cells with IL-1 or TNF-α induced E-selectin transcription within 1 hour. Maximal mRNA levels were attained within 2 to 4 hours with basal levels of expression occurring within 24 hours. Other studies indicated that there is little, if any, VCAM-1 or ICAM-1
mRNA detected in unstimulated endothelial cells, whereas TNF-α-induced VCAM-1 mRNA peaked 2 hours post-stimulation and persisted for at least 72 hours, whereas TNF-α-induced ICAM-1 mRNA peaked at 2 hours and then rapidly decreased over the next 24 hours. However, phorbol-ester-induced ICAM-1 mRNA was shown to peak later (4 hours) and to decline more slowly, demonstrating that agonist-induced expression of ICAM-1 may involve several pathways.

Reports using combinations of cytokines have provided further information regarding the regulation of E-selectin, ICAM-1, and VCAM-1 gene expression. Treatment of endothelial cells in vitro with the combination of IFNγ plus TNF-α or LPS prolonged the surface expression of E-selectin, whereas the combination of IL-1 and IFNγ had minimal effect. E-selectin accounts in large part for the short half-life of mRNA. However, the synergistic effect of the combination of TNF-α and IFNγ on E-selectin surface expression was not associated with marked changes in mRNA accumulation. Previous studies have shown that endocytosis of E-selectin accounts in large part for the short half-life of surface E-selectin. Whether IFNγ or other cytokines modulate this internalization process remains to be determined.

In contrast to the prolongation of E-selectin mRNA and surface protein expression produced by IFNγ, both TGF-β and IL-4 have been shown to inhibit cytokine-induced expression of E-selectin. Pretreatment of endothelial cells in vitro with TGF-β partially inhibited the expression of E-selectin induced by both TNF-α and IL-1, but did not affect the TNF-α-induced expression of ICAM-1 or VCAM-1. Concurrent treatment of endothelial cells with IL-4 also inhibited TNF-α-induced expression of E-selectin, and the combination of TGF-β and IL-4 produced additive inhibition. Whereas IL-4 antagonized the cytokine-induced expression of E-selectin, it augmented the induction of VCAM-1 by IL-1. These studies show that combinations of cytokines may increase (TNF-α + IFNγ for E-selectin and TNF-α + IL-4 for VCAM-1) or reduce (TNF-α + IL-4 for E-selectin and ICAM-1) the expression of endothelial adhesion proteins.

Finally, endothelial cells in different vascular beds may vary in their capacity to express adhesion proteins. As mentioned earlier, lysophosphatidylcholine induced the expression of ICAM-1 and VCAM-1 on endothelium derived from human iliac arteries but not umbilical veins. Other studies have shown that ICAM-1 was induced on cultured dexamethasone microvessel endothelium by either TNF-α and IL-1, whereas VCAM-1 was induced only by TNF-α. In contrast to responses observed with human umbilical vein endothelium, treatment of cultured human synovial microvascular endothelium with IL-1 or TNF-α led to minimal induction of ICAM-1. The basis for this differential response to agonists between endothelial cells derived from different vascular sites has not been defined.

In summary, expression of endothelial adhesion proteins is regulated at multiple levels. Some agonists are more selective for certain adhesion proteins (eg, IFNγ for ICAM-1, or IL-4 for VCAM-1); others (eg, IL-1, TNF-α, and LPS) are less specific, inducing several proteins. Combinations of cytokines may produce additive or synergistic (eg, IL-4 and TNF-α for VCAM-1) or antagonistic (eg, IL-4 and TNF-α for E-selectin) effects. These multiple levels of regulation provide for precise modulation of the expression of endothelial adhesion proteins that are involved in recruitment of leukocytes to sites of inflammation or immune reaction.

**REGULATION OF LEUKOCYTE ADHESION PROTEINS**

In contrast to endothelium, quantitative changes in leukocyte adhesion molecule expression may be less important than qualitative alterations in function (Fig 2). Activation of phagocytes provokes rapid translocation of CD11b/CD18 and CD11c/CD18 from intracellular granules to the plasma membrane. However, upregulation of CD11b/CD18 surface expression was not necessary for stimulated neutrophil adherence to endothelium in vitro. The functional importance of quantitative changes in CD11b/CD18 in neutrophil emigration has not yet been established. However, recent studies in vitro indicate that newly mobilized CD11b/CD18 receptors play an important role in subsequent adherence-dependent functions.

Most stimuli that produce upregulation of neutrophil CD11b/CD18 also induce a rapid, concomitant decrease in surface L-selectin. With appropriate activation, there is also a decrease in lymphocyte L-selectin. The downregulation of L-selectin is a result of shedding of surface L-selectin that is produced by cleavage of the extracellular portion of L-selectin by an unidentified endogenous protease. The functional significance of L-selectin shedding in leukocyte emigration is uncertain, but it may serve to limit leukocyte recruitment. Finally, in addition to these rapid (minutes) changes in cell-surface expression of adhesion molecules, quantitative alteration in integrin receptors have also been reported to occur with longer stimulation (hours to days).

Qualitative changes in adhesion receptor avidity (binding to multivalent ligands) play a critical role in leukocyte adhesion to endothelium or matrix components. Integrin receptors on circulating leukocytes are normally in an inactive or low-avidity state in that they do not bind, or bind only minimally, to their endothelial ligands. With appropriate activation of the leukocyte, the integrin receptors are transformed to an active state in which they bind avidly to surface ligand. Recent studies showed that increased leukocyte avidity may result from two distinct mechanisms—an increase affinity (as measured by binding to soluble monovalent ligands) or postreceptor events (eg, cytoskeletal associations). This avidity modulation or “inside-out” signaling can be triggered by a variety of mechanisms that range from the binding of peptide and lipid chemotactants to membrane receptors to the cross-linking of other surface molecules (eg, T-cell receptor, PECAM-1). The ability of integrin receptor to transform rapidly from a low-avidity to a high-avidity state allows leukocytes to circulate freely, but then stick firmly at sites of inflammation. It is equally important for leukocytes to modulate integrin receptors from the high to the low-avidity states as “freezing” receptors in a high-avidity state prevents migration. This topic of avidity modulation of leukocyte β1 and β2 integrin receptors has recently been reviewed.
LEUKOCYTE-ENDOTHELIAL ADHESION MOLECULES

in detail in this journal\textsuperscript{184} and elsewhere,\textsuperscript{14,15,18,207-309} and will not be considered further in this review article except to emphasize that it is a major determinant of integrin-mediated leukocyte adhesion to endothelium.

Similar to avidity modulation of leukocyte integrins, activation of neutrophils and lymphocytes was shown to produce a transient high-avidity state of L-selectin before shedding.\textsuperscript{312} Whether changes in avidity also occur with E- and P-selectin remains to be determined.

ADHESION CASCADE

Elegant studies by intravital microscopy, some dating back more than 100 years, have identified a sequence of adhesive interactions involved in leukocyte emigration from the bloodstream to extravascular sites of inflammation\textsuperscript{13} (Fig 3A). Because leukocytes do not have cilia, they cannot "swim" to the vessel wall in response to extravascular chemotactic stimuli. Initial contact with the vessel wall then is in large part a random event, perhaps enhanced by local alterations in flow characteristics. After initial contact some of the leukocytes are observed to "roll" along the vessel wall adjacent to the site of injury. Rolling is a phenomenon that is observed only under conditions of flow, and is the resultant of shear forces acting on the individual leukocyte and an adhesive interaction between the leukocyte and endothelium.\textsuperscript{314} Whether rolling is observed in normal vessels is a matter of controversy,\textsuperscript{315} but it has clearly been shown to be rapidly induced by only minimal perturbation of tissue.\textsuperscript{316,317}

Atherton and Born\textsuperscript{315} calculated that the adhesive force to which each neutrophil is subjected is \(~5\) dynes/cm\(^2\), and in vitro studies examining leukocyte adhesion to endothelium during shear force have used this estimate.\textsuperscript{318} However, others have suggested that adhesive forces of 17 to 29 dynes/cm\(^2\) may be better estimates of shear forces in vivo.\textsuperscript{319,320}

Interestingly, a consistent in vivo observation has been that leukocyte rolling seldom occurs along endothelium of arterioles.\textsuperscript{317,320-322} Although 39% of leukocytes were observed to roll along the endothelium of rat mesenteric venules, only 0.6% rolled along the endothelium of arterioles.\textsuperscript{322} It was initially reported that the mean velocity of rolling was directly proportional to mean blood velocity at flow rates less than 1 mm/s, but at higher rates was relatively constant.\textsuperscript{314} Hence, higher flow rates in the arteriolar circulation may be one explanation of this difference. However, with reduction

![Fig 3. Adhesive interactions during leukocyte emigration. (A) Studies using intravital microscopy have identified a series of events involved in leukocyte emigration from bloodstream to extravascular tissue. Under conditions of flow, leukocytes are first observed to roll along endothelium of postcapillary venules adjacent to the extravascular sites of inflammation. Initial contact with the vessel wall then is in large part a random event, perhaps enhanced by local alterations in flow characteristics. After initial contact some of the leukocytes are observed to "roll" along the vessel wall adjacent to the site of injury. Rolling is a phenomenon that is observed only under conditions of flow, and is the resultant of shear forces acting on the individual leukocyte and an adhesive interaction between the leukocyte and endothelium. Whether rolling is observed in normal vessels is a matter of controversy, but it has clearly been shown to be rapidly induced by only minimal perturbation of tissue. Atherton and Born calculated that the adhesive force to which each neutrophil is subjected is \(~5\) dynes/cm\(^2\), and in vitro studies examining leukocyte adhesion to endothelium during shear force have used this estimate. However, others have suggested that adhesive forces of 17 to 29 dynes/cm\(^2\) may be better estimates of shear forces in vivo. Interestingly, a consistent in vivo observation has been that leukocyte rolling seldom occurs along endothelium of arterioles. Although 39% of leukocytes were observed to roll along the endothelium of rat mesenteric venules, only 0.6% rolled along the endothelium of arterioles. It was initially reported that the mean velocity of rolling was directly proportional to mean blood velocity at flow rates less than 1 mm/s, but at higher rates was relatively constant. Hence, higher flow rates in the arteriolar circulation may be one explanation of this difference. However, with reduction
of flow rates in arterioles to that of venules, the frequency of leukocyte rolling on venular endothelium remained higher.

These studies suggest that there may be an intrinsic difference in the expression of endothelial adhesive components involved in leukocyte rolling, ie, the selectins and their counter-structures (vide supra) on venular versus arteriolar endothelium.

With sufficient tissue trauma or inflammation, a portion of the rolling leukocytes are observed to flatten and spread on the endothelium and then to 'stick' firmly. Occasionally, other leukocytes will stick to the leukocyte adherent to the vessel wall forming small aggregates of leukocytes attached to the endothelium. Some of the adherent leukocytes crawl over the endothelial surface, seeming to probe for an opening, and then diapedes or crawl between endothelial cells. Once in subendothelial tissue the extravasated leukocytes continue to migrate toward the inflammatory site.

In the past decade in vitro and in vivo studies have identified many of the adhesion molecules and locally generated inflammatory mediators that are involved in the adhesive interactions observed by intravital microscopy. The current model of leukocyte emigration proposes a cascade of events analogous to the complement and coagulation cascades (Fig 3B). The initial step involves local generation of mediators and initial activation of endothelium juxtaposed to the inflammatory site. This results in increased leukocyte rolling along the postcapillary endothelium, thereby 'tethering' the leukocyte to the vessel wall.

The next event in this cascade is continued activation of endothelium and now leukocytes by cytokines, chemokines, and chemoattractants that are produced locally. These agents may be specific for a particular endothelial adhesion protein (eg, IL-4 for VCAM-1) or a leukocyte subtype (eg, monocyte chemotactic protein-1 (MCP-1) for monocytes or IL-5 for eosinophils), thus promoting selective recruitment, or they may act nonspecifically to induce expression of several endothelial adhesion proteins (eg, TNF-α or IL-1 for E-selectin, ICAM-1, and VCAM-1) or activation of several leukocyte subtypes (eg, platelet-activating factor (PAF) for neutrophils, monocytes, and eosinophils), thus promoting selective recruitment, or they may act nonspecifically to induce expression of several endothelial adhesion proteins (eg, TNF-α or IL-1 for E-selectin, ICAM-1, and VCAM-1) or activation of several leukocyte subtypes (eg, platelet-activating factor (PAF) for neutrophils, monocytes, and eosinophils). Endothelial cells may further augment leukocyte activation by secretion (eg, IL-8, MCP-1, or GM-CSF) or surface expression (eg, PAF, MIP-1β, or IL-8) of proadhesive molecules. Additionally, endothelial adhesion proteins (eg, E-selectin, P-selectin, or PECAM-1) themselves may directly activate leukocytes. Events that occur during this phase of leukocyte activation strengthen adhesion and lead to firm sticking.

In the final steps of the cascade, some of the adherent leukocytes migrate between the inter-endothelial cell junctions, and then through the subendothelial extracellular matrix to accumulate finally at the site of inflammatory or immune reaction.

In general, the selectin family of adhesion proteins is primarily involved in the initial adhesive event manifested by rolling under conditions of flow, the binding of leukocyte integrins to endothelial Ig-like proteins mediates firm adhesion, and transendothelial migration involves leukocyte and endothelial PECAM-1 as well as leukocyte integrin and endothelial Ig-like proteins. Migration through subendothelial tissue is mediated in large part by interaction of integrins with components of extracellular matrix. We will consider leukocyte-endothelial adhesive interactions during rolling, activation-firm adherence, and transendothelial migration in greater detail.

Rolling

Convincing evidence from both in vitro and in vivo studies indicates that selectins are involved in the leukocyte rolling that is observed immediately after tissue injury. Models examining leukocyte adhesion to endothelium in vitro have shown that CD11/CD18-dependent adhesion, stimulated either by leukocyte activation with increased expression/avidity of the β2 integrins or with increased expression of ICAM-1 on activated endothelium, is seen only at low shear rates (<0.5 dynes/cm²) or in static adhesion systems. In contrast, with shear flow rates ~2 dynes/cm², either pretreatment of neutrophils or monocytes with anti-L-selectin MoAbs or with agonists that cause activation-induced shedding of L-selectin decreases neutrophil and monocyte adhesion to cytokine-stimulated endothelium. Agents previously shown to inhibit the adhesive function of L-selectin in vitro, including sulfated polysaccharides (fucoidan and dextran), were found to inhibit leukocyte rolling in vivo. The administration of MoAbs against L-selectin or a soluble, recombinant Ig-chimera of L-selectin significantly inhibited leukocyte rolling in vivo. By preventing leukocyte rolling, treatment with blocking antibodies or L-selectin-Ig chimera decreased leukocyte recruitment in vivo. Activity-induced L-selectin shedding or removal of glycosylated L-selectin by chymotrypsin-treatment of neutrophils ex vivo also abrogated rolling in vivo. Finally, transfection of a SLE-ε-negative murine lymphocyte line with human cDNA for L-selectin conferred the capacity to roll on inflamed rabbit venules.

The participation of P-selectin in leukocyte rolling and adhesion with higher shear forces was first shown in vitro. P-selectin in lipid bilayers mediated rolling of neutrophils at shear forces <4 dynes/cm², but ICAM-1 was unable to support adhesion at these shear forces. However, neutrophil binding to P-selectin was sensitive to greater shear forces. If neutrophil adhesion to ICAM-1 was first allowed to occur at low shear forces, subsequent increases in shear forces did not disrupt ICAM-1-dependent binding. These studies indicate that selectin-mediated adhesion remains sensitive to shear forces, whereas, once activated, integrin-mediated binding to ICAM-1 is resistant to increasing shear forces. Consistent with the in vitro studies, administration of a blocking anti-P-selectin MoAb was shown to decrease leukocyte rolling in vivo. Most importantly, 'spontaneous' rolling was reported to be virtually absent in mesenteric venules of P-selectin-deficient mice generated by gene targeting.

Evidence that E-selectin is also involved in leukocyte rolling in vitro has recently been reported. After binding to E-selectin, neutrophils do not undergo shape change and do not migrate under endothelial monolayers. A portion of this adhesion to E-selectin can be inhibited by treatment...
of neutrophils with blocking L-selectin antibodies, again suggesting E-selectin binding to SLe\(^\alpha\) expressed on L-selectin. In contrast to leukocyte rolling on P-selectin in vitro, adhesive binding to E-selectin was stronger.\(^{3,42}\) Because E-selectin is expressed only after several hours, it likely contributes to the later recruitment of leukocytes. In this regard, late neutrophil accumulation in inflamed peritoneum was not markedly reduced by treatment of normal animals with L-selectin Ig-chimeric protein or in the P-selectin-deficient mice, whereas an MoAb to E-selectin effectively inhibited neutrophil accumulation in the inflamed peritoneum and lungs of normal animals at 4 hours.\(^{3,43}\)

Thus, both L- and P-selectin have been shown to mediate rolling and recruitment of leukocytes in vivo, and all three selectins have been shown to support leukocyte rolling in vitro. The fact that both functional L-selectin and P-selectin are necessary for efficient rolling raises the question of whether they interact directly. L-selectin present on microvilli of neutrophils has been shown to present SLe\(^\alpha\) for recognition by P-selectin under conditions of shear forces in vitro.\(^{120}\) However, P-selectin has also been shown to bind with high affinity to a 120-kD protein distinct from L-selectin\(^{31}\) and to PSGL-1.\(^{3,2,2}\) Also, a SLe\(^\alpha\)-negative lymphocyte cell line transfected with human L-selectin cDNA was observed to roll in vivo,\(^{3,7,2}\) indicating that L-selectin (lacking carbohydrate ligands for P- or E-selectin) is able to mediate rolling, presumably by recognition of a rapidly induced carbohydrate counter-structure expressed on inflamed venules.\(^{3,7,2}\) Most likely then, both L-selectin and P-selectin are necessary for efficient rolling with each selectin interacting with a distinct counter-structure on the opposing cell (vide supra).

**Activation-Firm Adherence**

In the second phase of leukocyte recruitment, activation of leukocyte integrins and de novo expression of endothelial adhesion proteins occurs.\(^{1,6,18,3,2,2,3,2,4}\) As discussed previously, activation of leukocytes triggers an increase in avidity caused by a conformational change in the integrin heterodimer, resulting in greater affinity for ligands and/or postreceptor events.\(^{1,4,1,8,3,0,7,3,0,9}\) In the setting of leukocyte-endothelial interactions there are diverse mechanisms to modulate leukocyte integrin avidity. Binding of chemokines (eg, IL-8, MCP-1, MIP-1\(^\beta\)), cytokines (eg, GM-CSF, IL-5), or chemooattractants (eg, CSA, FMLP) to leukocytes expressing complementary receptors transduces signals that can augment \(\beta_1\) or \(\beta_2\) integrin-dependent adhesion. These activating agents may be derived from local tissue cells, infiltrating leukocytes, microorganisms, or, importantly, from the endothelium itself. Endothelial-derived IL-8 secreted into subendothelial matrix\(^{3,4,4}\) or bound to the surface\(^{3,4,5}\) was shown to promote neutrophil adherence and transmigration. Surface-expressed endothelial PAF also augmented neutrophil adhesion by activating \(\beta_2\) integrins.\(^{2,9,3,4}\) The chemokine MIP-1\(\beta\), bound to endothelial surface proteoglycans similarly augmented VLA-4-dependent T-cell adhesion to VCAM-1.\(^{3,4,7}\)

Activation of leukocyte integrins can also occur after the engagement of counter-structures by other leukocyte surface receptors. Cross-linking of several lymphocyte surface proteins, including CD2, CD3, CD43, and CD44, has been shown to induce high avidity binding of leukocyte CD11a/CD18.\(^{1,0}\) Moreover, the binding of leukocytes to endothelial adhesion proteins may also transduce activating signals. Cross-linking of the E- and P-selectin counter-structures, CD15 or its sialylated form, on neutrophils caused activation or upregulation of \(\beta_2\) integrins.\(^{3,4,8,3,1}\) Binding of neutrophils to E-selectin increased expression of CD11b/CD18 in vitro and augmented CD11/CD18-dependent adhesion to cultured endothelium.\(^{3,9}\) Binding of T cells to endothelial CD31 (PECAM-1) increased the function of \(\beta_1\) integrins.\(^{1,7,9}\) Lymphocyte adhesion to ICAM-1, ICAM-2, and VCAM-1 was shown to provide costimulatory signals for T-cell proliferation and IL-2 secretion,\(^{3,2,5,3,6}\) whereas monocye adhesion to P-selectin increased expression of tissue factor.\(^{3,5,9}\) Others have reported that neutrophil binding to P-selectin alone failed to provoke intracellular calcium transients, polarization, upregulation of \(\beta_2\) integrins, or priming of neutrophils.\(^{3,9,0}\)

Endogenous mediators may also function to reduce leukocyte-endothelial interactions. Although generally considered to be a proadhesive molecule promoting neutrophil emigration,\(^{3,4,4,2,3}\) under different assay conditions IL-8 reduced neutrophil adherence to endothelium in vitro,\(^{3,6,1}\) and high concentrations of intravascular IL-8 inhibited neutrophil emigration.\(^{3,6,2}\) Nitric oxide, presumably derived from endothelium, was shown to be an endogenous modulator of leukocyte adhesion because treatment with an inhibitor of endogenous nitric oxide synthesis resulted in neutrophil sticking to cat mesenteric venules observed by intravital microscopy.\(^{3,6,3}\) Also, the pleiotropic cytokine, TGF-\(\beta\), which is likely elaborated in an active form in the perivascular platelets,\(^{3,6,4,3,6,5}\) has been shown to decrease neutrophil and lymphocyte binding to basal and cytokine-stimulated endothelium, and to inhibit E-selectin expression on endothelial cells in vitro.\(^{3,6,1}\) Consistent with these in vitro studies, TGF-\(\beta\) was reported to suppress inflammatory responses in arthritis\(^{3,6,7}\) and in myocardial infarction,\(^{3,6,4}\) and mice that are deficient in TGF-\(\beta\) develop multifocal inflammatory disease with leukocyte infiltration.\(^{3,6,9}\)

If proadhesive factors predominate, the net result of the activation processes is to promote firm adhesion via integrin interactions with endothelial Ig-like ligands. Shear-sensitive, selectin-mediated tethering is replaced by shear-resistant, activated integrin-mediated adhesion. Recently, this scenario of activation-dependent adhesion was confirmed by intravital microscopy of lymphocyte binding to HEV of mouse Peyer's patches.\(^{3,7,0}\) In this model pertussis toxin, an inhibitor of G-protein-mediated signal transduction from a number of membrane receptors was found to inhibit activation-dependent sticking of lymphocytes to HEV without affecting rolling along HEV. Interestingly, lymphocyte sticking was observed to occur within 1 to 3 seconds of rolling, illustrating the rapidity with which activation events can occur.
The importance of $\beta_2$ integrins in the firm adhesion of phagocytes, and, hence, in phagocytosis, is established by LAD type I syndrome. As discussed previously, patients with this disorder have deficient or absent expression of CD11/CD18 because of heterogeneous mutations in the $\beta_2$ (CD18) subunit. Neutrophils and monocytes from these patients fail to migrate to skin windows or skin chambers and surgical biopsies of inflammatory sites are devoid of neutrophils. Studies in vitro initially demonstrated that stimulated neutrophil adhesion to endothelium under static conditions was dependent on CD11/CD18, and observations by intravital microscopy showed that administration of CD18 MoAbs prevented neutrophil sticking without affecting rolling.

Although neutrophil, and to some extent, monocyte migration is defective in LAD type I, lymphocytes, eosinophils, and plasma cells are able to emigrate to tissue. Moreover, cell-mediated immunity appears to remain relatively intact. From these observations it was apparent that an alternative adhesion pathway (s) existed. Studies in vitro identified the $\beta_1$ integrin VLA-4 and its endothelial ligand VCAM-1 as a candidate pathway of CD11/CD18-independent firm adhesion. More recently, blocking VLA-4 or VCAM-1 MoAbs have been shown to inhibit lymphocyte, monocyte, and eosinophil emigration in vivo. Although intravital microscopy studies have not been reported, it seems likely that inhibition of leukocyte emigration observed with the blocking VLA-4 or VCAM-1 MoAbs is caused in large part by a reduction in leukocyte adhesion.

Finally, $\alpha_{L}\beta_2$ binding to MAdCAM-1 has recently been identified as an adhesion pathway involved in lymphocyte homing to mucosal lymphoid tissue. By analogy to other integrin-Ig-like ligand interactions, it is presumed that activated $\alpha_{L}\beta_2$ will promote firm adhesion to MAdCAM-1 on mucosal HEV.

Recent studies provide compelling evidence in support of the current paradigm in which selectin-carbohydrate interactions initiate low-affinity adhesion, and subsequent activation-induced engagement of integrins with endothelial Ig-like ligands promotes firm adhesion. In these studies, the relative contribution of selectin and $\beta_2$ integrin was determined by intravital microscopy using fluorescent-labeled neutrophils from a normal donor, an LAD type I patient, and an LAD type II patient. Labeled cells were observed during interactions with venules in the peritoneum of rabbits treated with IL-1 to induce E-selectin (and likely the L-selectin ligand and perhaps P-selectin as well). Neutrophils from the LAD type I patient showed normal rolling, but were unable to stick and emigrate upon chemotactic stimulation. Neutrophils from the LAD type II patient rolled poorly, and failed to stick and emigrate under the shear forces provided by flow. The minimal residual rolling observed with LAD type II cells may have resulted from interaction of normal L-selectin protein with a normally fucosylated ligand on rabbit endothelium. However, when flow was reduced LAD type II cells adhered and emigrated in response to a chemoattractant. Thus, under conditions of flow (ie, with shear force) selectin binding to SLCE or related fucosylated carbohydrate structures is essential for rolling, and is prerequisite for subsequent integrin-mediated sticking and emigration.

Leukocyte Transmigration

Many of the leukocytes that are tightly bound to endothelium next crawl over the luminal surface, a process that requires reversible adhesion, ie, cyclic modulation of integrin receptor avidity. Upon encountering an intercellular junction some of the migratory leukocytes then squeeze between endothelial cells to enter extravascular tissue. The term "transmigration" has been used broadly to describe the process of leukocyte migration across endothelium from the luminal to the abluminal surface, or, more narrowly, to specify the actual penetration or diapedesis of leukocytes between endothelial cells. Migration across endothelial monolayers involves adherence to endothelium, movement over the endothelial luminal surface, and often some component of migration through subendothelial matrix in addition to penetration between endothelial cells. The distinction between the broad and narrow use of the term is important because many more adhesion molecules are involved in migration across endothelium than in diapedesis between endothelial cells.

Transendothelial migration has been studied in a number of in vitro models using endothelial cells grown on nitrocellulose or polycarbonate filters, glass coverslips, collagen gels, or amniotic membranes. It is important to note that most of these assays assess transmigration in the broader sense of net movement across endothelium rather than specific penetration between endothelial cells. Migration across endothelium has been assessed in these assays under basal, unactivated conditions (spontaneous) or in response to a chemotactic gradient or cytokine-treatment of the endothelium. Generally, there is little spontaneous migration of neutrophils or eosinophils, but monocytes, lymphocytes, and NK cells exhibit significant migration across unstimulated monolayers. With chemotactic stimulation, there is a marked increase in neutrophil and eosinophil transmigration. Pretreatment of endothelial monolayers with IL-1 or TNF-Î± also promotes neutrophil and eosinophil emigration. For neutrophils, endothelial-associated PAF and endothelial-derived IL-8 have been reported to play an important role in promoting diapedesis by activating (PAF) and guiding (IL-8) the neutrophil. Interestingly, endothelial cytosolic-free calcium was shown to regulate neutrophil transmigration in that clamping of endothelial intracellular calcium with a cell-permeant calcium buffer inhibited neutrophil migration across endothelium without affecting adherence.

Numerous studies have examined the adhesion molecules involved in transmigration. The profound defect in neutrophil emigration observed in LAD type I syndrome suggested that CD11/CD18 may be involved in neutrophil transmigration as well as adherence. Early studies showed that neutrophils from LAD type I patients or normal neutrophils treated with a blocking CD18 MoAb were unable to migrate across endothelial monolayers in response to a chemotactic stimulus. Subsequently, both CD11a/CD18 and CD11b/CD18 subunits were shown to be involved in transmigration in response to a chemotactic gradient. Chemoattractant-stimulated eosinophil migration across endothelial monolayers...
LEUKOCYTE-ENDOTHELIAL ADHESION MOLECULES

was also found to be largely CD11/CD18-dependent. CD11/CD18 also was reported to contribute significantly to spontaneous migration of monocytes, lymphocytes, and NK cells.

Several studies have established that CD11/CD18 and, to a lesser extent, ICAM-1 are important determinants of neutrophil transmigration across cytokine-activated endothelium. Pretreatment of neutrophils with a blocking MoAb against CD18 inhibited the majority (~75% to 95%) of transendothelial migration by neutrophils on 4-hour IL-1-treated endothelium. Whether CD11a/CD18 or CD11b/CD18 predominates in the interaction of the β2 leukocyte integrins with ICAM-1 during neutrophil migration is uncertain. However, MoAbs against ICAM-1 alone were noted to inhibit only a portion of neutrophil migration. The combination of E-selectin and ICAM-1 MoAbs was observed to be additive in their inhibition of neutrophil transendothelial migration on activated endothelium. Additive inhibition was also observed when MoAbs against CD11b/CD18 and ICAM-1 were combined. It has been suggested that these findings may be explained by upregulation of CD11b by initial binding of neutrophils to E-selectin on IL-1–activated endothelium at 4 hours (but not 24 hours). Because there is likely an unidentified endothelial ligand for CD11b/CD18202,241 that could circumvent ICAM-1–dependent migration, the combination of E-selectin (blocking upregulation of CD11b/CD18) and ICAM-1 (inhibiting CD11a-dependent migration) MoAbs would thus totally inhibit CD11-dependent migration.

As discussed previously, eosinophils, mononuclear phagocytes, and lymphocytes are present in surgical biopsies from LAD type I patients. Studies of monocyte and eosinophil transmigration in vitro have shown that both E-selectin and VCAM-1 are involved. Treatment of endothelium with IL-1, IL-4, or TNF-α increased eosinophil migration for greater than 24 to 48 hours. Although an anti-ICAM-1 MoAb alone inhibited 24% of IL-1 or TNF-α–induced eosinophil migration, the combination of anti-E-selectin, anti–VCAM-1, and anti–ICAM-1 MoAbs produced additive inhibition of transmigration. Similar results were obtained for monocyte migration across IL-1–activated endothelium where the combination of a CD18 MoAb with MoAbs against VLA-4 (CD49d) and E-selectin prevented 68% of monocyte migration, whereas the CD18 MoAb alone inhibited by only 51%.

The involvement of VCAM-1 in lymphocyte transendothelial migration remains to be shown definitively. Studies of lymphocyte migration on IL-1–activated endothelium have shown that a CD18 MoAb alone inhibited the majority of migration. Studies using an anti–VCAM-1 MoAb alone or a CD49d MoAb alone found little or no inhibition in lymphocyte transmigration. However, in view of the additive inhibition of transendothelial migration produced by blocking eosinophil and monocyte binding to both VCAM-1 and E-selectin in vitro and the synergistic inhibition of mononuclear leukocyte emigration produced by combined treatment with CD18 and CD49d MoAbs in vivo, the role of VCAM-1 in lymphocyte migration remains to be clarified in studies using combinations of MoAbs. Also, because VLA-4 binds to the CS-1 fragment of fibronectin that can be expressed on vascular lumen, it will be necessary to use MoAbs directed to VCAM-1 rather than CD49d, the α chain of VLA-4, to define the role of VCAM-1 in lymphocyte transmigration.

As noted previously, most transmigration assays assess some component of leukocyte migration through matrix components deposited by endothelium as well as adherence to and diapedesis between endothelial cells. Accordingly, in some studies transmigration was inhibited by MoAbs to the fibronectin receptor VLA-5 (α5β1, CD49e/CD29) and the laminin receptor VLA-6 (α6β1, CD49f/CD29) and to the CS-1 fragment of fibronectin.

In most assays it is difficult to distinguish between the contribution of a particular adhesion protein to adherence to endothelium versus diapedesis between endothelial cells during the process of transmigration. Because adherence to endothelium is prerequisite for subsequent diapedesis and transmigration, agents that inhibit adherence will also reduce diapedesis between endothelial cells and transmigration. However, studies have shown that only about half of neutrophils contacting the apical surface of endothelium eventually transmigrate under maximal stimulation. Thus, adherence alone is necessary, but not sufficient, for subsequent diapedesis between endothelial cells. The fact that neutrophil transmigration was inhibited by CD18 and ICAM-1 MoAbs, even when adherence was presumably dependent upon E-selectin, suggests that the interaction of CD11/CD18 with

![Fig 4. Selective recruitment of leukocyte subtypes. The selective recruitment of subpopulations of circulating leukocytes to sites of inflammation or immune reaction (eg, eosinophils to the lung in asthma) is a combinatorial process involving the coordinated interaction of various primary (selectin) and activation-dependent (integrin) adhesion receptors with different inflammatory stimuli (cytokines, chemoattractants, etc.) that activate leukocyte subtypes and/or endothelial cells.](https://www.bloodjournal.org)
ICAM-I may also play a role in neutrophil diapedesis between endothelial cells. Recent studies in vitro indicate that both leukocyte and endothelial PECAM-1 (CD31) are directly involved in the process of neutrophil and monocyte diapedesis between endothelial cells.\(^{181}\) In these studies an anti–PECAM-1 MoAb or recombinant soluble PECAM-1 blocked neutrophil and monocyte migration across cytokine-activated endothelial monolayers. Most importantly, light and electron microscopy showed that leukocytes blocked in transmigration by the process of neutrophil and monocyte diapedesis between endothelial cells versus adherence to endothelial cells. The fact that pretreatment of either the neutrophil or the endothelial cell was effective suggested that there was a homophilic interaction of leukocytes. Also, in synovial tissue in rheumatoid arthritis\(^{419}\) there was a permeability barrier.\(^{181}\) Because PECAM-1 is expressed only on a subpopulation of T cells,\(^{178}\) the molecules involved in the penetration of other lymphocytes between endothelial cells remain to be defined.\(^{181,182}\)

### SELECTIVE LEUKOCYTE RECRUITMENT

Experimental models of inflammation have shown that the recruitment of leukocyte subtypes follows a characteristic temporal sequence. Intradermal injection of chemotaxins or LPS induces an influx of neutrophils that peaks within 4 hours with minimal additional accumulation of neutrophils at 24 hours postinjection.\(^{407,408}\) In contrast, mononuclear leukocytes become the predominant leukocyte subtype by 12 hours postinjection, and continue to be recruited for at least 24 hours.\(^{408,409}\) By 48 hours postinjection the inflammatory infiltrate consists almost entirely of mononuclear leukocytes.\(^{408}\)

In addition to the pattern of early emigration of neutrophils versus the late accumulation of mononuclear leukocytes in inflammation, there is often selective recruitment of a leukocyte subtype in inflammatory or immune reactions. The most striking example is the marked accumulation of eosinophils at extravascular sites of allergic reactions, eg, the nasal mucosa in allergic rhinitis or alveolar spaces in asthma, although eosinophils represent only a small percentage of circulating leukocytes. Also, in synovial tissue in rheumatoid arthritis\(^{419}\) and in skin involved with inflammatory dermatoses\(^{411}\) there

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**Table 1. Animal Models of Anti-Adhesion Therapy. Models in Which Neutrophils Mediate Inflammation**

<table>
<thead>
<tr>
<th>MoAb Used</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ischemia/reperfusion</td>
<td></td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Reduced neurologic deficit with pretreatment in spinal cord ischemia/reperfusion but not irreversible ischemia(^ {411})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Reduced neurologic deficit with pretreatment in stroke model(^ {432})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Pretreatment or posttreatment in burn model improved microvascular perfusion in zone of stasis(^ {432})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Pretreatment of pulmonary artery occlusion decreased neutrophil accumulation in reperfused lung(^ {474})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Pretreatment in myocardial ischemia-reperfusion decreased neutrophil accumulation in ischemic area(^ {495,428})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Partial inhibition of human neutrophil recruitment in perfused lung model(^ {497})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Decreased neutrophilic infiltration, pulmonary hemorrhage and increased capillary permeability following limb ischemia/reperfusion injury(^ {428})</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Prevented vascular leak and tissue injury following partial ear transection(^ {429})</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Decreased myoccardial necrosis(^ {490})</td>
</tr>
<tr>
<td>L-selectin</td>
<td>Decreased myoccardial necrosis and neutrophilic infiltration(^ {471})</td>
</tr>
<tr>
<td>2. Acute inflammation</td>
<td></td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Prevention of Shwartzman response(^ {432,433})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>No inhibition of neutrophil recruitment in antigen-induced acute pulmonary inflammation(^ {534})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Decreased neutrophil recruitment in a model of phorbol ester-induced inflammation(^ {426})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Partial inhibition of neutrophil recruitment in complement-mediated acute lung injury(^ {495,437})</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Decreased leukocyte recruitment in models of IgG- and IgA-immune complex-induced acute pulmonary inflammation(^ {407})</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Decreased neutrophil recruitment in model of complement-mediated acute lung injury(^ {438})</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Decreased accumulation of neutrophils in model of IgG immune complex-mediated lung injury(^ {495})</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Decreased accumulation of total leukocytes and neutrophils in model of antigen-induced acute pulmonary inflammation(^ {494})</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Failed to decrease leukocytic infiltration in model of colitis(^ {440})</td>
</tr>
<tr>
<td>L-selectin</td>
<td>Decreased neutrophil migration into inflamed peritoneum(^ {434,435})</td>
</tr>
<tr>
<td>L-selectin</td>
<td>Decreased neutrophil migration into inflamed lung(^ {411})</td>
</tr>
<tr>
<td>L-selectin</td>
<td>Decreased neutrophil migration into inflamed lung, peritoneum and skin xenograft(^ {463})</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>Decreased neutrophil migration into inflamed lung, peritoneum and skin xenograft(^ {463})</td>
</tr>
</tbody>
</table>

For reviews of anti-adhesion therapy directed to CD11/CD18, see refs 185, 196, and 313.
is a preponderance of memory T cells that do not reflect their frequency in peripheral blood.

One possible mechanism to account for the selective recruitment of leukocyte subtypes is the expression of a specific combination of endothelial adhesion molecules that will preferentially bind certain leukocyte, i.e., an endothelial 'area code' in the inflamed systemic vasculature analogous to an address in lymphoid tissue. This possibility is supported by the observation that endothelial adhesion proteins exhibit some specificity, e.g., VCAM-1 binds mononuclear leukocytes but not neutrophils, E-selectin binds phagocytes but only a subset of memory T cells, and P-selectin binds phagocytes, NK cells, and some memory T but not B lymphocytes. However, the repertoire of known endothelial adhesion proteins in the systemic vasculature is somewhat limited, and analysis of tissue shows that several or all of the endothelial proteins may be expressed at sites of inflammation. More likely, additional levels of control are required to produce selective recruitment.

A model of leukocyte-endothelial cell recognition has been proposed that emphasizes combinatorial strategies to achieve diversity and specificity.

### Table 2. Animal Models of Anti-Adhesion Therapy. Models in Which Mononuclear Leukocytes Mediate Inflammation

<table>
<thead>
<tr>
<th>MoAb Used</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Organ transplantation</td>
<td></td>
</tr>
</tbody>
</table>
| ICAM-1 | Partial prevention of rejection in cardiac allograft 
| VCAM-1 | Prevention of graft rejection in cardiac allograft |
| L-selectin | Decreased leukocyte infiltration and vasculitis in cardiac allograft |
| 2. Autoimmune/chronic inflammatory disorder |
| ICAM-1 | Partial inhibition of leukocyte recruitment in collagen- or adjuvant-induced arthritis |
| ICAM-1 | Partial inhibition of mononuclear leukocyte recruitment in antipolymyositis membrane form of glomerulonephritis |
| ICAM-1 | Decreased leukocytic infiltration and suppressed the development of experimental autoimmune encephalitis (EAE) |
| ICAM-1 | Decreased the development of active, but not passively transferred, EAE |
| ICAM-1 | When administered with CD11a MoAb, prevented progression of crescentic glomerulonephritis |
| ICAM-1 | Reduced leukocytic infiltration and proteinuria in model of autoimmune nephritis |
| ICAM-1 | Partial reduction in number of mononuclear leukocytes at site of delayed-type hypersensitivity (DTH) reaction |
| VCAM-1 | Decreased leukocyte entry into CNS in EAE model |
| VLA-4 | Decreased migration to sites of DTH reaction |
| VLA-4 | Decreased lymphocyte accumulation into arthritic joints |
| VLA-4 | Decreased leukocytic infiltration in model of colitis |
| VLA-4 | Decreased accumulation of leukocytes in model of EAE |
| L-selectin | Decreased lymphocyte migration to sites of DTH reaction |

**Models in Which Eosinophils Mediate Inflammation**

<table>
<thead>
<tr>
<th>MoAb Used</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td>Decreased accumulation of eosinophils in model of antigen-induced asthma</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>No inhibition of eosinophil recruitment in primate model of asthma unless animals were withdrawn from dexamethasone</td>
</tr>
<tr>
<td>VLA-4</td>
<td>Decreased late phase airway hyperresponsiveness after antigen challenge</td>
</tr>
<tr>
<td>VLA-4</td>
<td>Decreased eosinophilic infiltration in passive cutaneous anaphylaxis reaction</td>
</tr>
</tbody>
</table>

For reviews of anti-adhesion therapy directed to CD11/CD18, see refs 185, 196, and 313.
and monocytes bind to the induced L-selectin ligand, P-selectin, E-selectin, and ICAM-1. Even when all of these endothelial adhesion molecules are expressed, selective recruitment of neutrophils should occur if IL-8 is the major local chemoattractant. Similarly, monocytes should accumulate preferentially when MCP-1 predominates. The traffic of eosinophils to sites of allergic reaction might result from the generation of IL-3, IL-4, IL-5, and GM-CSF by TH2 cells. As discussed previously, IL-4 induces VCAM-1 but not E-selectin, and would therefore augment the emigration of eosinophils and mononuclear leukocytes but not neutrophils that lack VLA-4. Selective activation of eosinophil integrins by IL-5 or other cytokines or chemokines would promote their adherence and transmigration, leading to eosinophil accumulation.

Experimental support for this combinatorial model will come from in vivo studies correlating the recruitment of leukocyte subclasses with the local expression of endothelial adhesion molecules, cytokines, and chemokines.

ANTI-ADHESION THERAPY IN ANIMAL MODELS OF HUMAN DISEASE STATES

In the past several years there have been numerous reports describing the systemic administration of MoAbs to inhibit leukocyte adhesion to endothelium in models of inflammation or immune reaction. The majority of these "anti-adhesion" studies have involved the use of MoAbs directed against CD11a, CD11b, or CD18 because these were some of the first reagents to be developed. Prior reviews have listed the results of these studies, and they will not be reiterated in this article. Instead, Tables 1 and 2 focus on animal models involving anti-adhesion therapy directed to endothelial adhesion proteins and leukocyte adhesion proteins other than CD11/CD18.

Animal models of human diseases in which neutrophils play a dominant role include models of ischemia/reperfusion and acute inflammation (Table 1). In the former, vascular occlusion initiates endothelial damage that is subsequently exacerbated after reperfusion by activation of the inflammatory system and the adherence of neutrophils.

Animal models of disorders in which mononuclear leukocytes play a dominant role include organ transplantation with alloimmune reaction, and chronic inflammatory disorders (eg, models of rheumatoid arthritis and demyelinating diseases) (Table 2).

Although most studies have used MoAbs to block leukocyte adherence to endothelium, other specific approaches have been tested in vivo. An L-selectin-Ig chimera was shown to inhibit early neutrophil accumulation in inflated mouse peritoneum. More recently, an SLE oligosaccharide was shown to inhibit cobra venom factor- and IgG-induced acute lung injury in rat models and myocardial reperfusion injury in the cat. Other potential approaches to specific "anti-adhesion" therapy that will likely soon be tested in vivo models include blocking peptides, soluble integrin receptors, antisense oligonucleotides, and drugs that selectively block the signaling pathways involved in the induction of endothelial adhesion proteins or the modulation of integrin avidity. Given the therapeutic potential, additional strategies will undoubtedly emerge.

SUMMARY

In the 9 years since the last review on leukocyte and endothelial interactions was published in this journal many of the critical structures involved in leukocyte adherence to and migration across endothelium have been elucidated. With the advent of cell and molecular biology approaches, investigations have progressed from the early descriptions by intravital microscopy and histology, to functional and immunologic characterization of adhesion molecules, and now to the development of genetically deficient animals and the first phase I trial of "anti-adhesion" therapy in humans. The molecular cloning and definition of the adhesive functions of the leukocyte integrins, endothelial members of the Ig gene superfamily, and the selectins has already provided sufficient information to construct an operant paradigm of the molecular basis of leukocyte emigration. The regulation of these adhesion molecules by chemoattractants, cytokines, or chemokines, and the interrelationships of adhesion pathways need to be examined in vitro and, particularly, in vivo. Additional studies are required to dissect the contribution of the individual adhesion molecules to leukocyte emigration in various models of inflammation or immune reaction. Certainly, new adhesion structures will be identified, and the current paradigm of leukocyte emigration will be refined. The promise of new insights into the biology and pathology of the inflammatory and immune response, and the potential for new therapies for a wide variety of diseases assures that this will continue to be an exciting area of investigation.

NOTE ADDED IN PROOF

Since the submission and revision of this manuscript the crystal structure of E-selectin has been reported (Graves et al: Nature 367:532, 1994). Mutagenesis studies of the lectin region confirmed the importance of arginine 97 and lysine 113 in ligand binding.

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Leukocyte-endothelial adhesion molecules

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