Atherosclerosis and Coronary Artery Disease,
edited by V. Fuster, R. Ross, and E. J. Topol.
Lippincott-Raven Publishers, Philadelphia © 1996.

CHAPTER 29

Traffic Signals on Endothelium for Leukocytes in Health, Inflammation, and Atherosclerosis

Timothy A. Springer and Myron I. Cybulsky

Key Words: Eosinophils; Granulocytes; High endothelial venules (HEV); Lipoproteins; Lymphocytes; Monocytes; Mononuclear leukocytes; Neutrophils; Tumor necrosis factor (TNF); Vascular cell adhesion molecule 1 (VCAM-1).

T. A. Springer: The Center for Blood Research, Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.

M. I. Cybulsky: Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts 02115.

INTRODUCTION

During atherogenesis, the emigration of mononuclear leukocytes from blood into the arterial intima is a key feature of initiation and progression of atherosclerotic plaques. This process may represent a protective inflammatory response of the host to intimal accumulation of oxidized lipoproteins. The mechanisms of leukocyte recruitment to the arterial wall during atherogenesis are likely shared by a variety of inflammatory and immune processes; however, leukocyte recruitment in the latter occurs primarily through postcapillary venules and veins. Because of differences in hemodynamics of

arterial and venous circulations, some of the steps in arterial leukocyte emigration, e.g., rolling, may differ from emigration in veins. In this chapter, we review the pathophysiological mechanisms of leukocyte emigration and lymphocyte recirculation and then, based on observations in animal models and in human specimens, speculate on the mechanisms that may be relevant to atherogenesis.

The circulatory and migratory properties of white blood cells have evolved to allow efficient surveillance of tissues for infectious pathogens and rapid accumulation at sites of injury and infection. Lymphocytes continually patrol the body for foreign antigens by recirculating from blood, through tissue, into lymph, and back to blood. Lymphocytes acquire a predilection, based on the environment in which they first encounter foreign antigen, to home to or recirculate through that same environment (1,2). Granulocytes and monocytes can emigrate from the bloodstream in response to molecular changes on the surface of blood vessels that signal injury or infection. Lymphocytes can similarly accumulate in response to inflammatory stimuli. The nature of the inflammatory stimulus determines whether lymphocytes, monocytes, neutrophils, or eosinophils predominate, and thus exercises specificity in the molecular signals or "area codes" that are displayed on endothelium and control traffic of particular leukocyte classes.

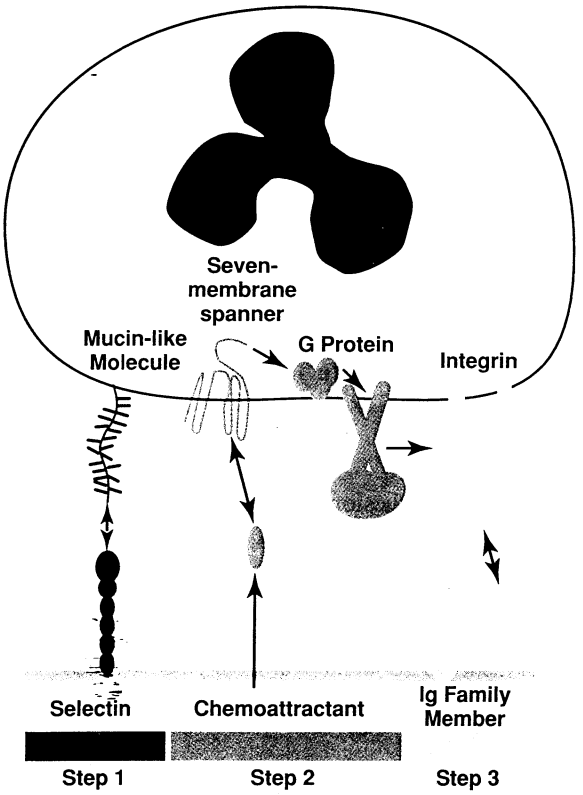


FIG. 1. Three sequential steps provide the traffic signals that regulate leukocyte localization in the vasculature. Selectin molecules that bind carbohydrate ligands, often displayed on mucin-like molecules, are responsible for the initial tethering of a flowing leukocyte to the vessel wall and labile, rolling adhesions (the green light). Tethering brings leukocytes into proximity with chemoattractants that are displayed on or released from the endothelial lining of the vessel wall. Chemoattractants bind to receptors that span the membrane seven times on the surface of leukocytes. These couple to G proteins, which transduce signals that activate integrin adhesiveness (the yellow light). The integrins can then bind to immunoglobulin superfamily (IgSF) members on the endothelium, increasing adhesiveness and resulting in arrest of the rolling leukocyte (the red light). Following directional cues from chemoattractants and using integrins for traction, leukocytes then cross the endothelial lining of the blood vessel and enter the tissue.

Recent findings show that the "traffic signals" for lymphocyte recirculation and for neutrophil and monocyte localization in inflammation are strikingly similar at the molecular level. These "traffic signal" or "area code" molecules are displayed together on endothelium but act on leukocytes in a sequence that was first defined for neutrophils and appears to hold true with slight modification for lymphocyte homing as well (Fig. 1). The selectin or green light allows cells to tether and roll, the chemoattractant or yellow light tells cells to activate integrin adhesiveness and put on the brakes, and the immunoglobulin (Ig) family member or red light binds integrins and causes cells to come to a full stop. These three steps, with multiple molecular choices at each step, provide great combinatorial diversity in signals. Accordingly, the selective responses of different leukocyte classes to inflammatory agents, as well as the preferential recirculation patterns of distinct lymphocyte subpopulations, can be explained by their distinct receptivity to combinations of molecular signals. Following an overview of leukocytes and endothelium, and of the molecules important in their interactions, we review the traffic signals that enable selective emigratory behavior of monocytes and neutrophils and then elaborate how a paradigm of three or four sequential signals can be extended to lymphocyte recirculation. This review updates and extends a previous one (3). (For recent reviews see refs. 4–20.)

THE FUNCTION OF LEUKOCYTE CLASSES CORRELATES WITH CIRCULATORY BEHAVIOR

Neutrophilic granulocytes are among the most abundant leukocytes in the bloodstream and the first to appear at sites of bacterial infection or injury. Neutrophils are produced at the prodigious rate of 10^9 cells/kg body weight per day in the bone marrow and have a half-life in the circulation of 7 h. Their life span after extravasation is hours or less (21). Their primary function is to phagocytose and eliminate foreign microorganisms and damaged tissue.

Monocytes are far less numerous than neutrophils in the blood, where their half-life is about 24 h (22). Like neutrophils, they are phagocytic and accumulate in response to traumatic injury or bacterial infection. However, monocytes differ from neutrophils in that they accumulate at sites where

T lymphocytes have recognized antigen, as in delayed-type hypersensitivity reactions and graft rejection. Monocytes are important effector cells in antigen-specific T-cell immunity, are activated by T-cell products such as interferon-γ (IFN-γ), and can organize around parasites into protective structures called granulomas. After extravasation, monocytes may also differentiate into longer-lived tissue macrophages or mononuclear phagocytes such as the Kupffer cells of the liver, which have a half-life of weeks to months.

In contrast to the neutrophil and monocyte, a lymphocyte may emigrate and recirculate many thousands of times during its life history. Recirculation of lymphocytes correlates with their role as antigen-receptor-bearing surveillance cells. Lymphocytes function as the reservoir of "immunologic memory" and recirculate through tissues to provide systemic memory. Few of the body's lymphocytes are present at any one time in the bloodstream, where their half-life is 2 h. Distinct subsets of lymphocytes extravasate through the microvasculature in tissues such as skin and gut, and through specialized high endothelial venules (HEV) in lymphoid organs (1,6,17). After migrating through tissue, lymphocytes find their way into the lymphatics. They percolate through draining lymph nodes in the lymphatic system and finally enter the thoracic duct, through which they return to the bloodstream. This journey is completed roughly every 1 to 2 days.

ENDOTHELIUM

By displaying specific signals, the endothelium is the most active player in controlling leukocyte traffic. Vascular endothelium is diversified at a number of levels. Large vessels differ from small vessels and capillaries; venular endothelium differs from arterial endothelium; and endothelial phenotype varies between tissues. The preferential migration of leukocytes from postcapillary venules may be related to factors such as shear stress, which is lower there, and hence more favorable for leukocyte attachment, than in capillaries or arterioles, or to events that occur when leukocytes pass through capillaries. However, when flow is controlled so that shear stress is equivalent in arterioles and venules (23), or when the direction of blood flow is reversed (24), attachment and emigration are far greater from venules, suggesting molecular differences in their endothelial surfaces. In agreement with this, P-selectin is much more abundant on postcapillary venules than on large vessels, arterioles, or capillaries (25), and induction of E-selectin and vascular cell adhesion molecule 1 (VCAM-1) expression in inflammation is most prominent on postcapillary venules (9). The mucin-like cluster of differentiation 34 (CD34) molecule is well expressed on capillaries and is absent from most large vessels (26), and CD36 is expressed on microvascular but not large-vessel endothelium (27). The extracellular matrix may exert an influence on endothelial differentiation, as exemplified by modulation of adhesiveness (28). The high endothelium in lymphoid tissue, which expresses addressins for lymphocyte recirculation, is one of the most dramatic examples of endothelial specialization (6).

Inflammatory cytokines dramatically and selectively modulate the transcription and expression of adhesion molecules and chemoattractants in endothelial cells (29). Tumor necrosis factor (TNF) and interleukin-1 (IL-1) increase adhesiveness of endothelium for both neutrophils and lymphocytes and induce ICAM-1, E-selectin, and VCAM-1; IL-4, synergistically with other cytokines, increases adhesion of lymphocytes and induces VCAM-1 (30,31). It is likely that the precise mixture of chemoattractants and cytokines produced at inflammatory sites in vivo determines which types of leukocytes emigrate. Thus, injection into skin of IL-1α induces emigration of neutrophils and monocytes, as do lipopolysaccharide (LPS) and TNF-α, but with more prolonged emigration of the monocytes. IFN-γ induces emigration of monocytes but not neutrophils (22). Interferon-γ and TNF-α, but not IL-1α or LPS, recruit lymphocytes, and IL-4 is ineffective by itself but synergizes with TNF (32–34).

Acting more quickly than cytokines, vasoactive substances such as histamine and thrombin modulate endothelial function in seconds or minutes. They induce secretion of the storage granules of endothelial cells and platelets. Furthermore, they dilate arterioles, increase plasma leakage and thereby raise the hematocrit within microvessels, and thus alter the rheology of blood so as to increase the collision of leukocytes with the vessel wall (35). Furthermore, arteriolar dilation and the ensuing increased blood flow in inflammatory sites are responsible for two of the cardinal signs of inflammation, rubor (redness) and calor (heat), and increased perfusion enhances the discharge and thus accelerates the accumulation of leukocytes.

AREA CODE MOLECULES

Selectins

Multiple protein families, each with a distinct function, provide the traffic signals for leukocytes. The selectin family of adhesion molecules (Fig. 2) has an N-terminal domain homologous to Ca^{2+}-dependent lectins (7,8,9,18,19,36,37). The name selectin capitalizes on the derivation of "lectin" and "select" from the same Latin root, meaning to separate by picking out. Selectins are limited in expression to cells of the vasculature (Fig. 2). L-Selectin is expressed on all circulating leukocytes except for a subpopulation of lymphocytes (38–40). P-Selectin is stored preformed in the Weibel–Palade bodies of endothelial cells and the α granules of platelets. In response to mediators of acute inflammation such as thrombin or histamine, P-selectin is rapidly mobilized to the plasma membrane to bind neutrophils and monocytes (25,41,42). E-Selectin is induced on vascular endothelial cells by cytokines such as IL-1, LPS, or TNF and requires de novo mRNA and protein synthesis (43).

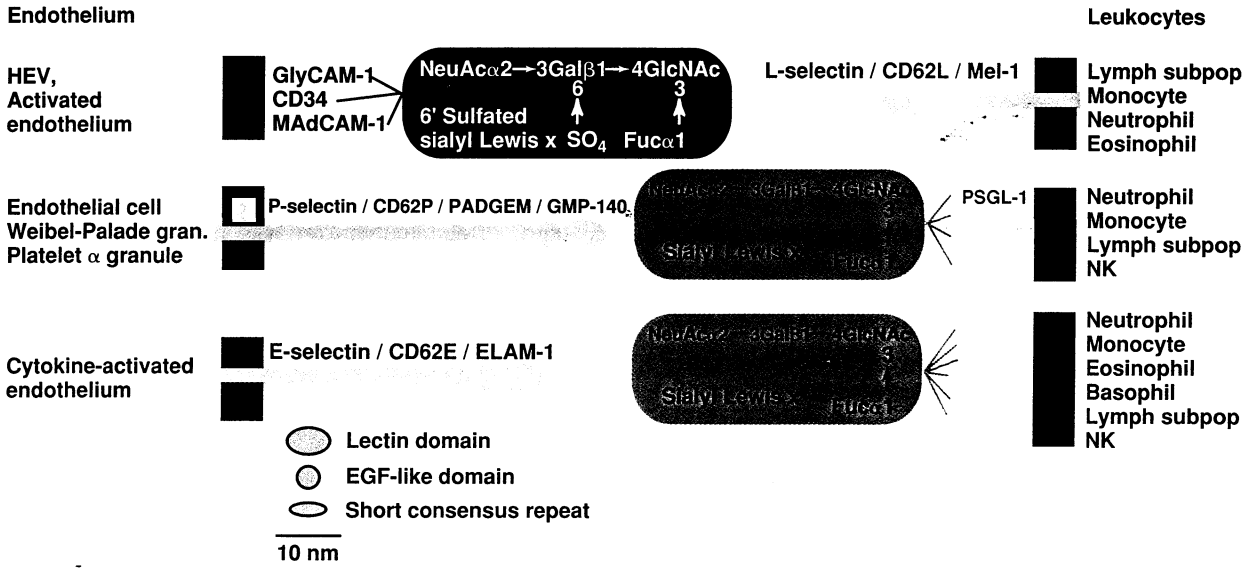


FIG. 2. Selectins and their ligands. The selectins are shown to scale, based on electron micrographs of P-selectin (47), the X-ray structure of E-selectin lectin and EGF domains (49), and estimates of the sizes of the short consensus repeats (SCR) (36). P-Selectin is shown palmitylated on a transmembrane cysteine (291). The carbohydrates are not to scale.

Carbohydrates and Mucin-Like Molecules

All selectins appear to recognize a sialylated carbohydrate determinant on their counterreceptors (7,8,19). E-Selectin and P-selectin recognize carbohydrate structures that are distinct but are both closely related to the tetrasaccharide sialyl Lewis^x and its isomer sialyl Lewis^a (Fig. 2). The actual ligand structures for E- and P-selectin are more complex, as shown by display of the ligand for E-selectin but not P-selectin on fucosyl-transferase-transfected cells that express sialyl Lewis^x (44). The affinity of E-selectin for soluble sialyl Lewis^x is quite low, with K_d = 0.2–0.8 mM (45), which suggests that a higher-affinity ligand may yet be identified. P-Selectin is specific for carbohydrate displayed on the P-selectin glycoprotein ligand (PSGL-1), suggesting either that PSGL-1 expresses a specific carbohydrate structure or that PSGL-1 protein forms part of the ligand binding site (46). The affinity of P-selectin for PSGL-1 is high, with a K_d = 70 nM (47). Structure–function studies suggest that the Ca^{2+}-binding site and a cluster of basic residues on E-selectin coordinate with the fucosyl and sialic acid carboxylate moieties, respectively, of sialyl Lewis^x (48,49).

The carbohydrate ligands for L- and P-selectin are O-linked to specific mucin-like molecules. Mucins are serine- and threonine-rich proteins that are heavily O-glycosylated and have an extended structure. L-Selectin recognizes at least two mucins in HEV (Fig. 3), glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1), which is secreted (37), and CD34, which is on the cell surface (50). The carbohydrate ligand for L-selectin is related to sialyl Lewis^a and sialyl Lewis^x (51,52), contains sialic acid and sulfate, and is O-linked to mucin-like structures of HEV (19). Structural studies on the carbohydrates of GlyCAM-1 show that 6′-sulfated sialyl Lewis^x (Fig. 2) is a major oligosaccharide capping group, and is a candidate for the ligand structure (53).

The mucin-like P-selectin glycoprotein ligand (PSGL-1) is a disulfide-linked dimer of 120-kDa subunits (46) that is sensitive to O-glycoprotease, which selectively cleaves mucin-like domains (54,55). The PSGL-1 (Fig. 3) was isolated by screening for cDNA that expressed ligand activity (56). COS cells must be transfected with both the PSGL-1 cDNA and α-3/4-fucosyl transferase cDNA to express P-selectin ligand activity. By contrast, COS cells cotransfected with cDNA for α-3/4-fucosyl transferase and another mucin-like molecule that is expressed by neutrophils, CD43, lack P-selectin ligand activity.

Function of Selectins and Their Ligands

Selectins mediate functions unique to the vasculature, the tethering of flowing leukocytes to the vessel wall and formation of labile adhesions with the wall that permit leukocytes subsequently to roll in the direct of flow. One study demonstrated this with purified P-selectin incorporated into supported planar lipid bilayers on one wall of a flow chamber (57). At wall shear stresses within the range of those found in postcapillary venules, neutrophils formed labile attachments to the P-selectin in the bilayer and rolled in response to fluid drag forces. In other studies, intravascular infusion of a soluble L-selectin/IgG chimera inhibited neutrophil rolling

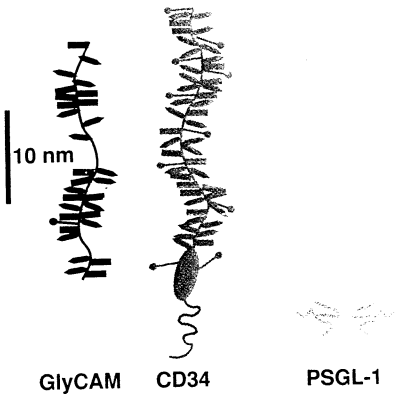


FIG. 3. Mucin-like carriers of selectin ligands. The GlyCAM (37) and CD34 (50,77) molecules synthesized by peripheral lymph node HEV and MAdCAM-1 molecule synthesized by mucosal HEV (see Fig. 5) bear O-linked carbohydrates that bind to L-selectin. CD34 has a globular domain that may be Ig-like (292) and is resistant to O-glycoprotease (293). The PSGL-1 molecule on neutrophils bears O-linked carbohydrates that bind to P-selectin (55,56). A cysteine in the transmembrane region is predicted to be palmitylated. O-Linked sites and N-linked sites are shown as bars and lollipops, respectively. The length of the mucin-like domains and the percentage of serines and threonines that are O-glycosylated are proportioned to measurements for CD43 (45 nm per 224 amino acids and 75% to 90% of O-glycosylation) (72).

attachments in vivo (58), as did infusion of anti-L-selectin monoclonal antibodies (59). More recent studies have shown that neutrophils roll on E-selectin in purified form (60) or on the endothelial cell surface both in vitro (61) and in vivo (62), that monoclonal antibody (mAb) to P-selectin decreases neutrophil rolling in vivo (63), and that neutrophil rolling in the microvasculature of mice genetically deficient in P-selectin is almost completely absent (64).

P- and L-selectin may cooperate with one another, because inhibition of either almost completely inhibits neutrophil rolling in vivo (58,59,64,65). E- and L-selectin also appear to cooperate (60,66–68). A class of ligand that is closely associated with L-selectin on the neutrophil surface is required for the initial tethering during flow to E-selectin bilayers, after which another class of ligands that mediates rolling takes over (69).

Selectins can mediate tethering of a flowing cell in the span of a millisecond. The integrin LFA-1 and the IgSF member CD2 require minutes to develop similar adhesive strength and do not mediate rolling (57,70). It has been hypothesized that selectins differ from other adhesion molecules not in affinity (K_{eq}) but in having much more rapid association (k_{on}) and dissociation (k_{off}) rate constants (57), as has recently been confirmed (Table 1). Rolling is intermittent and appears mediated by random association and dissociation of selectin–ligand bonds, a small number of which tether a leukocyte to the vessel wall at any one time. A rapid association rate facilitates the initial tethering in flow. A rapid dissociation rate ensures that even with multiple selectin–ligand bonds, it will not take long before the bond that is most upstream randomly dissociates, allowing the cell to roll forward a small distance until it is held by the next most upstream bond. (57,71).

The elongated molecular structure of selectins and mucins and their segmental flexibility (47,72) are predicted to enhance their accessibility for binding to counterstructures on closely opposed cells (57). P-Selectin and PSGL-1 are currently the most elongated adhesion molecules known (Figs. 2 and 3) and could bridge together two cells with plasma membranes about 0.1 μm apart. Expression on cytoplasmic protrusions further enhances accessibility. L-Selectin is clustered on microvilli of neutrophils (67,73), which project about 0.3 μm above the surface of a cell with a diameter of 7 μm and contain 90% of the L-selectin (D. Bainton, D. Hammer, and T Springer, *unpublished data*). In keeping with this topographic distribution, rolling in vivo requires the integrity of the L-selectin cytoplasmic domain and is inhibited by cytochalasin B (74). Lymphocytes bind through microvilli to HEV (75,76). Conversely, the mucin-like CD34 molecule (77) is concentrated on filopodia of nonspecialized endothelial cells found in the microvasculature of most tissues (26). These filopodia are concentrated near junctions between endothelial cells, and electron micrographs of granulocytes binding to the microvasculature in inflammatory sites suggest that the earliest binding event is to these filopodia (78).

TABLE 1. *Fast on and off rates of a selectin, and affinity modulation of an integrin*

	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (μM)
P-Selectin	1.4×10^{7} [a]	1 [b]	0.07 [c]
LFA-1 low affinity [d]	3×10^{2}	0.03	100
LFA-1 high affinity [e]	ND [f]	ND	0.6

[a] Calculated from $k_{on} = k_{off}/K_d$.

[b] At very low P-selectin densities in lipid bilayers, neutrophils attach transiently; i.e., they subsequently detach rather than roll. Measurements of the cellular dissociation rate suggest that the $t_{1/2}$ for dissociation of a single selectin–ligand bond is about 0.7 s (315).

[c] For binding of monomeric, truncated P-selectin to neutrophils (47).

[d] k_{on}, k_{off}, and K_d were measured by competitive inhibition by monomeric, truncated ICAM-1 of binding of Fab to LFA-1 on resting lymphocytes (111).

[e] Same as *d*, but for phorbol-ester-stimulated lymphocytes. Approximately 20% of the cell surface LFA-1 was in the high-affinity state (111).

[f] ND, not determined.

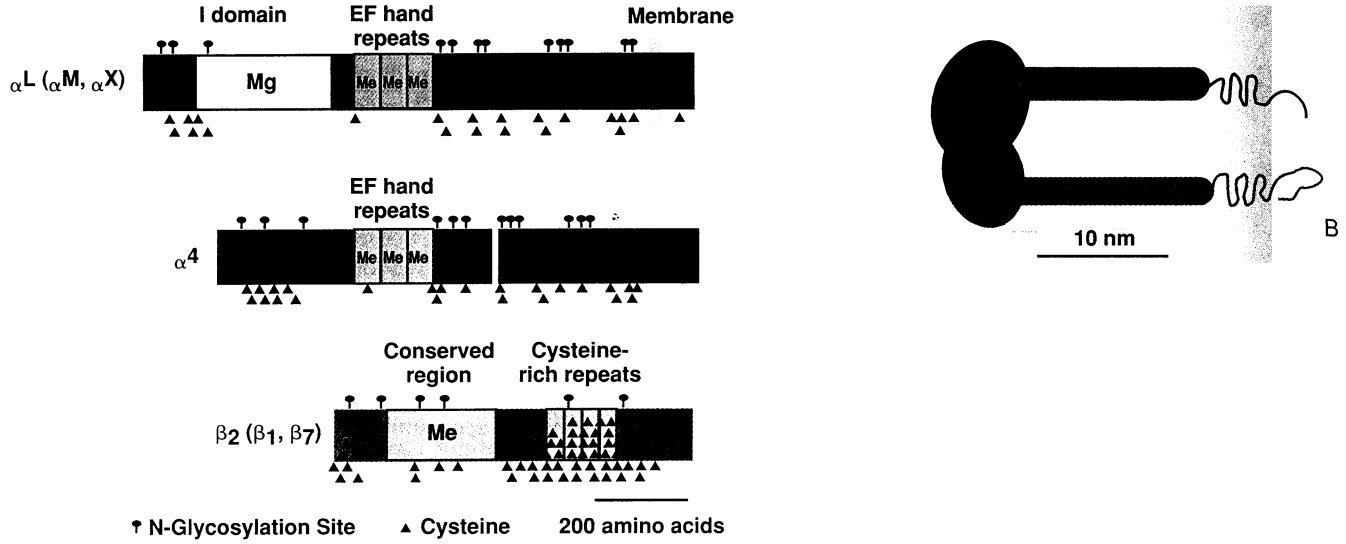


FIG. 4. Integrins that bind endothelial ligands. **A:** Schematics of representative integrin α and β subunits. The structures of α^L (294) and β_2 (295) integrin subunits are shown as representative of α^M and α^X or β_1 and β_7, respectively; cysteines are identical, and glycosylation sites vary but are sparse in the I domain and EF hand repeats. The EF hand repeats are divalent metal-binding motifs that may bind Ca^{2+} or Mg^{2+} (labeled "Me"). A binding site for Mg^{2+} and Mn^{2+} but not Ca^{2+} has been identified in the I domain (110). The α^4 integrin subunit has a posttranslational proteolytic cleavage site (296). A putative divalent cation binding site has been defined in the conserved domain of the integrin β_3 subunit and is shown for β_2 (297). **B:** Scale model of an integrin, based on electron micrographs of the integrins gpIIbIIIa (298) and VLA-5 ($\alpha^5\beta_1$) (299).

Chemoattractants

Chemoattractants are important in activation of integrin adhesiveness and in directing the migration of leukocytes. In chemotaxis, cells move in the direction of increasing concentration of a chemoattractant, which typically is a soluble molecule that can diffuse away from the site of its production, where its concentration is highest (79,80). Leukocytes, which can sense a concentration difference of 1% across their diameter, move steadily in the direction of the chemoattractant. There is much interplay between adhesion molecules and chemoattractants because adhesion to a surface is required to provide the traction necessary for migration directed by chemoattractants, and chemoattractants can activate adhesiveness.

The alternative mechanism to chemotaxis is haptotaxis. In "haptotaxis," cells migrate to the region of highest adhesiveness (81). Thus, on a gradient of an adhesive ligand affixed to the surface of other cells or to the extracellular matrix, and in the absence of a chemotactic gradient, motile cells will tend to accumulate in the region of highest ligand density. Both chemotaxis and haptotaxis can contribute to cell localization, but haptotaxis has yet to be demonstrated in vivo.

Classical leukocyte chemoattractants act broadly, on neutrophils, eosinophils, basophils, and monocytes (Table 2). A recently described family of chemoattractive cytokines, termed chemokines, are 70- to 80-residue polypeptides and have specificity for leukocyte subsets (11,12). Two subfamilies of chemokines have been defined by sequence homology and by the sequence around two cysteine residues (Table 2). The CXC or α chemokines tend to act on neutrophils and nonhematopoietic cells involved in wound healing, whereas the CC or β chemokines tend to act on monocytes and in some cases on eosinophils and lymphocyte subpopulations.

It has long been debated whether chemoattractants can act in the circulation, where they would be rapidly diluted and swept downstream by blood flow. Tethering and rolling of leukocytes through selectins would enhance exposure to chemoattractants by prolonging contact with the vessel wall. However, retention of chemoattractants at their site of production by noncovalent interactions with molecules on the vessel wall and within the inflammatory site may also be important. Heparin-binding sites on chemokines provide a mechanism for retention in the extracellular matrix (82), to enhance concentration gradients, and perhaps to present chemokines on the endothelium to circulating leukocytes (83,84).

Chemoattractant Receptors

Leukocyte chemoattractant receptors have multiple functions. They not only direct migration but also activate integrin adhesiveness and stimulate degranulation, shape change, actin polymerization, and the respiratory burst (85). Chemoattractant receptors are G-protein-coupled receptors that span the membrane seven times. Ligand binding to the

TABLE 2. *Leukocyte chemoattractants*

Chemoattractant	Origin	Responding cells
Classical chemoattractants[a]		
N-Formyl peptides	Bacterial protein processing	Monocyte, neutrophil, eosinophil, basophil
C5a	Complement activation	Monocyte, neutrophil, eosinophil, basophil
Leukotriene B_4	Arachidonate metabolism	Monocyte, neutrophil
Platelet-activating factor (PAF)	Phosphatidylcholine metabolism	Monocyte, neutrophil, eosinophil
CXC chemokines[b]		
IL-8/NAP-1	T lymphocyte, monocyte, endothelial cell, fibroblast, keratinocyte, chondrocyte, mesothelial cell	Neutrophil, basophil
CTAP-III/β-thromboglobulin/ NAP-2	Successive N-terminal cleavage of platelet basic protein released from α-granules	Neutrophil, basophil, fibroblast
gro/MGSA	Fibroblast, melanomas, endothelial cell, monocyte	Neutrophil, melanomas, fibroblast
ENA-78	Epithelium	Neutrophil
CC chemokines[c]		
MCP-1	T lymphocyte, monocyte, fibroblast, endothelial cell, smooth muscle	Monocyte, T lymphocyte subpopulation, basophil
MIP-1α	Monocyte, B and T lymphocyte	Monocyte, T lymphocyte subpopulation, basophil, eosinophil
RANTES	T lymphocyte, platelets	Monocyte, T lymphocyte subpopulation, eosinophil
I-309	T lymphocyte, mast cell	Monocyte

[a] References 80,85.
[b] References 11,12,173,174,255.
[c] References 11,12,84,250–252,255,306–309.

membrane-spanning receptor is coupled to exchange of GTP for GDP bound to the associated G protein heterotrimer and results in activation by the G protein α and $\beta\gamma$ subunits of signaling effectors such as phospholipase $C_{\beta 2}$ (86). This results in release of diacylglycerol and inositol phosphates and mobilization of Ca^{2+}. Neutrophils and lymphocytes express $G\alpha_{i2}$ and $G\alpha_{i3}$ subunits (85,87). The $G\alpha$ subunits of the α_i class are ADP-ribosylated and irreversibly inactivated by pertussis toxin. All of the biological effects of leukocyte chemoattractants are inhibited by pertussis toxin. Coupling through $G\alpha_i$ subunits has been confirmed by reconstitution in transfected cells (86). The lipid mediators LTB_4 and PAF are as active as formylated bacterial peptides, C5a, and IL-8 in stimulating chemotaxis but less active in stimulating the respiratory burst and other functions of neutrophils (85); this correlates with their ability to couple to distinct $G\alpha$ subunits in transfected cells (88).

Cloning of the receptors for formylated bacterial peptides, C5a, and platelet-activating factor (PAF) has shown that they are expressed on both neutrophils and monocytes, whereas the receptor for IL-8 is expressed only on neutrophils (89). The receptor for MCP-1 is expressed on monocytic cells but not on neutrophils (90). Thus, the specificity of chemoattractants is regulated by the cellular distribution of their receptors.

Integrins

Integrins are perhaps the most versatile of the adhesion molecules. Integrin adhesiveness can be rapidly regulated by the cells on which they are expressed. Each integrin contains noncovalently associated α and β subunits with characteristic structural motifs (Fig. 4). Five integrins are important in the interaction of leukocytes with endothelial cells. Their cellular distribution, ligand specificity, and structure are summarized in Table 3 and Fig. 4.

Activation of Integrins

The adhesiveness of LFA-1 and VLA-4 on T lymphocytes is activated by cross-linking of the antigen receptor and other surface molecules (13,20,36). Increased adhesiveness occurs within a few minutes, is not accompanied by any change in quantity of surface expression, and appears to result from both conformational changes that increase affinity for ligand and altered interaction with the cytoskeleton (20,91,92). However, it is unlikely that recognition by T-cell receptors of antigen on endothelial cells (93) is a step in lymphocyte trafficking, because traffic of both lymphocytes that can and cannot recognize specific antigen is increased in antigen-induced inflammation. Although evidence has been presented that binding of neutrophils to selectins can activate adhesiveness of integrins (94), other evidence has failed to confirm this (60,95; T. G. Diacovo and T. A. Springer, *unpublished data*).

Thus far the best candidates for activation of integrin adhesiveness within the vasculature are chemoattractants. Adhesiveness of Mac-1 and LFA-1 on neutrophils and monocytes is activated by N-formylated peptide and IL-8

TABLE 3. *Integrins in leukocyte–endothelial interactions*

Subunits	Names	Distribution	Ligands
Leukocyte integrins[a]			
$\alpha^L\beta_2$	LFA-1, CD11a/CD18	B and T lymphocyte, monocyte, neutrophil	ICAM-1, ICAM-2, ICAM-3
$\alpha^M\beta_2$	Mac-1, CR3, CD11b/CD18	Monocyte, neutrophil	ICAM-1, iC3b, fibrinogen, factor X
$\alpha^x\beta_2$	p150,95, CR4, CD11c/CD18	Monocyte, neutrophil, eosinophil	iC3b, fibrinogen
α^4 Integrins[b]			
$\alpha^4\beta_1$	VLA-4, CD49d/CD29	B and T lymphocyte, monocyte, neural crest-derived cells, fibroblast, muscle	VCAM-1, fibronectin
$\alpha^4\beta_7$	LPAM-1, CD49d/CD$^-$	B and T lymphocyte subpopulations	MAdCAM-1, VCAM-1, fibronectin

[a] References 36,151.
[b] References 36,123,124,131,132,199,310–313.

(96–100). In contrast to LFA-1 on lymphocytes and neutrophils, Mac-1 on neutrophils is increased about tenfold on the surface by chemoattractant-stimulated fusion of secretory granules with the plasma membrane (101); however, this is neither sufficient or necessary for increased adhesiveness (102,103). The transient nature of the activation of integrin adhesiveness (96,104) provides a mechanism for de-adhesion and perhaps for retraction of the trailing edge of a leukocyte from the substrate during cell migration.

Conformational changes in LFA-1 and Mac-1 that are associated with increased adhesiveness are suggested by mAb and Fab that react only with these molecules after cellular activation (105–108). After chemoattractant activation of neutrophils, saturation binding shows that 10% of the surface Mac-1 molecules express an activation epitope, yet mAb to this epitope completely blocks binding to ligands such as ICAM-1 and fibrinogen. This suggests that ligand binding is mediated by a subpopulation of activated Mac-1 molecules (108). The I domain of leukocyte integrins is important in ligand binding (109,110) and expresses activation epitopes (107,108). Recent measurements of the affinity of cell surface LFA-1 for soluble, monomeric ICAM-1 (Table 1) have directly demonstrated that cellular activation increases the affinity of a subpopulation of LFA-1 molecules by approximately 200-fold (111).

Surprisingly, the integrin VLA-4, in contrast to LFA-1 and Mac-1, has recently been found to be capable of supporting rolling. Lymphocytes can tether in flow and subsequently roll on VCAM-1. If activated while rolling by phorbol ester or TS2/16 mAb to the β_1 subunit, the lymphocytes arrest and develop firm adhesion. Activated lymphocytes tether as efficiently as resting lymphocytes but do not roll. Fibronectin can support development of firm adhesion in static conditions but not tethering or rolling in flow. VCAM-1 is less efficient than selectins in mediating tethering and rolling (112).

Immunoglobulin Superfamily Members on Endothelium as Integrin Ligands

In a paradigm first established with ICAM-1 binding to LFA-1, several immunoglobulin superfamily (IgSF) members, expressed on endothelium, bind to integrins expressed on leukocytes (Fig. 5). ICAM-1, ICAM-2, and ICAM-3 are products of distinct and homologous genes and were all initially identified by their ability to interact with LFA-1 (113–115). ICAM-1 has also been found to bind to Mac-1 through a distinct site in its third Ig domain (99,116,117) (Fig. 5). Induction of ICAM-1 on endothelium and other cells by inflammatory cytokines may increase cell–cell in-

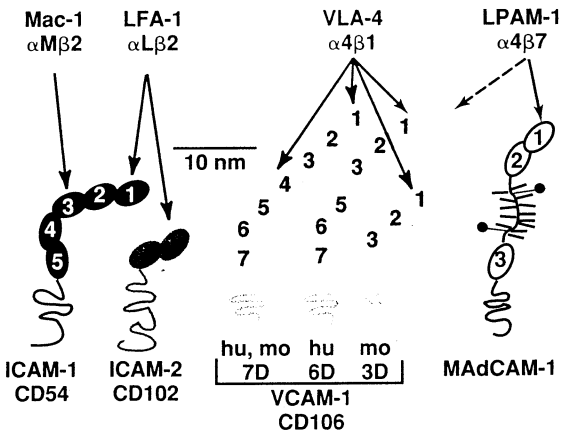


FIG. 5. Immunoglobulin superfamily adhesion receptors on endothelium and their integrin-binding sites. Members of the Ig superfamily share the immunoglobulin domain, composed of 90 to 100 amino acids arranged in a sandwich of two sheets of antiparallel β-strands that is stabilized by one or (in the N-terminal domain of the molecules shown) two disulfide bonds. The immunoglobulins and T-cell receptors are the only known members of this family that undergo somatic diversification. The function of the IgSF in adhesion evolutionarily predates specialization for antigen recognition. The shape and size of the ICAM-1 molecule, with its unpaired Ig domains and bend, were determined by electron microscopy (300,301), as were those of VCAM-1 (128). Immunoglobulin domains are ellipsoids with a length of 4 nm parallel to the β-strands and 2.5 nm in the other dimensions. The mucin-like region of MAdCAM-1 is modeled as described in the legend to Fig. 3; N-linked glycosylation sites in the Ig domains of this and the other molecules are not shown. References for structures (in parentheses) and for localization (in brackets) follow: ICAM-1 (302,303); [117,300]; ICAM-2 (114); VCAM-1 (119,304,305); [125–128]; MAdCAM-1 (130).

teractions and leukocyte extravasation at inflammatory sites, whereas constitutive expression of ICAM-2 may be important for leukocyte trafficking in uninflammed tissues, as in lymphocyte recirculation. ICAM-3 is restricted to leukocytes. All three of the ICAMs contribute to antigen-specific interactions, so that inhibition with mAb to all three is required to completely block LFA-1-dependent antigen-specific T-cell responses (118).

VCAM-1 is inducible by cytokines on endothelial cells and on a more restricted subset of nonvascular cells than ICAM-1 (9). A single VCAM-1 gene gives rise through alternative splicing to a seven-domain isoform and to a second isoform that contains either six domains or three domains and glycosyl phosphatidylinositol membrane anchor (119–121) (Fig. 5). VCAM-1 is a ligand for the integrin $\alpha^4\beta_1$ (VLA-4) and binds weakly to $\alpha^4\beta_7$ (122–124). In contrast to the shorter isoforms, the seven-domain isoform of VCAM-1 has two binding sites for VLA-4, in highly homologous domains 1 and 4 (125–128).

An addressin for lymphocyte recirculation to mucosa is expressed on Peyer's patch HEV and on other venules (129). Now termed mucosal addressin cell adhesion molecule (MAdCAM-1), it contains three Ig-like domains and a mucin-like region interposed between domains 2 and 3 (130) (Fig. 5). The MAdCAM-1 molecule binds the integrin $\alpha^4\beta_7$ but not $\alpha^4\beta_1$ (131,132). Furthermore, carbohydrates attached to the mucin-like domain of MAdCAM-1 bind L-selectin and mediate lymphocyte rolling (133). Thus, MAdCAM-1 has a dual function as an integrin and selectin ligand.

Other Molecules

CD31 is an IgSF member expressed on leukocytes, platelets, and at cell–cell junctions on endothelium (134–140). CD31 can bind homophilically to itself and also heterophilically to an uncharacterized counterreceptor. The mAb cross-linking of CD31, similarly to many but not all other lymphocyte surface molecules, can trigger integrin adhesiveness (140). Interaction between CD31 on endothelial junctions and CD31 on leukocytes appears to be required for transmigration but not for integrin-mediated binding of leukocytes to endothelium (141). CD31–CD31 interaction may represent a fourth step in transendothelial migration that overlaps the integrin-mediated step and may contribute to the maintenance of the permeability barrier function of endothelia during transmigration.

CD44 is a widely distributed molecule in the body that is homologous with cartilage link protein, is extensively alternatively spliced, and can bear heparin sulfate or chondroitin sulfate side chains (142). The best understood function of CD44 is as a major surface receptor for hyaluronate (143,144). Alternatively spliced forms of CD44 are important in tumor metastasis (145) and in localization of antibody-secreting cells (146). CD44 (H-CAM, Hermes) was at one time mistakenly thought to be the human equivalent of murine mel-14 (L-selectin). It participates in vitro in lymphocyte interaction with HEV and activated endothelium (147,148). However, lack of cell surface CD44 has no effect on lymphocyte recirculation in vivo (149).

TOWARD A MULTISTEP MODEL OF NEUTROPHIL EMIGRATION IN INFLAMMATION

Integrins and Selectins

Patients who are genetically deficient in the leukocyte integrins because of mutations in the common β_2 integrin CD18 subunit provided early evidence that adhesion molecules were required for leukocyte extravasation in vivo (150,151). Leukocyte adhesion deficiency I (LAD-I) patients have life-threatening bacterial infections, and neutrophils in these patients fail to cross the endothelium and accumulate at inflammatory sites despite higher than normal levels of neutrophils in the circulation. In vitro, LAD-I neutrophils or normal neutrophils treated with mAb to the leukocyte integrins are deficient in binding to and migrating across resting or activated endothelial monolayers (152,153). Even though capable of binding to activated endothelium through selectins, LAD-I neutrophils fail to transmigrate (153). Monoclonal antibodies to the leukocyte integrin β_2 subunit, and in some cases the integrin α^M subunit, have been found to have profound effects in vivo (15). These mAb prevent the neutrophil-mediated injury that occurs when ischemic tissue is reperfused and thus can prevent death from shock after blood loss, limb necrosis after frostbite or after amputation and replantation, and tissue necrosis from myocardial ischemia and reperfusion. Monoclonal Abs to leukocyte integrins and to ICAM-1 can also inhibit lymphocyte- and monocyte-mediated antigen-specific responses in vivo, including delayed-type hypersensitivity, granuloma formation, and allograft rejection (15).

Whereas mAb to the leukocyte integrin β_2 subunit blocked accumulation of leukocytes in tissue in response to chemoattractants, and stable adhesion of leukocytes in the local vasculature, it had no effect on the number of rolling leukocytes on the vessel wall (154). Furthermore, leukocyte integrins were found to mediate binding of neutrophils to endothelial monolayers in a parallel wall flow chamber at subphysiological but not at physiological shear stresses found in postcapillary venules (153,155).

Parallel studies showed that selectins were required for leukocyte accumulation in vivo and acted at an early step. Antagonists of L-selectin and E-selectin inhibit neutrophil and monocyte influx into skin, peritoneal cavity, and lung in response to inflammatory agents (40,156–159). Monoclonal Ab to L-selectin was shown to inhibit neutrophil accumulation on cytokine-stimulated endothelium at physiological shear stress (100). Stimulation of neutrophils with chemoattractants results within minutes in shedding into the medium of L-selectin, with kinetics similar to up-regulation of sur-

face expression of the integrin Mac-1. Based on this, and the evidence reviewed above, it was hypothesized that selectins might act at a step prior to integrins (160).

Further studies showed that selectins mediate rolling, and function before development of firm adhesion through integrins. At sites of inflammation, leukocytes first attach to the vessel wall in a rolling interaction, then become arrested or firmly adherent at a single location on the vessel wall before diapedesis (161). This process was fully reconstituted with purified components of the endothelial surface (57). At physiological shear stresses, neutrophils attach to and form labile rolling adhesions on phospholipid bilayers containing purified P-selectin but not on bilayers containing ICAM-1. Chemoattractants stimulate strong, integrin-mediated adhesion to bilayers containing ICAM-1 under static conditions but not in shear flow. At physiological shear stresses, if both P-selectin and ICAM-1 are present in the phospholipid bilayer, resting neutrophils attach and roll identically as on bilayers containing P-selectin alone. However, when chemoattractant is added to the buffer flowing through the chamber, the rolling neutrophils arrest, spread, and firmly adhere through the integrin–ICAM-1 interaction. Chemoattractant does not enhance and actually inhibits interactions of neutrophils with bilayers containing P-selectin alone. These findings show that purified adhesion molecules and chemoattractants representing the endothelial signals can reproduce the key events in leukocyte localization in vivo and prove that the selectin-mediated step is a prerequisite for the chemoattractant- and integrin-mediated steps (57). Complementary studies in vivo showed that mAb to L-selectin, or L-selectin/IgG chimeras, decreased both the number of rolling leukocytes (58,59) and the number of leukocytes that subsequently became firmly adherent, whereas mAb to the β_2 integrin subunit only decreased firm adherence of leukocytes. This suggested that L-selectin acts at a step prior to leukocyte integrins (59). In static assays, a factor derived from cytokine-stimulated endothelium induced shedding of L-selectin and, if transmigration was blocked with CD18 mAb, induced release of neutrophils from inverted endothelial monolayers. This also suggested that L-selectin acted before leukocyte integrin-mediated emigration (100). In elegant confirmation of a three-step model in a static assay of neutrophil adhesion to histamine-stimulated endothelium, juxtacrine cooperation between P-selectin and platelet-activating factor (PAF) was found (95). P-selectin tethered neutrophils to endothelium and thereby augmented stimulation by PAF of CD18-dependent neutrophil adhesion. Stimulation of adhesiveness was by PAF and not by P-selectin, as shown with PAF receptor antagonists.

The requirement for the carbohydrate ligands of selectins for leukocyte emigration in vivo has received strong support from studies of two patients with a genetic defect in biosynthesis of fucose and who therefore lack the ligands for E-selectin and P-selectin (162,163). The defect, designated LAD-II, has many clinical similarities to LAD-I, including strikingly depressed neutrophil emigration into inflammatory sites.

Chemoattractants

Chemoattractants appear to be required for transendothelial migration in vitro and in vivo and can induce all steps required for transmigration in vivo. Injection of chemoattractants into skin or muscle leads to robust emigration of neutrophils from the vasculature and accumulation at the injection site (164). Injection of lipopolysaccharide or cytokines that induce IL-8 synthesis also elicits neutrophil emigration. Moreover, mAb to IL-8 markedly inhibits neutrophil emigration into lung and skin in several models of inflammation (165,166).

The effect of pertussis toxin provides further evidence for the importance of $G\alpha_i$-protein-coupled receptors in leukocyte emigration in vivo. Pretreatment of neutrophils with pertussis toxin inhibits emigration into inflammatory sites (167,168).

Chemoattractants impart directionality to leukocyte migration. By contrast to intradermal injection, intravascular injection of IL-8 does not lead to emigration (169). Cytokine-stimulated endothelial monolayers grown on filters secrete IL-8 into the underlying collagen layer. Neutrophils added to the apical compartment emigrate into the basilar compartment, but not when the IL-8 gradient is disrupted by addition of IL-8 to the apical compartment (82). Although IL-8 acts as an adhesion inhibitor in some assays (170), this result may be partially attributable to disruption of a gradient of IL-8 on activated endothelial monolayers when exogenous IL-8 is added on the same side as the neutrophils.

Chemoattractants act on the local tissue as well as on leukocytes. Neutrophil chemoattractants injected into the same skin site hours apart will stimulate neutrophil accumulation the first but not the second time, whereas a second injection into a distant site will stimulate accumulation. Desensitization occurs for homologous chemoattractants only (171,172). Thus, chemoattractants must act on and homologously desensitize a cell type that is localized in tissue. In some cases this localized cell may be the mast cell. Some chemoattractants stimulate the mast cell (which localizes in tissue adjacent to the vasculature) or its better-studied relative, the basophil, to release histamine (173,174) and TNF (175). Histamine induces P-selectin, and TNF induces E-selectin on endothelium. Thus, chemoattractants may indirectly increase selectin expression on endothelium as well as directly activate integrin adhesiveness on leukocytes.

A THREE-STEP AREA CODE FOR SIGNALING NEUTROPHIL AND MONOCYTE TRAFFIC

The above evidence has shown that emigration from the vasculature of neutrophils and monocytes is regulated by at least three distinct molecular signals (Figs. 1 and 6A). A

key feature is that selectin–carbohydrate, chemoattractant–receptor, and integrin–Ig family interactions act in sequence, not in parallel. This concept has been confirmed by the observation that inhibition of any one of these steps gives essentially complete, rather than partial inhibition of neutrophil and monocyte emigration. An important consequence of a sequence of steps, at any one of which there are choices of multiple receptors or ligands that have distinct distributions on leukocyte subpopulations or endothelium, is that it provides great combinatorial diversity for regulating the selectivity of leukocyte localization in vivo, as has been emphasized in several reviews (3,7,10,13,14).

"Area code" models for cell localization in the body (176,177) are particularly apt, because it is now known that at least three sequential steps are involved. The concepts of area codes and traffic signals can be combined by thinking of how telephone traffic is routed by digital signals. Each type of leukocyte responds to a particular set of area code signals. Inflammation alters the expression and location of the signals on vascular endothelium. It is as if leukocytes carry "cellular phones." An example of how this model works is shown in Fig. 6B for the two cell types for which the signals are best understood, neutrophils and monocytes. Chemoattractants provide the greatest number of molecular choices (or "digits") and the greatest cellular specificity.

Refinements to the three-step model are in order. First, selectins actually mediate two steps, initial tethering to the vessel wall and rolling (Fig. 6A), which can be distinguished for E-selectin by dependence on different classes of neutrophil ligands (69). Thus, selectins can cooperate, and some selectin–ligand combinations may be more important in tethering and others in rolling. Second, the steps are overlapping rather than strictly sequential (Fig. 6A). Although L-selectin is shed from neutrophils soon after activation (160), the kinetics of shedding by neutrophils in whole blood (minutes) are much slower than the transition from rolling to integrin-mediated attachment (milliseconds to seconds) (59). L-Selectin is shed more slowly from lymphocytes than from neutrophils (178,179). Furthermore, ligands for P-selectin (46) and E-selectin (69) remain on the neutrophil surface after activation. Thus, interactions with selectins will continue after activation of integrins, probably persisting until transendothelial migration is completed. Chemoattractants are required not only for activation of integrin adhesiveness but also for directional cues during the subsequent step of transendothelial migration. Finally, β_1 integrins that bind to extracellular matrix components are undoubtedly required during migration through the subendothelial basement membrane.

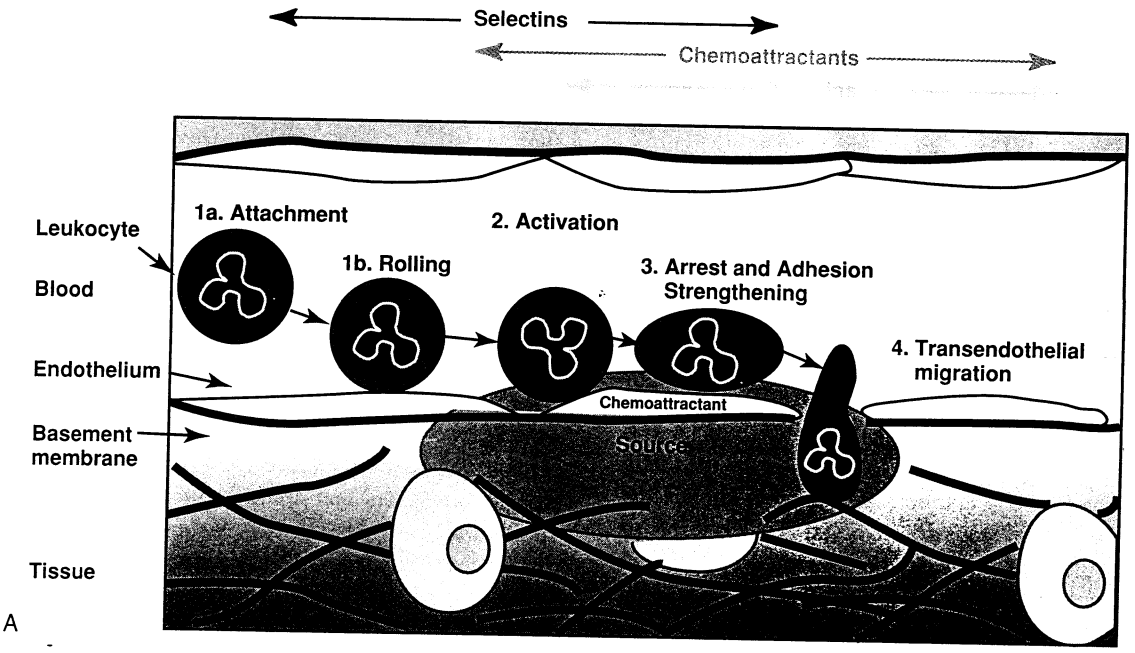
LYMPHOCYTE RECIRCULATION: DISTINCT TRAFFIC PATTERNS FOR NAIVE AND MEMORY LYMPHOCYTES

Patrolling the body in search of foreign antigen, lymphocytes follow circuits through both nonlymphoid and lymphoid tissues (Fig. 7). The peripheral lymph nodes draining skin and muscle, and the gut-associated lymphoid tissues such as Peyer's patch, differ in the types of antigens to which lymphocytes are exposed. When collected from lymph draining gut or skin, lymphocytes from adult animals, but not newborns, show a twofold or higher preference to recirculate to the type of organ from which they came and to reappear in the draining lymph (1,2,17,180). This suggests that priming by specific antigen in a particular environment may induce expression of surface receptors that enable preferential recirculation to the type of secondary organ where specific antigen was first encountered. Evidence exists for separate streams of lymphocytes that recirculate through the skin, gut, and lung and that drain into their associated lymphoid tissues (6,17).

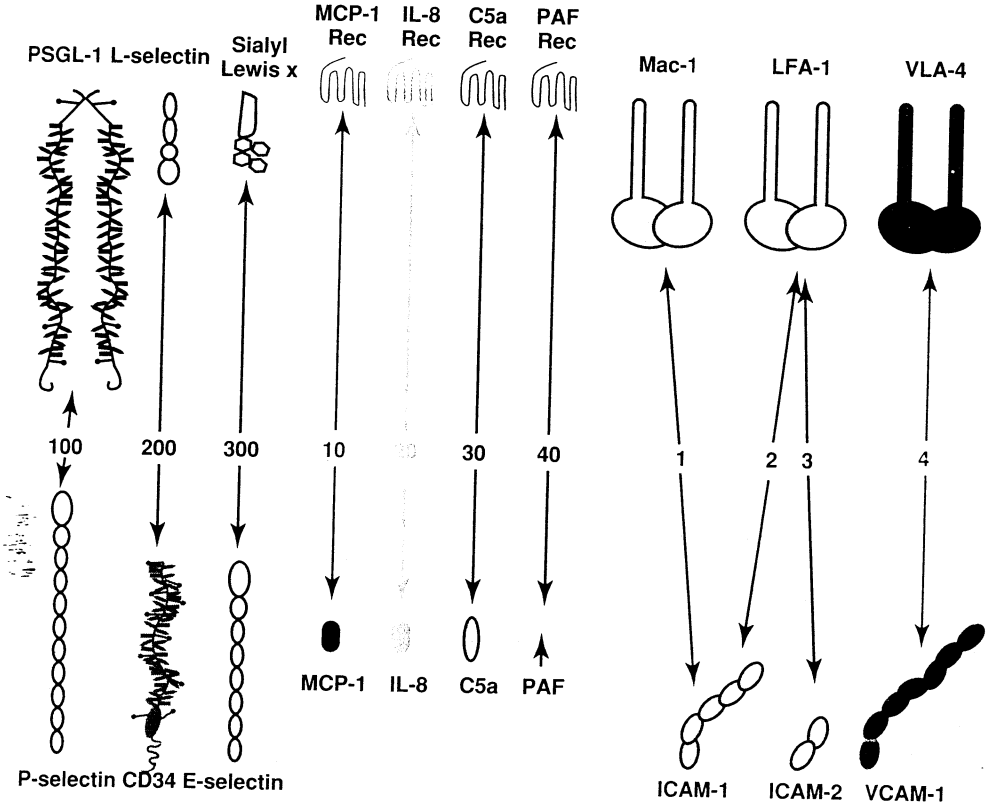
Our understanding of the mechanisms of this selectivity has been advanced by the discovery that "naive" and "memory" lymphocytes prefer different recirculation pathways (181). When naive lymphocytes encounter antigen, those lymphocytes with receptors specific for the antigen are stimulated to expand clonally and are converted to memory lymphocytes that have altered expression of adhesion receptors and circulatory patterns. Lymphocytes that emigrate in the hind leg of a sheep through "flat" endothelium in the skin and drain through the afferent lymphatics to the popliteal lymph node are all of the memory phenotype. By contrast, lymphocytes in the efferent lymph from the popliteal lymph node, derived mostly from traffic through HEV, are predominantly of the naive phenotype. Thus, at least for peripheral tissues and lymph nodes, memory lymphocytes emigrate preferentially through tissue endothelium, whereas naive lymphocytes enter the lymph node through HEV (Fig. 7). Memory lymphocytes are more sensitive to specific antigen than naive lymphocytes and thus are better able to respond to antigen in peripheral tissues, which have fewer antigen-presenting cell than lymph nodes (16).

TRAFFIC THROUGH HEV

The "high" or cuboidal-shaped endothelial cells found in HEV are specialized for emigration of lymphocytes into peripheral lymph nodes that drain skin and the lymphoid tissues of the mucosa: Peyer's patches, tonsils, and appendix. Emigration into the spleen, by contrast, involves sinusoidal endothelia and molecular mechanisms that are distinct and not yet characterized. About 25% of lymphocytes that circulate through a HEV will bind and emigrate, a much higher percentage than through nonspecialized flat venules (182,183). The HEV phenotype is developmentally regulated. The carbohydrate ligands for L-selectin are absent from peripheral lymph node HEV at birth but are displayed at adult levels by 6 weeks (6). If peripheral lymph nodes are deprived of afferent lymph, the HEV convert from a high to a flat-walled endothelial morphology, lose expression of



A



	Monocyte Area Codes	111, 211, 311, 112, 212, 312, 113, 213, 313, 114, 214, 314, 134, 234, 334, 144, 244, 344
	Neutrophil Area Codes	121, 221, 321, 122, 222, 322, 123, 223, 323
	Monocyte and Neutrophil Area Codes	131, 231, 331, 132, 232, 332, 133, 233, 333, 141, 241, 341, 142, 242, 342, 143, 243, 343
B	Null Area Codes	124, 224, 324

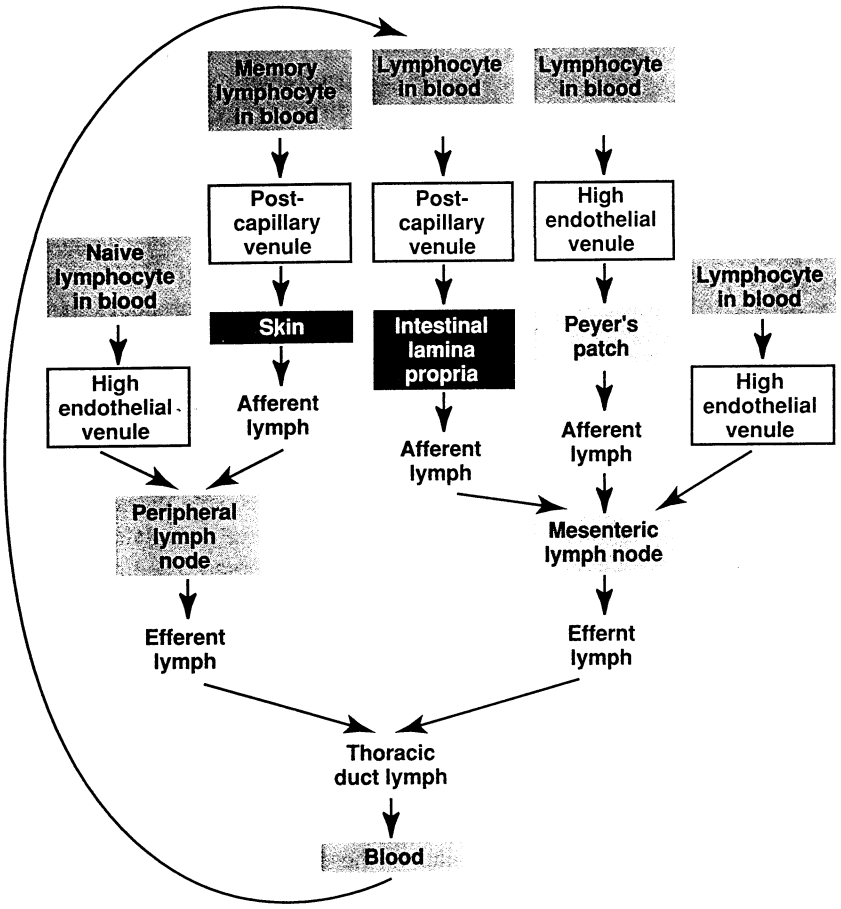


FIG. 7. Lymphocyte recirculation routes.

L-selectin ligands, and lose ability to support lymphocyte traffic (184,185). Introduction of antigen into the node leads to a full restoration of HEV phenotype and function. Furthermore, intense antigenic stimulation can induce formation of HEV in diverse nonlymphoid tissues (6,186).

Molecular Mechanisms Defined by the HEV Binding Assay

When lymphocyte suspensions are overlaid on thin sections cut from frozen lymph nodes, the lymphocytes specifically bind to the morphologically distinct HEV (187). Specific differences have been demonstrated between binding to peripheral lymph node and Peyer's patch HEV (183,188). T lymphocytes bind 1.5-fold better than B lymphocytes to peripheral lymph node HEV in vitro and show a similar preference to recirculate to this site in vivo. B lymphocytes bind two- to threefold better to Peyer's patch than to peripheral lymph node HEV and show similar preference in recirculation in vivo. These preferences are reflected in the preponderance of T cells in peripheral lymph nodes and the preponderance of B lymphocytes in Peyer's patch, where they are important in secretion of IgA and IgM into the mucosa (189). Certain lymphoma cells possess marked preference for binding to Peyer's patch or peripheral lymph node HEV in vitro (188) and for metastasis in vivo to mucosal or peripheral lymphoid tissue, respectively (190). Assay of lymphoma cell binding to HEV in the Stamper–Woodruff assay has led to the identification of two important adhesion pathways.

FIG. 6. The three-step area code model. **A:** Selectins, chemoattractants, and integrins act in sequence, with some overlap. **B:** Combinatorial use of different molecules at each step can generate a large number of different area codes and specificity for distinct leukocyte subpopulations. All of the known selectin and integrin interactions are shown in the hundreds and ones places, respectively; however, only a subset of the chemoattractants is shown in the tens place (see Table 1) because of space limitations. The area codes symbolize how specificity for monocytes, neutrophils, or both can be generated at inflammatory sites. Interactions that are monocyte or neutrophil specific are shown in dark red and light red, respectively.

Molecules Involved in Binding to Peripheral Node HEV

The L-selectin molecule was initially defined in the mouse with the Mel-14 mAb as a molecule on lymphocytes required for binding to peripheral lymph node, but not Peyer's patch, HEV (38). Conversely, the MECA-79 carbohydrate antigen was defined with mAb that bound specifically to peripheral lymph node HEV and blocked lymphocyte binding. The isolated MECA-79 antigen, termed the peripheral node addressin (191), bind to L-selectin on lymphocytes (192). An L-selectin/IgG chimera was also found to bind specifically to HEV in peripheral lymph node and to block lymphocyte binding (193). The L-selectin/IgG chimera was used to isolate two distinct mucin-like ligands, GlyCAM-1, which is secreted by HEV (37), and CD34, a surface molecule on HEV (50). MECA-79 mAb recognizes a carbohydrate determinant that is expressed on multiple protein species in HEV, including GlyCAM-1 and CD34, and compared to L-selectin recognizes an overlapping but distinct set of glycoproteins (37,192). Sialylation and sulfation of the O-linked side chains of the GlyCAM-1 and CD34 molecules are required for activity in binding to L-selectin (19,192,194). The HEV differ from other tissues in carbohydrate processing; GlyCAM-1 and CD34 expressed in transfectants and CD34 in other vascular endothelia do not bind L-selectin chimera under conditions in which binding to HEV is detectable (37). However, an L-selectin ligand with a presumably lower affinity is certainly present on most endothelia, as shown by L-selectin-dependent rolling in vivo and binding in vitro (58,59,68,74,100,195,196).

Molecules Involved in Binding to Peyer's Patch HEV

Elegant screens for mAb with specificity for Peyer's patch HEV and ability to block lymphocyte binding to HEV yielded mAb MECA-367 to the mucosal addressin now termed MAdCAM-1 (129). The MAdCAM-1 molecule is expressed on endothelia in mucosal tissues not only on HEV in Peyer's patch but also on venules in intestinal lamina propria and in the lactating mammary gland (129,197). The MAdCAM-1 molecule has both IgSF domains and a mucin-like domain (130) (Fig. 5).

Similar elegant screens for mAbs with specificity for lymphoma cells that bound to Peyer's patch HEV and with ability to block binding to HEV in the Stamper–Woodruff assay yielded mAbs to the α^4 subunit of the Peyer's patch homing receptor (198). The α^4 subunit was found to be associated with a novel β subunit, β_p (199), which is identical to β_7 (131). The integrin $\alpha^4\beta_7$ but not $\alpha^4\beta_1$ binds to Peyer's patch HEV (131), and $\alpha^4\beta_7$ binds directly to MAdCAM-1 (132).

An Area Code Model for Lymphocyte Migration Through HEV

Peripheral Lymph Node HEV

Although the L-selectin–mucin and $\alpha^4\beta_7$–MAdCAM-1 interactions were identified in parallel assays, recent studies suggest that multiple steps are involved in lymphocyte interaction with HEV and raise the possibility that these interactions may function in distinct, rather than parallel, steps in this process. Soon after its discovery as a lymphocyte homing receptor, L-selectin also was found to be present on neutrophils and eosinophils and to be important in emigration of at least neutrophils (40). As expected from their strong expression of L-selectin, neutrophils and other leukocytes can bind avidly to HEV in the Stamper–Woodruff assay yet do not normally home to peripheral lymph nodes in vivo. Injection of *E. coli* supernatant induces acute emigration of neutrophils through HEV of the draining lymph node. Thus, signals other than those mediated by L-selectin can regulate the class of leukocytes that home into a lymph node (40). Although peripheral node HEV is far richer than any other site in the body in expression of the carbohydrate receptor for L-selectin (200), this is insufficient to explain the specificity of lymphocyte homing to this organ. The findings suggest that L-selectin is required for lymphocyte emigration through peripheral lymph node HEV and may help regulate recirculation of the L-selectin$^+$ subset of lymphocytes; however, L-selectin is insufficient to determine the specificity of the cell types that emigrate, and other, currently undefined molecules are required to achieve specificity.

In vivo studies strongly suggest that lymphocyte emigration through HEV is a multistep process that utilizes area code models similar to those of other leukocytes. Monoclonal Ab to L-selectin almost completely blocks emigration of lymphocytes from blood into peripheral lymph nodes (38,201). However, mAb to the integrin LFA-1 also markedly reduces or almost completely abolishes lymphocyte migration into peripheral lymph nodes (149,202). Thus, molecules of steps 1 and 3 are required for homing to peripheral lymph nodes in vivo. LFA-1 on blood lymphocytes requires activation for binding to its counterstructures ICAM-1 and ICAM-2 (36,104), which are expressed on HEV (203). Binding of L-selectin does not trigger activation of LFA-1 because lymphocytes attach and roll in flow identically on purified peripheral node addressin whether or not purified ICAM-1 is present on the substrate; an additional stimulus is required before lymphocytes will arrest and strengthen adhesion through LFA-1 (316).

G-protein-coupled receptors are required for lymphocyte recirculation and likely provide the signals required to activate the adhesiveness of LFA-1. Pertussis toxin causes lymphocytosis and profoundly depresses lymphocyte recirculation (204). Murine lymphocytes treated with pertussis toxin in vitro and reinfused fail to emigrate into either peripheral lymph nodes or Peyer's patches (205). This suggests that G-protein-coupled receptors of the α_i class are required for lymphocyte emigration through HEV. Results with mice with a transgene for the ADP-ribosylating subunit of pertussis toxin selectively expressed in the T lineage suggest that $G\alpha_i$ proteins are required not only for emigration from the bloodstream but also for emigration from the thymus

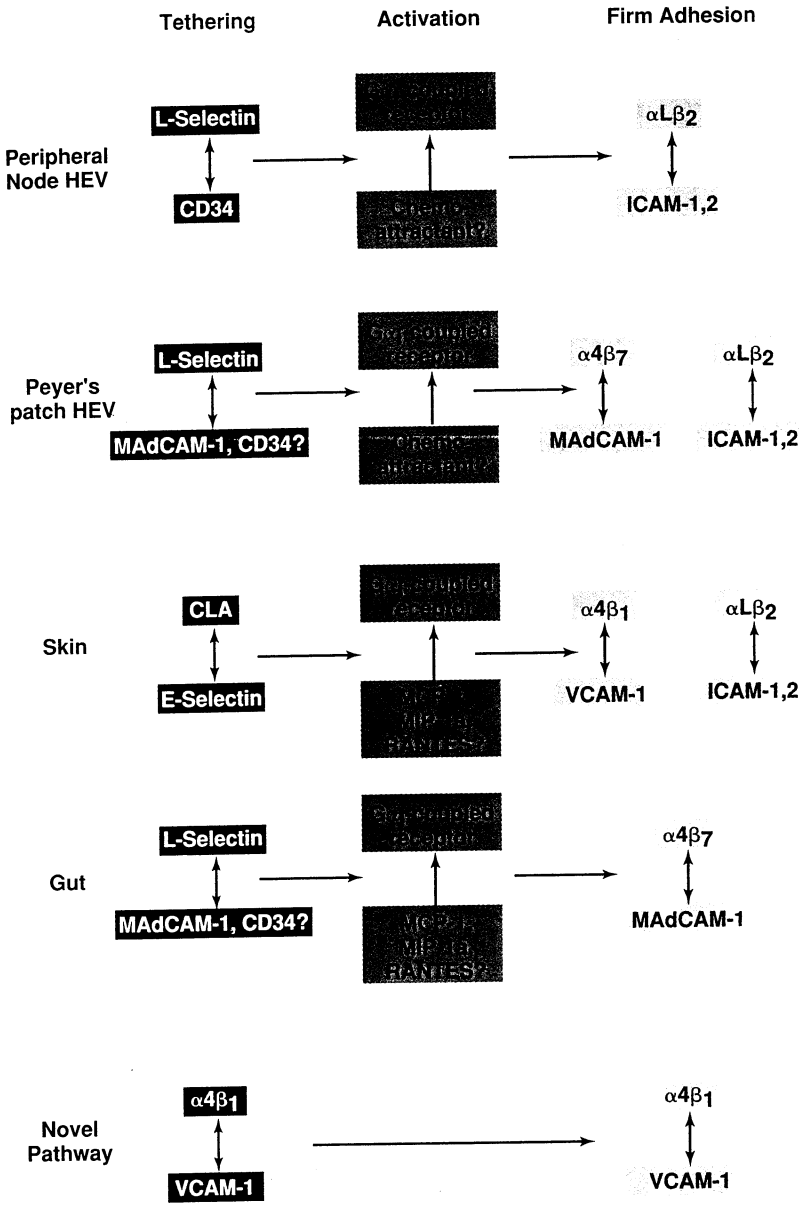


FIG. 8. The three-step or four-step area code paradigm for lymphocytes. For skin and gut, the pathways shown may mediate both recirculation and increased accumulation in inflammation. The novel pathway shown at the bottom may be important when VCAM-1 expression on endothelium is induced by cytokines and may cooperate with the other illustrated pathways. For each organ, the interacting molecules are shown on the top for lymphocytes and on the bottom for endothelia. See text for support for the molecular assignments at each step, based primarily on in vivo data.

(206,207). Despite lack of emigration, pertussis-toxin-treated lymphocytes bind normally to lymph node HEV in vitro. These findings provided the basis for an early proposal for a two-step model in which G-protein-coupled receptors function subsequent to binding of lymphocytes to HEV (208).

Thus, emigration of lymphocytes through peripheral node HEV requires three sequential area code signals that are analogous to those involved in neutrophil emigration from the bloodstream (Fig. 8). Identification of a putative lymphocyte chemoattractant secreted by peripheral lymph node HEV, and a chemoattractant receptor that is predicted to be selectively expressed on the naive subset of lymphocytes that recirculate through peripheral node HEV, will be a subject of intense research interest in coming years.

Peyer's Patch

Monoclonal antibodies to L-selectin block 50% of lymphocyte emigration from blood to Peyer's patch and to the remainder of the intestine (201,209). This is consistent with the lower level of L-selectin ligand in Peyer's patch HEV than in peripheral lymph node HEV (193,210,211). Monoclonal antibodies to certain epitopes on the integrin α^4 and β_7 subunits inhibit by approximately 50% recirculation of lymphocytes to Peyer's patch and intestine but have no effect on recirculation to peripheral lymph node; furthermore, mAb specific for the $\alpha^4\beta_7$ complex are equally effective as mAb to α^4 (209). Moreover, recirculation is inhibited by mAb to MAdCAM-1 (129), implicating $\alpha^4\beta_7$ binding to MAdCAM-1 in recirculation to mucosal tissue. The mAbs to LFA-1

block recirculation to Peyer's patch by 50% to 80% but have no effect on recirculation to the remainder of the intestine (149,202). Thus, both LFA-1 and $\alpha^4\beta_7$ contribute to emigration into mucosal lymphoid tissue.

G-protein-coupled receptors act subsequent to a rolling interaction in Peyer's patch HEV. In contrast with peripheral lymph nodes, Peyer's patches may be visualized by intravital microscopy (182). Normally, lymphocytes roll along Peyer's patch HEV only for a few seconds, then arrest and emigrate. However, prior treatment of lymphocytes with pertussis toxin completely blocks arrest and emigration and prolongs the rolling indefinitely, so that the lymphocytes pass out of the Peyer's patch rather than emigrate (212). It remains to be established, but seems likely, that a chemoattractant presented or secreted by Peyer's patch binds to a $G\alpha_i$-coupled receptor on lymphocytes and activates LFA-1 and $\alpha^4\beta_7$ to mediate arrest and emigration (Fig. 8). Lymphoma cells or lymph node lymphocytes can bind to Peyer's patch HEV or purified MAdCAM-1 without any apparent need for activation; however, activation increases the strength of binding to MAdCAM-1 (131,132). The pertussis toxin studies suggest that activation of blood lymphocytes is required for the last step of arrest and emigration (208,212). Truncation of the cytoplasmic domain of β_7 greatly decreases binding to HEV. Thus, interactions with the cytoplasmic domain can regulate the avidity of $\alpha^4\beta_7$ for MAdCAM-1 (213), similar to regulation of the avidity of LFA-1 for ICAM-1 by the β_2 integrin subunit cytoplasmic domain (214,215).

RECIRCULATION OF MEMORY LYMPHOCYTES

Distinct Pathways Through Skin and Gut

Memory lymphocytes are imprinted so that they are more likely to return to the type of tissue, such as skin or mucosa, where they first encountered antigen (1,2,17). The surface phenotypes of gut- and skin-homing memory cells are distinct (211). Furthermore, staining of lymphocytes in sections of skin and gut with mAb shows distinctive expression of adhesion molecules that may contribute to selective extravasation in these tissues or to subsequent localization within these tissues in specific anatomic compartments (Table 4).

Skin-Homing Lymphocytes

Lymphocytes that extravasate in the skin and appear in afferent lymph have a distinct pattern of expression of adhesion molecules (211) (Table 4). Furthermore, as shown by staining of tissue sections, T lymphocytes localized in the skin but not in the gut express a carbohydrate termed cutaneous lymphocyte-associated antigen (CLA) (216). The CLA antigen is closely related to sialyl Lewis^a and Lewis^x (217) and is a ligand for E-selectin (218). Binding of a subpopulation of memory lymphocytes that bears CLA to E-selectin may contribute to the tropism of this subset to the skin (219–221). E-selectin is induced on dermal endothelial cells in delayed type hypersensitivity (222) and in chronically inflamed skin (220). Cloned T cells derived from challenged skin express high levels of CLA and bind to E-selectin, whereas T cell clones derived from blood lymphocytes do not (223). Both types of clones bind to P-selectin.

TABLE 4. *Naive and memory lymphocyte subsets*[a]

Molecule	Naive lymphocytes	Memory lymphocytes
CD45R0	Negative	Positive
CD45RA	High	Low
CD2	Low	High
LFA-3	Negative	Positive
L-selectin	Positive	Positive and negative subsets
α^4	Low	High

	Gut associated	Skin associated
Memory lymphocyte subsets		
CLA	Negative	Positive
$\alpha^E\beta_7$ (HML-1)	Positive	Negative
$\alpha^4\beta_7$[b]	High	Low
$\alpha^4\beta_1$	Low	High
α^6	Low	High

[a] References 211,224,313.
[b] But see ref. 314.

Gut-Homing Lymphocytes

The most organized lymphoid structures in the wall of the gut are the Peyer's patches, which underlie follicle-associated epithelia that contain M cells, which are specialized for uptake of antigen from the gut lumen. Other lymphocytes localize more diffusely in the lamina propria underlying the digestive epithelium and in the epithelial layer. Studies on gut afferent lymph reveal the presence of both memory and naive lymphocytes (211); whether there is differential migration of naive and memory lymphocytes through Peyer's patch HEV and lamina propria postcapillary venules, both of which contribute to gut afferent lymph (Fig. 7), remains unclear. Gut-homing memory lymphocytes display a surface phenotype distinct from skin-homing lymphocytes (Table 4). When injected into the bloodstream, memory lymphocytes from gut afferent lymph display a strong preference to return to gut afferent lymph, whereas naive lymphocytes redistribute randomly (211). Gut afferent memory lymphocytes display an α^4-high, β_1-integrin-low phenotype, suggesting they are $\alpha^4\beta_7^+$ (211) in common with a subpopulation of memory lymphocytes in blood (224). Expression of MAdCAM-1 on both Peyer's patch HEV and postcapillary venules in lamina propria (129), and 50% inhibition by mAb to α^4 and β_7 of migration into both Peyer's patch and intestine (209), suggest a role for $\alpha^4\beta_7$ interaction with MAdCAM-1 in both sites.

A subpopulation of gut lymphocytes distinct from those in lamina propria localize within the epithelium on the external surface of the basement membrane and express the human mucosal lymphocyte (HML-1) integrin $\alpha^E\beta_7$ (224–227). The α^E integrin subunit contains an I domain and a novel proteolytic cleavage site preceded by a stretch of acidic residues, just N-terminal to the I domain (228). Binding of intraepithelial lymphocytes (IEL) to epithelial cell monolayers in vitro is inhibited by mAb to α^E, suggesting that $\alpha^E\beta_7$ may help mediate localization of IEL in epithelia in vivo (229). Intraepithelial T lymphocytes may undergo thymus-independent differentiation in situ, and their recirculation pattern is undefined. The HML-1 integrin is expressed on a subpopulation of 2% to 6% of blood T cells, which are in the memory subset and are CLA^- and L-selectin$^-$ (230). Transforming growth factor β (TGF-β) together with mitogen induces expression of HML-1 on peripheral T cells and increases expression on IEL (226,227). The TGF-β also induces switching of B lymphocytes to production of the IgA class of immunoglobulin (231) the predominant class secreted in the mucosa. These dual effects on differentiation of mucosal lymphocytes suggest the possibility that TGF-β may be an environment-specific cytokine that imprints lymphocytes, when first exposed to antigen, to recirculate selectively to the gut.

ALTERATION OF LYMPHOCYTE TRAFFICKING IN INFLAMMATION

Antigen injected into the tissue of sensitized individuals induces localized accumulation of lymphocytes. These lymphocytes, and those accumulating in tissues in autoimmune disease, are almost all memory cells (232,233). The phenotype of these cells is quite similar to that of lymphocytes trafficking through these sites under basal conditions. This suggests that the signals for lymphocyte trafficking may be qualitatively the same in the basal and inflammatory states and are up-regulated in inflammation. Accumulation of lymphocytes induced by specific antigen or by injection of IFN-γ or TNF-α is significantly inhibited by mAb to either the LFA-1α or the integrin α^4 subunit (234–238). A combination of mAb to LFA-1 and α^4 gives almost complete inhibition of lymphocyte emigration and the resulting induration and plasma leakage (239). Monoclonal Abs to E-selectin and VCAM-1 also inhibit lymphocyte accumulation in delayed-type hypersensitivity in skin (240). Multiple signals are thus required for augmented trafficking of lymphocytes into skin in inflammation (Fig. 8). Both antigen-responsive and -nonresponsive lymphocytes traffic into sites of antigenic stimulation (241). Antigen-specific lymphocytes may accumulate in the site because stimulation through their antigen receptors increases adhesiveness of integrins and causes them to be retained, whereas nonresponsive lymphocytes more rapidly enter the lymphatics and leave the site.

The interaction between VCAM-1 and VLA-4 can mediate both rolling and firm adhesion (112); thus, it does not fit neatly into the three-step paradigm established for neutrophils. The mAbs to LFA-1 or VLA-4 alone do not completely inhibit lymphocyte accumulation in inflammation, and patients with LAD-I show delayed-type hypersensitivity reactions. This suggests that the functions of VLA-4 and LFA-1 are partially overlapping in the step of firm adhesion, but they may also act in series, as in VLA-4-mediated rolling following by LFA-1-mediated firm adhesion. VLA-4 may act together with selectins to augment T-lymphocyte tethering and rolling in the vasculature. All or most memory T lymphocytes lack L-selectin (39,211,242,243). The CLA^+ subset can bind E-selectin, and T lymphocytes can also bind P-selectin (223,244,245). Peripheral blood T lymphocytes are substantially less efficient than neutrophils in tethering in hydrodynamic flow to E-selectin and P-selectin (T. Diacovo, R. Alon, and T. Springer, *unpublished data*); therefore, cooperation of VCAM-1 with E-selectin or P-selectin, or among all three molecules, may be important in enhancing lymphocyte accumulation in inflammation.

Inflammation also affects traffic through HEV. Antigen injected into tissue drains to the regional lymph node and greatly increases blood flow to the node and traffic of naive lymphocytes through HEV (186). Furthermore, memory lymphocytes now appear to enter the node directly; this is associated with induction of VCAM-1 on non-HEV vascular endothelia within the node (186). Entry is inhibited by mAb to α^4, and this suggests a role for interaction of VCAM-1 with $\alpha^4\beta_1$ (186,234).

Lymphocyte chemoattractants are interesting candidates for the step 2 signal for lymphocyte accumulation at inflammatory sites. Pertussis toxin treatment inhibits lymphocyte emigration in response to antigen in delayed-type hypersensitivity (167). Identification of lymphocyte chemoattractants has been hampered by the low motility of lymphocytes compared to monocytes or neutrophils (246) and by the low signal-to-background ratio, typically less than 2 in most chemotaxis assays. Recent interest has focused on chemokines (Table 2). A number of chemokines, all of which were isolated based on chemoattractive activity for neutrophils or monocytes or by cloning genes of unknown function, have subsequently been tested and found to be chemoattractive for lymphocyte subpopulations (11,12). These include IL-8 (247) (but see refs. 248,249), RANTES (250), MIP-1β (84), MIP-1α and β (251,252), and IP-10 (253). There are differences among reports in the subsets found to be chemoattracted, and some reports use lymphocytes preactivated by T-cell receptor cross-linking, which may be relevant to migration within inflammatory sites but not emigration from blood. Of interest, MIP-1β can induce binding of the naive $CD8^+$ subset to VCAM-1, either in solution or when immobilized on a substrate, mimicking presentation by an endothelial cell surface (84,254); the specific effect is modest, equal to background binding. The RANTES cytokine, by contrast

to MIP-1β, selectively attracts the memory T lymphocyte subset (250).

Vascular endothelium may function to present chemoattractants to lymphocytes in a functionally relevant way as well as to provide a permeability barrier that stabilizes the chemoattractant gradient. A transendothelial chemotaxis assay more accurately simulates lymphocyte emigration from the bloodstream than filter chemotaxis assays and yields signals over ten times background (255). Because lymphocytes, responding to specific antigen in tissue, signal emigration of further lymphocytes into the site, a chemoattractant was sought in material secreted by mitogen-stimulated mononuclear cells. Purification to homogeneity guided by the transendothelial lymphocyte chemotaxis assay revealed that MCP-1, previously thought to be solely a monocyte chemoattractant, is a major lymphocyte chemoattractant (255). Subsequent studies using the transendothelial chemotaxis assay have confirmed that lymphocytes respond to RANTES and MIP-1α (CC chemokines) but do not respond to IL-8 or IP-10 (CXC chemokines) (256). MCP-1, RANTES, and MIP-1α all selectively attract the memory T-lymphocyte subset and both the CD4 and CD8 subsets. All also attract monocytes but not neutrophils, with MCP-1 being more potent than RANTES or MIP-1α as a monocyte chemoattractant. The physiologically relevant transendothelial assay suggests that CC chemokines tend to attract both monocytes and lymphocytes, in agreement with the long-standing clinical observation that lymphocyte emigration into inflammatory sites is always accompanied by emigration of monocytes. The converse is not true. Monocytes sometimes emigrate in the absence of lymphocytes, correlating with activity of chemoattractants such as C5a and PAF on monocytes but not on lymphocytes. Teleologically, it is important that monocytes accompany lymphocytes into inflammatory sites in order to present antigen and to carry out effector functions in which monocytes are activated by T lymphocytes. MCP-1 is abundantly expressed at sites of antigen challenge and autoimmune disease (12,257,258) and, together with MIP-1α and RANTES, is an excellent candidate to provide the step 2 signal required to activate integrin adhesiveness and emigration of both monocytes and lymphocytes in vivo (Fig. 8).

The finding that resting T lymphocytes that tether and roll on VCAM-1 can spontaneously arrest and develop firm adhesion on VCAM-1 (112) has provocative implications for the multistep model. It suggests that the VLA-4–VCAM-1 interaction not only can mediate the steps of rolling and firm adhesion but may also short-circuit the step of stimulation by chemoattractants of firm adhesion through integrins. This is intriguing, because although a twofold stimulation of adhesiveness of VLA-4 to VCAM-1 has been demonstrated by MIP-1β in one system (84), with the chemoattractant that is most effective in eliciting transendothelial chemotaxis of T lymphocytes, MCP-1, it is difficult to detect stimulation of integrin adhesiveness for ICAM-1 or VCAM-1 on lymphocytes (M. W. Carr, and T. A. Springer, *unpublished data*). Therefore, an alternative pathway may exist in which VCAM-1 can mediate both tethering and arrest of lymphocytes, perhaps in cooperation with other endothelial molecules, before stimulation by chemoattractants. After arrest, chemoattractants would guide transendothelial migration and perhaps stimulate further increases in the adhesiveness of the integrins VLA-4 and LFA-1, important in migration across the endothelium and basement membrane.

ATHEROGENESIS

Introduction

Careful ultrastructural and immunohistochemical studies in various animal models and human tissues have established that the adherence of blood monocytes and lymphocytes to endothelial cells lining large arteries is one of the earliest detectable events in atherosclerosis (259,260). The subsequent transendothelial migration of monocytes and their accumulation in the intima and development into lipid-engorged "foam cells" appear to be important steps in the initiation of atherosclerotic lesions. Monocytes and macrophage foam cells may also contribute to the progression of atherosclerotic lesions by producing cytokines and growth factors (259,260). These, in turn, may amplify mononuclear leukocyte recruitment, induce migration of smooth muscle cells into the intima, and stimulate cell replication. The formation of foam-cell-rich lesions during hypercholesterolemia appears to be a highly regulated process during which the vascular endothelium remains intact and may participate in regulating leukocyte recruitment into the intima by expressing leukocyte adhesion molecules.

Expression of Adhesion Molecules in Animal Models of Atherogenesis

The expression patterns of inducible endothelial leukocyte adhesion molecules have been examined in rabbit hypercholesterolemic models. Initially, VCAM-1 expression was found selectively in arterial endothelial cells covering early foam cell lesions of both dietary and Watanabe heritable hyperlipidemic rabbits (261). VCAM-1 expression in endothelium over foam cell lesions was not uniform but appeared particularly elevated at edges of lesions and extended several cells beyond the edge. In this region, scanning electron microscopy showed that mononuclear leukocyte recruitment through an intact endothelial monolayer was increased and presumably contributed to lateral expansion of lesions (262). The induction of endothelial VCAM-1 expression was an early event, occurring approximately 1 week following the initiation of a hypercholesterolemic diet in rabbits and preceding detectable intimal monocyte/macrophage accumulation (263). Endothelium not involved by foam cell lesions did not express VCAM-1 (261,263). In normocholesterolemic rabbits, VCAM-1 was not expressed by aortic endothe-

lium except at sites that are predilected for foam cell lesion formation—the aortic arch and downstream of arterial ostia.

VCAM-1 was also expressed within neointimal smooth muscle cells near the surface and base of intimal lesions and in the medial smooth muscle cells adjacent to the internal elastic lamina (264,265). In cultured rabbit and human arterial vascular smooth muscle cells, VCAM-1 expression could be induced with appropriate cytokine treatment (265). The pathophysiological function of VCAM-1 in smooth muscle cells remains unknown. One possibility is that VCAM-1 may promote the retention of mononuclear leukocytes within atherosclerotic lesions. Alternatively, smooth muscle cell VCAM-1 may interact with VLA-4 on mononuclear leukocytes, thus activating these cells and initiating cytokine cascades or protease production leading to matrix degradation. Smooth muscle cell VCAM-1 may not have an important biological function and may be a marker of smooth muscle cell migration, activation, or differentiation.

In addition to VCAM-1, ICAM-1 expression was also detected by immunohistochemical staining in rabbit models of atherosclerosis. ICAM-1 was expressed in endothelium over lesions contemporaneously with VCAM-1 (264). Its expression was more uniform than that of VCAM-1, and unlike VCAM-1, ICAM-1 expression extended into noninvolved regions. Extensive ICAM-1 expression was found within intimal foam cell lesions, within macrophages and smooth muscle cells. E-Selectin expression over foam cell lesions was low (264); however, increased expression was found in an alloxan diabetic model (266).

Expression of Adhesion Molecules in Human Atherosclerotic Plaques

Several recent studies utilized immunohistochemistry to examine the expression patterns of leukocyte adhesion molecules in human atherosclerotic plaques obtained at autopsy or from hearts of transplant recipients. Unlike the rabbit models, where early lesions were examined, the human atherosclerotic plaques were generally advanced. In all cases, ICAM-1 expression was found in endothelial cells over plaques and in intimal smooth muscle cells and macrophages (267–271). E-Selectin expression was variable and restricted to vascular endothelium (269–271). Caution should be applied to the interpretation of these data, since some E-selectin antibodies acquired from British Biotechnology have subsequently been shown to cross-react with P-selectin. In advanced human coronary artery plaques, VCAM-1 was expressed focally by luminal endothelial cells, usually in association with inflammatory infiltrates (271,272). Focal endothelial VCAM-1 expression was also found in uninvolved vessels with diffuse intimal thickening. Within plaques, VCAM-1 was expressed by subsets of smooth muscle cells and macrophages and by endothelial cells of neovasculature. The variability of VCAM-1 expression in human atherosclerotic lesions, apart from possible technical difficulties with detection, may reflect states of plaque activity or quiescence with regard to leukocyte recruitment. In contrast to rabbit models, where relatively high levels of hypercholesterolemia are maintained by an atherogenic diet and intimal lesion growth is progressive, humans with atherosclerosis generally have low levels of hypercholesterolemia, and human plaque expansion as a result of leukocyte recruitment may develop at intervals.

Chemoattractants in Atherosclerotic Plaques

Mononuclear leukocytes can respond chemotactically to numerous substances, including peptides, lipids, and modified plasma components. Of particular relevance to atherosclerosis are chemoattractants selective for monocytes and lymphocytes, because these cells and not neutrophils are found in atherosclerotic plaques. Monocyte chemotactic activities have been isolated from atherosclerotic lesions in hypercholesterolemic swine and pigeons (273,274). These activities may result from modification of plasma lipoproteins and from chemokines produced locally in the arterial wall by endothelium, smooth muscle, and infiltrating leukocytes.

The accumulation of LDL in the arterial intima is an early event in atherogenesis and continues in advanced lesions. Its modification in the arterial wall by oxidation (275,276) likely has important pathological consequences. Minimally oxidized LDL can stimulate vascular cells to produce the chemoattractant MCP-1 (277) and cytokine/growth factors (278). Lysophosphatidylcholine, a component of highly oxidized LDL is a monocyte chemoattractant (270) and can induce the expression of VCAM-1 and ICAM-1 on arterial endothelial cells (280).

A number of groups have described chemotactic activity specific for monocytes but not neutrophils produced by cultured vascular endothelial and smooth muscle cells (281,282). This activity was biologically and biochemically characterized, and the bulk was attributed to MCP-1 after its cloning and the development of neutralizing antibody reagents. In an endothelial–smooth muscle cell coculture system, MCP-1 was a key mediator of monocyte transmigration (283), and its expression has been detected in human and rabbit atherosclerotic plaques (284). Another monocyte-specific factor produced in atherosclerotic lesions is M-CSF (CSF-1) (285,286). In addition to acting locally, M-CSF may stimulate increased monocyte production by the bone marrow and account for the monocytosis in hypercholesterolemic animals (287).

Mechanisms of Mononuclear Leukocyte Recruitment to Atherosclerotic Plaques

The induction of leukocyte adhesion molecule expression by arterial endothelium and local production of chemoattractants suggest that these molecules are important in the re-

cruitment of mononuclear leukocytes to an atherosclerotic plaque. The specificity of this process, as in other inflammatory reactions, likely is regulated by the repertoire of adhesion molecules and chemoattractants. Mononuclear leukocytes, but not neutrophils, express VLA-4 and can interact with VCAM-1. Chemokines of the CC family, including MCP-1, whether secreted or bound to proteoglycans on the endothelial surface, can up-regulate mononuclear leukocyte integrins. To date, all of the potential mediators have not been identified, and the in vivo relevance of those that have has not been firmly established.

The initial step in leukocyte emigration, whether in atherosclerosis or inflammation, is the interaction of the circulating leukocyte with the endothelial lining. In venules and veins, this results in leukocyte rolling, mediated by selectins. Although E- and P-selectins can be expressed by arterial endothelium, the rheological factors in these vessels are different. Wall shear stress is significantly higher in arteries. In venules it varies depending on the organ but can be as high as 36 dynes/cm^2 in 30- to 40-μm venules of the cat mesentery (288). Even in venules, hemodynamic parameters that affect leukocyte–endothelial interactions during inflammation are complex (289). Arterial shear stresses depend on the anatomic location and phase of the cardiac cycle, and shear stresses up to 100 dynes/cm^2 can be encountered (290). In experimental settings, arterial segments at different anatomic locations of various animal species will alter their diameters to achieve an average wall shear stress of approximately 15 dynes/cm^2 (290). Atherosclerotic lesions form initially in arterial regions where hemodynamics are complex and shear forces are variable and may even oscillate as a result of flow reversal during different stages of the cardiac cycle. Arterial curvature, branching, and pulsatile blood flow are the main factors generating complex flow patterns (290). Direct visualization of leukocyte–endothelial interactions in large arteries has not been feasible; therefore, it is not known whether leukocyte rolling occurs. An alternative possibility is that firm mononuclear leukocyte adhesion occurs without prior leukocyte rolling, and leukocyte integrins may participate if their functional state is up-regulated in the circulation by hypercholesterolemia and/or circulating monocyte-specific cytokines and growth factors. In vitro interactions between VCAM-1 and VLA-4 can mediate firm adhesion and rolling of leukocytes (112).

When the mechanisms of mononuclear leukocyte recruitment to atherosclerotic plaques are understood, it is likely that clinicians will consider inhibiting this process in order to minimize plaque development. However, in addition to contributing to atherosclerotic plaque initiation, progression, and development of complications, monocytes may have a protective role in the arterial wall. Monocytes engulf lipoproteins and thus prevent excessive extracellular accumulation and oxidation of lipids, which can be toxic to vascular wall cells. This potentially important function should not be overlooked in future therapies for atherosclerosis in which mononuclear leukocyte recruitment to atherosclerotic plaques will be inhibited. In conjunction with these therapies, it may be necessary to utilize lipid-lowering or antioxidant drugs to reduce the accumulation of extracellular lipids in the arterial wall.

ACKNOWLEDGMENTS

We thank the NIH for supporting most of the cited work, and Uli von Andrian for comments on the manuscript.

REFERENCES

1. Cahill RNP, Poskitt DC, Frost H, Trnka Z. Two distinct pools of recirculating T lymphocytes: Migratory characteristics of nodal and intestinal T lymphocytes. *J Exp Med* 1977;145:420–428.
2. Cahill RNP, Poskitt DC, Hay JB, Heron I, Trnka Z. The migration of lymphocytes in the fetal lamb. *Eur J Immunol* 1979;9:251–253.
3. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: The multi-step paradigm. *Cell* 1994;76:201–314.
4. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994;84:2068–2101.
5. Granger DN, Kubes P. The microcirculation and inflammation: Modulation of leukocyte–endothelial cell adhesion. *J Leukocyte Biol* 1994; 55:662–675.
6. Picker LJ, Butcher EC. Physiological and molecular mechanisms of lymphocyte homing. *Annu Rev Immunol* 1992;10:561–591.
7. Lasky LA. Selectins: Interpreters of cell-specific carbohydrate information during inflammation. *Science* 1992;258:964–969.
8. Bevilacqua MP, Nelson RM. Selectins. *J Clin Invest* 1993;91: 379–387.
9. Bevilacqua MP. Endothelial–leukocyte adhesion molecules. *Annu Rev Immunol* 1993;11:767–804.
10. Butcher EC. Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. *Cell* 1991;67:1033–1036.
11. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Adv Immunol* 1994;55: 97–179.
12. Miller MD, Krangel MS. Biology and biochemistry of the chemokines: A family of chemotactic and inflammatory cytokines. *Crit Rev Immunol* 1992;12:17–46.
13. Shimizu Y, Newman W, Tanaka Y, Shaw S. Lymphocyte interactions with endothelial cells. *Immunol Today* 1992;13:106–112.
14. Zimmerman GA, Prescott SM, McIntyre TM. Endothelial cell interactions with granulocytes: Tethering and signaling molecules. *Immunol Today* 1992;13:93–100.
15. Harlan JM, Winn RK, Vedder NB, et al. *In vivo* models of leukocyte adherence to endothelium. In: Harlan JR, Liu D, eds. *Adhesion: Its Role in Inflammatory Disease*. New York: W.H. Freeman; 1992: 117–150.
16. Mackay CR. Immunological memory. *Adv Immunol* 1993;53: 217–265.
17. Mackay CR. Migration pathways and immunologic memory among T lymphocytes. *Semin Immunol* 1992;4:51–58.
18. McEver RP. Selectins: Novel receptors that mediate leukocyte adhesion during inflammation. *Thromb Haemostas* 1991;65:223–228.
19. Rosen SD. Cell surface lectins in the immune system. *Semin Immunol* 1993;5:237–247.
20. Diamond MS, Springer TA. The dynamic regulation of integrin adhesiveness. *Curr Biol* 1994;4:506–517.
21. Cline MJ. *The White Cell*. Cambridge: Harvard University Press; 1975.
22. Issekutz AC, Issekutz RB. Quantitation and kinetics of blood monocyte migration to acute inflammatory reactions, and IL-1α, TNFα, and IFN-γ. *J Immunol* 1993;151:2105–2115.
23. Ley K, Gaehtgens P. Endothelial, not hemodynamic, differences are responsible for preferential leukocyte rolling in rat mesenteric venules. *Circ Res* 1991;69:1034–1041.
24. Nazziola E, House SD. Effects of hydrodynamics and leukocyte-endo-

thelium specificity on leukocyte–endothelium interactions. *Microvasc Res* 1992;44:127–142.
25. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel–Palade bodies. *J Clin Invest* 1989;84:92–99.
26. Fina L, Molgaard HV, Robertson D, Bradley NJ, Monaghan P, Delia D, Sutherland DR, Baker MA, Greaves MF. Expression of the CD34 gene in vascular endothelial cells. *Blood* 1990;75:2417–2426.
27. Swerlick RA, Lee KH, Wick TM, Lawley TJ. Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 *in vivo* and *in vitro*. *J Immunol* 1992;148:78–83.
28. Zhu D, Cheng CF, Pauli BU. Mediation of lung metastasis of murine melanomas by a lung-specific endothelial cell adhesion molecule. *Proc Natl Acad Sci USA* 1991;88:9568–9572.
29. Pober JS, Cotran RS. Cytokines and endothelial cell biology. *Physiol Rev* 1990;70:427–452.
30. Thornhill MH, Wellicome SM, Mahiouz DL, Lanchbury JSS, Kyan-Aung U, Haskard DO. Tumor necrosis factor combines with IL-4 or IFN-gamma to selectively enhance endothelial cell adhesiveness for T cells: The contribution of vascular cell adhesion molecule-1-dependent and -independent binding mechanisms. *J Immunol* 1991;146: 592–598.
31. Masinovsky B, Urdal D, Gallatin WM. IL-4 acts synergistically with IL-1β to promote lymphocyte adhesion to microvascular endothelium by induction of vascular cell adhesion molecule-1. *J Immunol* 1990; 145:2886–2895.
32. Colditz IG, Watson DL. The effect of cytokines and chemotactic agonists on the migration of T lymphocytes into skin. *Immunology* 1992; 272:278.
33. Briscoe DM, Cotran RS, Pober JS. Effects of tumor necrosis factor, lipopolysaccharide, and IL-4 on the expression of vascular cell adhesion molecule-1 in vivo. *J Immunol* 1992;149:2954–2960.
34. Issekutz TB, Stoltz JM, Meide PVD. Lymphocyte recruitment in delayed-type hypersensitivity: The role of IFN-γ. *J Immunol* 1988;140: 2989–2993.
35. Schmid-Schönbein GW, Usami S, Skalak R, Chien S. The interaction of leukocytes and erthrocytes in capillary and postcapillary vessels. *Microvasc Res* 1980;19:45–70.
36. Springer TA. Adhesion receptors of the immune system. *Nature* 1990; 346:425–433.
37. Lasky LA, Singer MS, Dowbenko D, Imai Y, Henzel WJ, Grimley C, Fennie C, Gillett N, Watson SR, Rosen SD. An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell* 1992;69:927–938.
38. Gallatin WM, Weissman IL, Butcher EC. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* 1983;304: 30–34.
39. Kansas GS, Wood GS, Fishwild DM, Engleman EG. Functional characterization of human T lymphocyte subsets distinguished by monoclonal anti-leu-8. *J Immunol* 1985;134:2995–3002.
40. Lewinsohn DM, Bargatze RF, Butcher EC. Leukocyte-endothelial cell recognition: Evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. *J Immunol* 1987;138: 4313–4321.
41. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, Wagner DD, Furie B. PADGEM protein: A receptor that mediates the interaction of activated platelets with neutrophils and monocytes. *Cell* 1989;59:305–312.
42. Geng J-G, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA, McEver RP. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* 1990;343:757–760.
43. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA. Identification of an inducible endothelial–leukocyte adhesion molecule, E-LAM 1. *Proc Natl Acad Sci USA* 1987;84:9238–9242.
44. Larsen GR, Sako D, Ahern TJ, Shaffer M, Erban J, Sajer SA, Gibson RM, Wagner DD, Furie BC, Furie B. P-Selectin and E-selectin: Distinct but overlapping leukocyte ligand specificities. *J Biol Chem* 1992; 267:11104–11110.
45. Nelson RM, Dolich S, Aruffo A, Cecconi O, Bevilacqua MP. Higher-affinity oligosaccharide ligands for E-selectin. *J Clin Invest* 1993;91: 1157–1166.
46. Moore KL, Stults NL, Diaz S, Smith DF, Cummings RD, Varki A, McEver RP. Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. *J Cell Biol* 1992;118:445–456.
47. Ushiyama S, Laue TM, Moore KL, Erickson HP, McEver RP. Structural and functional characterization of monomeric soluble P-selectin and comparison with membrane P-selectin. *J Biol Chem* 1993;268: 15229–15237.
48. Erbe DV, Wolitzky BA, Presta LG, Norton CR, Ramos RJ, Burns DK, Rumberger JM, Rao BNN, Foxall C, Brandley BK, Lasky LA. Identification of an E-selectin region critical for carbohydrate recognition and cell adhesion. *J Cell Biol* 1992;119:215–227.
49. Graves BJ, Crowther RL, Chandran C, Rumberger JM, Li S, Huang K-S, Presky DH, Familletti PC, Wolitzky BA, Burns DK. Insight into E-selectin/ligand interaction from the crystal structure and mutagenesis of the lec/EGF domains. *Nature* 1994;367:532–538.
50. Baumhueter S, Singer MS, Henzel W, Hemmerich S, Renz M, Rosen SD, Lasky LA. Binding of L-selectin to the vascular sialomucin, CD34. *Science* 1993;262:436–438.
51. Foxall C, Watson SR, Dowbenko D, Fennie C, Lasky LA, Kiso M, Hasegawa A, Asa D, Brandley BK. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis^x oligosaccharide. *J Cell Biol* 1992;117:895–902.
52. Berg EL, Magnani J, Warnock RA, Robinson MK, Butcher EC. Comparison of L-selectin and E-selectin ligand specificities: The L-selectin can bind the E-selectin ligands sialyl Le^x and sialyl Le^a. *Biochem Biophys Res Commun* 1992;184:1048–1055.
53. Hemmerich S, Rosen SD. 6′-Sulfated sialyl Lewis x is a major capping group of GlyCAM-1. *Biochemistry* 1994;33:4830–4835.
54. Steininger CN, Eddy CA, Leimgruber RM, Mellors A, Welply JK. The glycoprotease of *Pasteurella haemolytica* A1 eliminates binding of myeloid cells to P-selectin but not to E-selectin. *Biochem Biophys Res Commun* 1992;188:760–766.
55. Norgard KE, Moore KL, Diaz S, Stults NL, Ushiyama S, McEver RP, Cummings RD, Varki A. Characterization of a specific ligand for P-selectin on myeloid cells: A minor glycoprotein with sialylated *O*-linked oligosaccharides. *J Biol Chem* 1993;268:12764–12774.
56. Sako D, Chang XJ, Barone KM, Vachino G, White HM, Shaw G, Veldman GM, Bean KM, Ahern TJ, Furie B, Cumming DA, Larsen GR. Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell* 1993;75:1179–1186.
57. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: Distinction from and prerequisite for adhesion through integrins. *Cell* 1991;65:859–873.
58. Ley K, Gaehtgens P, Fennie C, Singer MS, Lasky LA, Rosen SD. Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo. *Blood* 1991;77:2553–2555.
59. von Andrian UH, Chambers JD, McEvoy LM, Bargatze RF, Arfors KE, Butcher EC. Two-step model of leukocyte-endothelial cell interaction in inflammation: Distinct roles for LECAM-1 and the leukocyte β_2 integrins *in vivo*. *Proc Natl Acad Sci USA* 1991;88:7538–7542.
60. Lawrence MB, Springer TA. Neutrophils roll on E-selectin. *J Immunol* 1993;151:6338–6346.
61. Abbassi O, Kishimoto TK, McIntire LV, Anderson DC, Smith CW. E-selectin supports neutrophil rolling in vitro under conditions of flow. *J Clin Invest* 1993;92:2719–2730.
62. Olofsson AM, Arfors KE, Ramezani L, Wolitzky BA, Butcher EC, von Andrian UH. E-selectin mediates leukocyte rolling in interleukin-1 treated rabbit mesentery venules. *Blood* 1994;84:2749–2758.
63. Bienvenu K, Granger DN. Molecular determinants of shear rate-dependent leukocyte adhesion in postcapillary venules. *Am J Physiol* 1993;264:H1504–H1508.
64. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P-selectin-deficient mice. *Cell* 1993;74:541–554.
65. von Andrian UH, Hansell P, Chambers JD, Berger EM, Filho IT, Butcher EC, Arfors KE. L-selectin function is required for β_2-integrin-mediated neutrophil adhesion at physiological shear rates in vivo. *Am J Physiol* 1992;263:H1034–H1044.
66. Kishimoto TK, Warnock RA, Jutila MA, Butcher EC, Lane C, Anderson DC, Smith CW. Antibodies against human neutrophil LECAM-1 (LAM-1/Leu-8/DREG-56 antigen) and endothelial cell ELAM-1 inhibit a common CD18-independent adhesion pathway in vitro. *Blood* 1991;78:805–811.
67. Picker LJ, Warnock RA, Burns AR, Doerschuk CM, Berg EL, Butcher EC. The neutrophil selectin LECAM-1 presents carbohydrate ligands

to the vascular selectins ELAM-1 and GMP-140. *Cell* 1991;66: 921–933.
68. von Andrian UH, Chambers JD, Berg EL, Michie SA, Brown DA, Karolak D, Ramezani L, Berger EM, Arfors KE, Butcher EC. L-Selectin mediates neutrophil rolling in inflamed venules through sialyl Lewis^x-dependent and -independent recognition pathways. *Blood* 1993;82:182–191.
69. Lawrence MB, Bainton DF, Springer TA. Neutrophil tethering to and rolling on E-selectin are separable by requirement for L-selectin. *Immunity* 1994;1:137–145.
70. Chan P-Y, Lawrence MB, Dustin ML, Ferguson LM, Golan DE, Springer TA. Influence of receptor lateral mobility on adhesion strengthening between membranes containing LFA-3 and CD2. *J Cell Biol* 1991;115:245–255.
71. Hammer DA, Apte SM. Simulation of cell rolling and adhesion on surfaces in shear flow: General results and analysis of selectin-mediated neutrophil adhesion. *Biophys J* 1992;63:35–57.
72. Cyster JG, Shotton DM, Williams AF. The dimensions of the T lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation. *EMBO J* 1991;10: 893–902.
73. Erlandsen SL, Hasslen SR, Nelson RD. Detection and spatial distribution of the β_2 integrin (Mac-1) and L-selectin (LECAM-1) adherence receptors on human neutrophils by high-resolution field emission SEM. *J Histochem Cytochem* 1993;41:327–333.
74. Kansas GS, Ley K, Munro JM, Tedder TF. Regulation of leukocyte rolling and adhesion to high endothelial venules through the cytoplasmic domain of L-selectin. *J Exp Med* 1993;177:833–838.
75. Van Ewijk W, Brons NHC, Rozing J. Scanning electron microscopy of homing and recirculating lymphocyte populations. *Cell Immunol* 1975;19:245–261.
76. Anderson AO, Anderson ND. Lymphocyte emigration from high endothelial venules in rat lymph nodes. *Immunology* 1976;31:731–748.
77. Simmons DL, Satterthwaite AB, Tenen DG, Seed B. Molecular cloning of a cDNA encoding CD34, a sialomucin of human hematopoietic stem cells. *J Immunol* 1992;148:267–271.
78. Cross AH, Raine CS. Central nervous system endothelial cell–polymorphonuclear cell interactions during autoimmune demyelination. *Am J Pathol* 1992;139:1401–1409.
79. Wilkinson PC. *Chemotaxis and Inflammation*. London: Churchill Livingstone; 1982.
80. Devreotes PN, Zigmond SH. Chemotaxis in eukaryotic cells: A focus on leukocytes and *dictyostelium*. *Annu Rev Cell Biol* 1988;4:649–686.
81. Carter SB. Haptotaxis and the mechanism of cell motility. *Nature* 1967;213:256–260.
82. Huber AR, Kunkel SL, Todd RF III, Weiss SJ. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science* 1991;254:99–102.
83. Rot A. Endothelial cell binding of NAP-1/IL-8: Role in neutrophil emigration. *Immunol Today* 1992;13:291–294.
84. Tanaka Y, Adams DH, Hubscher S, Hirano H, Siebenlist U, Shaw S. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1β. *Nature* 1993;361:79–82.
85. Snyderman R, Uhing RJ. Chemoattractant stimulus-response coupling. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: Basic Principles and Clinical Correlates*. New York: Raven Press; 1992:421–439.
86. Wu D, LaRosa GJ, Simon MI. G protein-coupled signal transduction pathways for interleukin-8. *Science* 1993;261:101–103.
87. Beals CR, Wilson CB, Perlmutter RM. A small multigene family encodes G_i signal-transduction proteins. *Proc Natl Acad Sci USA* 1987;84:7886–7890.
88. Amatruda TT, Gerard NP, Gerard C, Simon MI. Specific interactions of chemoattractant factor receptors with G-proteins. *J Biol Chem* 1993;268:10139–10144.
89. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. *Ann Rev Immunol* 1994;12:593–633.
90. Charo IF, Myers SJ, Herman A, Franci C, Connolly AJ, Coughlin SR. Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails. *Proc Natl Acad Sci USA* 1994;91:2752–2756.
91. Faull RJ, Kovach NL, Harlan HM, Ginsberg MH. Stimulation of integrin-mediated adhesion of T lymphocytes and monocytes: Two mechanisms with divergent biological consequences. *J Exp Med* 1994;179: 1307–1316.
92. Ginsberg MH, Du X, Plow EF. Inside-out integrin signalling. *Curr Opin Cell Biol* 1992;4:766–771.
93. Pober JS, Doukas J, Hughes CCW, Savage COS, Munro JM, Cotran RS.The potential roles of vascular endothelium in immune reactions. *Hum Immunol* 1990;28:258–262.
94. Lo SK, Lee S, Ramos RA, Lobb R, Rosa M, Chi-Rosso G, Wright SD. Endothelial–leukocyte adhesion molecule 1 stimulates the adhesive activity of leukocyte integrin CR3 (CD11b/CD18, Mac-1, $\alpha_m\beta_2$) on human neutrophils. *J Exp Med* 1991;173:1493–1500.
95. Lorant DE, Patel KD, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: A juxtacrine system for adhesion and activation of neutrophils. *J Cell Biol* 1991;115:223–234.
96. Lo SK, Detmers PA, Levin SM, Wright SD. Transient adhesion of neutrophils to endothelium. *J Exp Med* 1989;169:1779–1793.
97. Wright SD, Meyer BC. Phorbol esters cause sequential activation and deactivation of complement receptors on polymorphonuclear leukocytes. *J Immunol* 1986;136:1759–1764.
98. Buyon JP, Abramson SB, Philips MR, Slade SG, Ross GD, Weissman G, Winchester RJ. Dissociation between increased surface expression of Gp165/95 and homotypic neutrophil aggregation. *J Immunol* 1988; 140:3156–3160.
99. Diamond MS, Staunton DE, deFougerolles AR, Stacker SA, Garcia-Aguilar J, Hibbs ML, Springer TA. ICAM-1 (CD54): A counter-receptor for Mac-1 (CD11b/CD18). *J Cell Biol* 1990;111:3129–3139.
100. Smith CW, Kishimoto TK, Abbass O, Hughes B, Rothlein R, McIntire LV, Butcher E, Anderson DC. Chemotactic factors regulate lectin adhesion molecule 1 (LECAM-1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. *J Clin Invest* 1991;87: 609–618.
101. Sengelov H, Kjeldsen L, Diamond MS, Springer TA, Borregaard N, Subcellular localization and dynamics of Mac-1 ($\alpha_m\beta_2$) in human neutrophils. *J Clin Invest* 1993;92:1467–1476.
102. Philips MR, Buyon JP, Winchester R, Weissman G, Abramson SB. Up-regulation of the iC3b receptor (CR3) is neither necessary nor sufficient to promote neutrophil aggregation. *J Clin Invest* 1988;82: 495–501.
103. Vedder NB, Harlan JM. Increased surface expression of CD11b/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. *J Clin Invest* 1988;81:676–682.
104. Dustin ML, Springer TA. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989;341:619–624.
105. Pircher H, Groscurth P, Baumhutter S, Aguet M, Zinkernagel RM, Hengartner H. A monoclonal antibody against altered LFA-1 induces proliferation and lymphokine release of cloned T cells. *Eur J Immunol* 1986;16:172–181.
106. Keizer GD, Visser W, Vliem M, Figdor CG. A monoclonal antibody (NKI-L16) directed against a unique epitope on the alpha-chain of human leukocyte function-associated antigen 1 induces homotypic cell-cell interactions. *J Immunol* 1988;140:1393–1400.
107. Landis RC, Bennett RI, Hogg N. A novel LFA-1 activation epitope maps to the I domain. *J Cell Biol* 1993;120:1519–1527.
108. Diamond MS, Springer TA. A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. *J Cell Biol* 1993;120:545–556.
109. Diamond MS, Garcia-Aguilar J, Bickford JK, Corbi AL, Springer TA. The I domain is a major recognition site on the leukocyte integrin Mac-1 (CD11b/CD18) for four distinct adhesion ligands. *J Cell Biol* 1993;120:1031–1043.
110. Michishita M, Videm V, Arnaout MA. A novel divalent cation-binding site in the A domain of the β2 integrin CR3 (CD11b/CD18) is essential for ligand binding. *Cell* 1993;72:857–867.
111. Lollo BA, Chan KWH, Hanson EM, Moy VT, Brian AA. Direct evidence for two affinity states for lymphocyte function-associated antigen 1 on activated T cells. *J Biol Chem* 1993;268:21693–21700.
112. Alon R, Kassner PD, Carr MW, Finger EB, Hemler ME, Springer TA. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J Cell Biol* 1995;128:1243–1253.
113. Rothlein R, Dustin ML, Marlin SD, Springer TA. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J Immunol* 1986;137:1270–1274.
114. Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-

2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 1989;339:61–64.
115. deFougerolles AR, Springer TA. Intercellular adhesion molecule 3, a third adhesion counter-receptor for lymphocyte function-associated molecule 1 on resting lymphocytes. *J Exp Med* 1992;175:185–190.
116. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 1989;83:2008–2017.
117. Diamond MS, Staunton DE, Marlin SD, Springer TA. Binding of the integrin Mac-1 (CD11b/CD18) to the third Ig-like domain of ICAM-1 (CD54) and its regulation by glycosylation. *Cell* 1991;65:961–971.
118. deFougerolles AR, Qin X, Springer TA. Characterization of the function of ICAM-3 and comparison to ICAM-1 and ICAM-2 in immune responses. *J Exp Med* 1994;179:619–629.
119. Moy P, Lobb R, Tizard R, Olson D, Hession C. Cloning of an inflammation-specific phosphatidyl inositol-linked form of murine vascular cell adhesion molecule-1. *J Biol Chem* 1993;268:8835–8841.
120. Terry RW, Kwee L, Levine JF, Labow MA. Cytokine induction of an alternatively spliced murine vascular cell adhesion molecule (VCAM) mRNA encoding a glycosylphosphatidylinositol-anchored VCAM protein. *Proc Natl Acad Sci USA* 1993;90:5919–5923.
121. Kinashi T, St. Pierre Y, Springer TA. Expression of glycophosphatidylinositol (GPI)-anchored and non GPI-anchored isoforms of vascular cell adhesion molecule 1 (VCAM-1) in stromal and endothelial cells. *Blood* 1995;57:168–173.
122. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, Lobb RR. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990;60:577–584.
123. Rüegg C, Postigo AS, Sikorski EE, Butcher EC, Pytela R, Erle DJ. Role of integrin $\alpha 4\beta 7/\alpha 4\beta P$ in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J Cell Biol* 1992;117: 179–189.
124. Chan BMC, Elices MJ, Murphy E, Hemler ME. Adhesion to vascular cell adhesion molecule 1 and fibronectin: Comparison of $\alpha^4\beta_1$ (VLA-4) and $\alpha^4\beta_7$ on the human B cell line JY. *J Biol Chem* 1992;267: 8366–8370.
125. Osborn L, Vassallo C, Benjamin CD. Activated endothelium binds lymphocytes through a novel binding site in the alternately spliced domain of vascular cell adhesion molecule-1. *J Exp Med* 1992;176: 99–107.
126. Vonderheide RH, Springer TA. Lymphocyte adhesion through VLA-4: Evidence for a novel binding site in the alternatively spliced domain of VCAM-1 and an additional $\alpha 4$ integrin counter-receptor on stimulated endothelium. *J Exp Med* 1992;175:1433–1442.
127. Vonderheide RH, Tedder TF, Springer TA, Staunton DE. Residues within a conserved amino acid motif of domains 1 and 4 of VCAM-1 are required for binding to VLA-4. *J Cell Biol* 1994;125:215–222.
128. Osborn L, Vassallo C, Browning BG, Tizard R, Haskard DO, Benjamin CD, Douglas I, Kirchhausen T. Arrangement of domains, and amino acid residues required for binding of vascular cell adhesion molecule-1 to its counter-receptor VLA-4 ($\alpha 4\beta 1$). *J Cell Biol* 1994; 124(4):601–608.
129. Streeter PR, Lakey-Berg E, Rouse BTN, Bargatze RF, Butcher EC. A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature* 1988;331:41–46.
130. Briskin MJ, McEvoy LM, Butcher EC. MAdCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgA1. *Nature* 1993;363:461–464.
131. Hu MC-T, Crowe DT, Weissman IL, Holzmann B. Cloning and expression of mouse integrin $\beta_p(\beta_7)$: A functional role in Peyer's patch-specific lymphocyte homing. *Proc Natl Acad Sci USA* 1992; 89:8254–8258.
132. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A, Butcher EC. $\alpha 4\beta 7$ integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 1993;74:185–195.
133. Berg EL, McEvoy LM, Berlin C, Bargatze RF, Butcher EC. L-Selectin-mediated lymphocyte rolling on MAdCAM-1. *Nature* 1993;366: 695–698.
134. Muller WA, Ratti CM, McDonnell SL, Cohn ZA. A human endothelial cell-restricted, externally disposed plasmalemmal protein enriched in intercellular junctions. *J Exp Med* 1989;170:399–414.
135. Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): A novel vascular cell–cell adhesion molecule. *J Cell Biol* 1991;114:1059–1068.
136. Newman PJ, Berndt MC, Gorski J, White GC, Lyman S, Paddock C, Muller WA. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. *Science* 1990; 247:1219–1222.
137. Simmons DL, Walker C, Power C, Pigott R. Molecular cloning of CD31, a putative intercellular adhesion molecule closely related to carcinoembryonic antigen. *J Exp Med* 1990;171:2147–2152.
138. Albelda SM, Oliver PD, Romer LH, Buck CA. EndoCAM. A novel endothelial cell–cell adhesion molecule. *J Cell Biol* 1990;110: 1227–1237.
139. Stockinger H, Gadd SJ, Eher R, Majdic O, Schreiber W, Kasinrerk W, Strass B, Schnabl E, Knapp W. Molecular characterization and functional analysis of the leukocyte surface protein CD31. *J Immunol* 1990;145:3889–3897.
140. Tanaka Y, Albelda SM, Horgan KJ, Van Seventer GA, Shimizu Y, Newman W, Hallam J, Newman PJ, Buck CA, Shaw S. CD31 expressed on distinctive T cell subsets is a preferential amplifier of $\beta 1$ integrin-mediated adhesion. *J Exp Med* 1992;176:245–253.
141. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 1993;178: 449–460.
142. Haynes BF, Liao H-X, Patton KL. The transmembrane hyaluronate receptor (CD44): Multiple functions, multiple forms. *Cancer Cell* 1991;3:347–350.
143. Culty M, Miyake K, Kincade PW, Sikorski E, Butcher EC, Underhill C. The hyaluronate receptor is a member of the CD44 (H-CAM) family of cell surface glycoproteins. *J Cell Biol* 1990;111:2765–2774.
144. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 1990;61: 1303–1313.
145. Günthert U. CD44: A multitude of isoforms with diverse functions. *Curr Top Microbiol Immunol* 1993;184:47–63.
146. Arch R, Wirth K, Hofmann M, Ponta H, Matzku S, Herrlich P, Zöller M. Participation in normal immune responses of a metastasis-inducing splice variant of CD44. *Science* 1992;257:682–685.
147. Jalkanen S, Bargatze RF, de los Toyos J, Butcher EC. Lymphocyte recognition of high endothelium: Antibodies to distinct epitopes of an 85–95 kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal and synovial endothelial cells. *J Cell Biol* 1987;105:983–993.
148. Oppenheimer-Marks N, Davis LS, Lipsky PE. Human T lymphocyte adhesion to endothelial cells and transendothelial migration: Alteration of receptor use relates to the activation status of both the T cell and the endothelial cell. *J Immunol* 1990;145:140–148.
149. Camp RL, Scheynius A, Johansson C, Puré E. CD44 is necessary for optimal contact allergic responses but is not required for normal leukocyte extravasation. *J Exp Med* 1993;178:497–507.
150. Anderson DC, Springer TA. Leukocyte adhesion deficiency: An inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu Rev Med* 1987;38:175–194.
151. Kishimoto TK, Larson RS, Corbi AL, Dustin ML, Staunton DE, Springer TA. The leukocyte integrins: LFA-1, Mac-1, and p150,95. *Adv Immunol* 1989;46:149–182.
152. Buchanan MR, Crowley CA, Rosin RE, Gimbrone MA, Babior BM. Studies on the interaction between GP-180-deficient neutrophils and vascular endothelium. *Blood* 1982;60:160–165.
153. Smith CW, Rothlein R, Hughes BJ, Mariscalco MM, Schmalstieg FC, Anderson DC. Recognition of an endothelial determinant for CD18-dependent neutrophil adherence and transendothelial migration. *J Clin Invest* 1988;82:1746–1756.
154. Arfors KE, Lundberg C, Lindborm L, Lundberg K, Beatty PG, Harlan JM. A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. *Blood* 1987;69:338–340.
155. Lawrence MB, Smith CW, Eskin SG, McIntire LV. Effect of venous shear stress on CD18-mediated neutrophil adhesion to cultured endothelium. *Blood* 1990;75:227–237.
156. Jutila MA, Lewinsohn D, Berg EL, et al. Homing receptors in lymphocyte, neutrophil, and monocyte interaction with endothelial cells. In: Springer TA, Anderson DC, Rosenthal AS, et al, eds. *Leukocyte Adhesion Molecules*. New York: Springer-Verlag; 1988:227–235.

157. Jutila MA, Rott L, Berg EL, Butcher EC. Function and regulation of the neutrophil MEL-14 antigen in vivo: Comparison with LFA-1 and MaC-1. *J Immunol* 1989;143:3318–3324.
158. Watson SR, Fennie C, Lasky LA. Neutrophil influx into an inflammatory site inhibited by a soluble homing receptor–IgG chimaera. *Nature* 1991;349:164–167.
159. Mulligan MS, Varani J, Dame MK, Lane CL, Smith CW, Anderson DC. Ward PA. Role of endothelial–leukocyte adhesion molecule 1 (ELAM-1) in neutrophil-mediated lung injury in rats. *J Clin Invest* 1991;88:1396–1406.
160. Kishimoto TK, Jutila MA, Berg EL, Butcher EC. Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science* 1989;245:1238–1241.
161. Cohnheim J. *Lectures on General Pathology: A Handbook for Practitioners and Students*. London: The New Sydenham Society; 1889.
162. Etzioni A, Frydman M, Pollack S, Avidor I, Phillips ML, Paulson JC, Gershoni-Baruch R. Recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N Engl J Med* 1992;327:1789–1792.
163. von Andrian UH, Berger EM, Ramezani L, Chambers JD, Ochs HD, Harlan JM, Paulson JC, Etzioni A, Arfors KE. *In vivo* behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. *J Clin Invest* 1993;91:2893–2897.
164. Colditz IG. Sites of antigenic stimulation: Role of cytokines and chemotactic agonists in acute inflammation. In: Beh KJ, ed. *Animal Health and Production in the 21st Century*. Melbourne: CSIRO; 1992.
165. Mulligan MS, Jones ML, Bolanowski MA, Baganoff MP, Deppeler CL, Meyers DM, Ryan US, Ward PA. Inhibition of lung inflammatory reactions in rats by an anti-human IL-8 antibody. *J Immunol* 1993; 150:5585–5595.
166. Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. *Nature* 1993;365:654–657.
167. Spangrude GH, Sacchi F, Hill HR, Van Epps DE, Daynes RA. Inhibition of lymphocyte and neutrophil chemotaxis by pertussis toxin. *J Immunol* 1985;135:4135–4143.
168. Nourshargh S, Williams TJ. Evidence that a receptor-operated event on the neutrophil mediates neutrophil accumulation in vivo. *J Immunol* 1990;145:2633–2638.
169. Hechtman DH, Cybulsky MI, Fuchs HJ, Baker JB, Gimbrone MA Jr. Intravascular IL-8: Inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation. *J Immunol* 1991;147: 883–892.
170. Gimbrone MA, Obin MS, Brock AF, Luis EA, Hass PE, Hebert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Baker JB. Endothelial interleukin-8: A novel inhibitor of leukocyte–endothelial interactions. *Science* 1989;246:1601–1603.
171. Colditz IG, Movat HZ. Desensitization of acute inflammatoy lesions to chemotaxins and endotoxin. *J Immunol* 1984;144:2163–2168.
172. Colditz IG. Desensitisation mechanisms regulating plasma leakage and neutrophil emigration. In: Gordon JL, ed. *Vascular Endothelium: Interactions with Circulating Cells*. New York: Elsevier; 1991: 175–187.
173. Kuna P, Reddigari SR, Schall TJ, Rucinski D, Sadick M, Kaplan AP. Characterization of the human basophil response to cytokines, growth factors, and histamine releasing factors of the intercrine/chemokine family. *J Immunol* 1993;150:1932–1943.
174. Bischoff SC, Krieger M, Brunner T, Rot A, Tscharner V, Baggiolini M, Dahinden CA. RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors. *Eur J Immunol* 1993;23:761–767.
175. Walsh LJ, Lavker RM, Murphy GF. Biology of disease. Determinants of immune cell trafficking in the skin. *Lab Invest* 1990;63:592–600.
176. Hood L, Huang HV, Dreyer WJ. The area-code hypothesis: The immune system provides clues to understanding the genetic and molecular basis of cell recognition during development. *J Supramol Struct* 1987;7:531–559.
177. Springer TA. Area code molecules of lymphocytes. In: Burger MM, Sordat B, Zinkernagel RM, eds. *Cell to Cell Interaction: A Karger Symposium*. Basel: S Karger; 1990:16–39.
178. Jung TM, Gallatin WM, Weissman IL, Dailey MO. Down-regulation of homing receptors after T cell activation. *J Immunol* 1988;141: 4110–4117.
179. Spertini O, Kansas GS, Munro JM, Griffin JD, Tedder TF. Regulation of leukocyte migration by activation of the leukocyte adhesion molecule (LAM-1) selectin. *Nature* 1991;349:691–694.
180. Issekutz TB, Chin W, Hay JB. The characterization of lymphocytes migrating through chronically inflamed tissues. *Immunology* 1982; 46:59–66.
181. Mackay CR, Marston WL, Dudler L. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990;171: 801–817.
182. Bjerknes M, Cheng H, Ottaway CA. Dynamics of lymphocyte–endothelial interactions in vivo. *Science* 1986;231:402–405.
183. Woodruff JJ, Clarke LM, Chin YH. Specific cell-adhesion mechanisms determining migration pathways of recirculating lymphocytes. *Annu Rev Immunol* 1987;5:201–222.
184. Mebius RE, Streeter PR, Breve J, Duijvestijn AM, Kraal G. The influence of afferent lymphatic vessel interruption on vascular addressin expression. *J Cell Biol* 1991;115:85–95.
185. Mebius RE, Dowbenko D, Williams A, Fennie C, Lasky LA, Watson SR. Expression of GlyCAM-1, an endothelial ligand for L-selectin, is affected by afferent lymphatic flow. *J Immunol* 1993;151:6769–6776.
186. Mackay CR, Marston W, Dudler L. Altered patterns of T cell migration through lymph nodes and skin following antigen challenge. *Eur J Immunol* 1992;22:2205–2210.
187. Stamper HB Jr, Woodruff JJ. Lymphocyte homing into lymph nodes: In vitro demonstration of the selective affinity of recirculating lymphocytes for high-endothelial venules. *J Exp Med* 1976;144:828.
188. Butcher EC, Scollay RG, Weissman IL. Organ specificity of lymphocyte migration: Mediation by highly selective lymphocyte interaction with organ-specific determinants on high endothelial venules. *Eur J Immunol* 1980;10:556–561.
189. Stevens SK, Weissman IL, Butcher EC. Differences in the migration of B and T lymphocytes: Organ-selective localization in vivo and the role of lymphocyte–endothelial cell recognition. *J Immunol* 1982; 844–851.
190. Bargatze RF, Wu NW, Weissman IL, Butcher EC. High endothelial venule binding as a predictor of the dissemination of passaged murine lymphomas. *J Exp Med* 1987;166:1125–1131.
191. Streeter PR, Rouse BTN, Butcher EC. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J Cell Biol* 1988;107: 1853–1862.
192. Berg EL, Robinson MK, Warnock RA, Butcher EC. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J Cell Biol* 1991;114: 343–349.
193. Watson S, Imai Y, Fennie C, Geoffroy JS, Rosen SD, Lasky LA. A homing receptor–IgG chimera as a probe for adhesive ligands of lymph node high endothelial venules. *J Cell Biol* 1990;110: 2221–2229.
194. Imai Y, Lasky LA, Rosen SD. Sulphation requirement for GlyCAM-1, an endothelial ligand for L-selectin. *Nature* 1993;361:555–557.
195. Spertini O, Luscinskas FW, Kansas GS, Munro JM, Griffin JD, Gimbrone MA Jr, Tedder TF. Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. *J Immunol* 1991;147:2565–2573.
196. Spertini O, Luscinskas FW, Gimbrone MA Jr, Tedder TF. Monocyte attachment to activated human vascular endothelium in vitro is mediated by leukocyte adhesion molecule-1 (L-selectin) under nonstatic conditions. *J Exp Med* 1992;175:1789–1792.
197. San Gabriel-Masson C. *Adhesion of lymphocytes to the lactating mammary gland in the mouse*. [Dissertation]. Hershey: Pennsylvania State University; 1992.
198. Holzmann B, McIntyre BW, Weissman IL. Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an alpha chain homologous to human VLA-1 alpha. *Cell* 1989;56:37–46.
199. Holzmann B, Weissman IL. Peyer's patch-specific lymphocyte homing receptors consist of a VLA-4-like α chain associated with either of two integrin β chains, one of which is novel. *EMBO J* 1989;8: 1735–1741.
200. Imai Y, Singer MS, Fennie C, Lasky LA, Rosen SD. Identification of a carbohydrate based endothelial ligand for a lymphocyte homing receptor. *J Cell Biol* 1991;113:1213–1221.
201. Hamann A, Jablonski-Westrich D, Jonas P, Thiele HG. Homing receptors reexamined: Mouse LECAM-1 (MEL-14 antigen) is involved

in lymphocyte migration into gut-associated lymphoid tissue. *Eur J Immunol* 1991;21:2925–2929.
202. Hamann A, Westrich DJ, Duijevstijn A, Butcher EC, Baisch H, Harder R, Thiele HG. Evidence for an accessory role of LFA-1 in lymphocyte–high endothelium interaction during homing. *J Immunol* 1988; 140:693–699.
203. deFougerolles AR, Stacker SA, Schwarting R. Springer TA. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J Exp Med* 1991;174:253–267.
204. Wardlaw AC, Parton R. *Bordetella pertussis* toxins. *Pharmacol Ther* 1983;19:1–53.
205. Morse SI, Barron BA. Studies on the leukocytosis and lymphocytosis induced by bordetella pertussis. III. The distribution of transfused lymphocytes in pertussis-treated and normal mice. *J Exp Med* 1970; 132:663–672.
206. Chaffin KE, Beals CR, Wilkie TM, Forbush KA, Simon MI, Perlmutter RM. Dissection of thymocyte signaling pathways by *in vivo* expression of pertussis toxin ADP-ribosyltransferase. *EMBO J* 1990;9: 3821–3829.
207. Chaffin KE, Perlmutter RM. A pertussis toxin-sensitive process controls thymocyte emigration. *Eur J Immunol* 1991;21:2565–2573.
208. Spangrude GJ, Braaten BA, Daynes RA. Molecular mechanisms of lymphocyte extravasation. I. Studies of two selective inhibitors of lymphocyte recirculation. *J Immunol* 1984;132:354–362.
209. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. The role of $\alpha 4$ integrins in lymphocyte homing to mucosal tissues *in vivo*. *J Immunol* 1993;152:3283–3293.
210. Bargatze RF, Streeter PR, Butcher EC. Expression of low levels of peripheral lymph node-associated vascular addressin in mucosal lymphoid tissues: Possible relevance to the dissemination of passaged *akr* lymphomas. *J Cell Biochem* 1990;42:219–227.
211. Mackay CR, Marston WL, Dudler L, Spertini O, Tedder TF, Hein WR. Tissue-specific migration pathways by phenotypically distinct subpopulations of memory T cells. *Eur J Immunol* 1992;22:887–895.
212. Bargatze RF, Butcher EC. Rapid G protein-regulated activation events involved in lymphocyte binding to high endothelial venules. *J Exp Med* 1993;178:367–372.
213. Crowe DT, Chiu H, Fong S, Weissman IL. Regulation of the avidity of integrin $\alpha_4\beta_7$ by the β_7 cytoplasmic domain. *J Cell Biol* 1994; 269:14411–14418.
214. Hibbs ML, Xu H, Stacker SA, Springer TA. Regulation of adhesion to ICAM-1 by the cytoplasmic domain of LFA-1 integrin beta subunit. *Science* 1991;251:1611–1613.
215. Hibbs ML, Jakes S, Stacker SA, Wallace RW, Springer TA. The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 β subunit: Sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. *J Exp Med* 1991;174:1227–1238.
216. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* 1990;136:1053–1068.
217. Berg EL, Robinson MK, Mansson O, Butcher EC, Magnani JL. A carbohydrate domain common to both sialyl Le^a and sialyl Le^x is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J Biol Chem* 1991;266:14869–14872.
218. Berg EL, Yoshino T, Rott LS, Robinson MK, Warnock RA, Kishimoto TK, Picker LJ, Butcher EC. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. *J Exp Med* 1991;174: 1461–1466.
219. Graber N, Gopal TV, Wilson D, Beall LD, Polte T, Newman W. T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. *J Immunol* 1990;145:819–830.
220. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 1991;349:796–798.
221. Shimizu Y, Shaw S, Graber N, Gopal TV, Horgan KJ, Van Seventer GA, Newman W. Activation-independent binding of human memory T cells to adhesion molecule ELAM-1. *Nature* 1991;349:799–802.
222. Cotran RS, Gimbrone MA Jr, Bevilacqua MP, Mendrick DL, Pober JS. Induction and detection of a human endothelial activation antigen in vivo. *J Exp Med* 1986;164:661–666.
223. Alon R, Rossiter H, Springer TA, Kupper TS. Distinct cell surface ligands mediate T lymphocyte attachment and rolling on P- and E-selectin under physiological flow. *J Cell Biol* 1994;127:1485–1495.
224. Schweighoffer T, Tanaka Y, Tidswell M, Erle DJ, Horgan KJ, Luce GEG, Lazarovits AI, Buck D, Shaw S. Selective expression of integrin $\alpha 4\beta 7$ on a subset of human $CD4^+$ memory T cells with hallmarks of gut-trophism. *J Immunol* 1993;151:717–729.
225. Cerf-Bensussan N, Jarry A, Brousse N, Lisowska-Grospierre B, Guy-Grand D, Griscelli C. A monoclonal antibody (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *Eur J Immunol* 1987;17:1279–1285.
226. Kilshaw PJ, Murant SJ. A new surface antigen on intraepithelial lymphocytes in the intestine. *Eur J Immunol* 1990;20:2201–2207.
227. Parker CM, Cepek K, Russell GJ, Shaw SK, Posnett D, Schwarting R, Brenner MB. A family of β_7 integrins on human mucosal lymphocytes. *Proc Natl Acad Sci USA* 1992;89:1924–1928.
228. Shaw SK, Cepek KL, Murphy EA, Russell GJ, Brenner MB, Parker CM. Molecular cloning of the human mucosal lymphocyte integrin α^E subunit. *J Biol Chem* 1994;269:6016–6025.
229. Cepek KL, Parker CM, Madara JL, Brenner MB. Integrin $\alpha^E\beta_7$ mediates adhesion of T lymphocytes to epithelial cells. *J Immunol* 1993; 150:3459–3470.
230. Picker LJ, Terstappen LWMM, Rott LS, Streeter PR, Stein H, Butcher EC. Differential expression of homing-associated adhesion molecules by T cell subsets in man. *J Immunol* 1990;145:3247–3255.
231. Coffman RL, Lebman DA, Shrader B. Transforming growth factor β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. *J Exp Med* 1989;170:1039–1044.
232. Pitzalis C, Kingsley G, Haskard D, Panayi G. The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: Evidence for regulation by selective endothelial and homotypic adhesion. *Eur J Immunol* 1988;18:1397–1404.
233. Janossy G, Bofill M, Rowe D, Muir J, Beverley PC. The tissue distribution of T lymphocytes expressing different CD45 polypeptides. *Immunology* 1989;66:517–525.
234. Issekutz TB. Inhibition of *in vivo* lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 monoclonal antibody: A likely role for VLA-4 *in vivo*. *J Immunol* 1991;147: 4178–4184.
235. Issekutz TB. Inhibition of lymphocyte endothelial adhesion and in vivo lymphocyte migration to cutaneous inflammation by TA-3, a new monoclonal antibody to rat LFA-1. *J Immunol* 1992;149:3394–3402.
236. Chisholm PL, Williams CA, Lobb RR. Monoclonal antibodies to the integrin $\alpha 4$ subunit inhibit the murine contact hypersensitivity response. *Eur J Immunol* 1993;23:682–688.
237. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin. *Nature* 1992;356:63–66.
238. Scheynius A, Camp RL, Puré E. Reduced contact sensitivity reactions in mice treated with monoclonal antibodies to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. *J Immunol* 1993;150:655–663.
239. Issekutz TB. Dual inhibition of VLA-4 and LFA-1 maximally inhibits cutaneous delayed type hypersensitivity-induced inflammation. *Am J Pathol* 1993;143:1286–1293.
240. Silber A, Newman W, Sasseville VG, Pauley D, Beall D, Walsh DG, Ringler DJ. Recruitment of lymphocytes during cutaneous delayed hypersensitivity in nonhuman primates is dependent on E-selectin and VCAM-1. *J Clin Invest* 1994;93:1554–1563.
241. McCluskey RT, Benacerraf B, McClusky JW. Studies on the specificity of the cellular infiltrate in delayed type hypersensitivity reactions. *J Immunol* 1963;90:466.
242. Tedder TF, Matsuyama T, Rothstein D, Schlossman SF, Morimoto C. Human antigen-specific memory T cells express the homing receptor (LAM-1) necessary for lymphocyte recirculation. *Eur J Immunol* 1990;20:1351–1355.
243. Bradley LM, Atkins GG, Swain SL. Long-term $CD4^+$ memory T cells from the spleen lack MEL-14, the lymph node homing receptor. *J Immunol* 1992;148:324–331.
244. Moore KL, Thompson LF. P-Selectin (CD62) binds to subpopulations of human memory T lymphocytes and natural killer cells. *Biochem Biophys Res Commun* 1992;186:173–181.
245. Damle NK, Klussman K, Dietsch MT, Mohagheghpour N, Aruffo A. GMP-140 (P-selectin/CD62) binds to chronically stimulated but not

resting CD4+ T lymphocytes and regulates their production of proinflammatory cytokines. *Eur J Immunol* 1992;22:1789–1793.
246. Parrott DMV, Wilkinson PC. Lymphocyte locomotion and migration. *Prog Allergy* 1982;18:193–284.
247. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* 1989;241:1464–1466.
248. Kudo C, Araki A, Matsushima K, Sendo F. Inhibition of IL-8 induced W3/25+ (CD4+) T lymphocyte recruitment into subcutaneous tissues of rats by selective depletion of *in vivo* neutrophils with a monoclonal antibody. *J Immunol* 1991;174:2196–2201.
249. Leonard EJ, Yoshimura T, Tanaka S, Raffeld M. Neutrophil recruitment by intradermally injected neutrophil attractant/activation protein-1. *J Invest Dermatol* 1991;96:690–694.
250. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 1990;347:669–671.
251. Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1α and MIP-1β. *Science* 1993;360:355–358.
252. Schall TJ, Bacon K, Camp RDR, Kaspari JW, Goeddel DV. Human macrophage inflammatory protein α (MIP-1α) and MIP-1β chemokines attract distinct populations of lymphocytes. *J Exp Med* 1993; 177:1821–1825.
253. Taub DD, Lloyd AR, Conlon K, Wang JM, Ortaldo JR, Harada A, Matsushima K, Kelvin DJ, Oppenheim JJ. Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J Exp Med* 1993;177:1809–1814.
254. Adams DH, Harvath L, Bottaro DP, Interrante R, Catalano G, Tanaka Y, Strain A, Hubscher SG, Shaw S. Hepatocyte growth factor and macrophage inflammatory protein 1β: Structurally distinct cytokines that induce rapid cytoskeletal changes and subset-preferential migration in T cells. *Proc Natl Acad Sci USA* 1994;91:7144–7148.
255. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA. Monocyte chemoattractant protein-1 is a major T lymphocyte chemoattractant. *Proc Natl Acad Sci USA* 1994;91:3652–3656.
256. Roth SJ, Carr MW, Rose SS, Springer TA. Characterization of transendothelial chemotaxis of T lymphocytes. *Am J Pathol [submitted]*.
257. Leonard EJ, Yoshimura T. Human monocyte chemoattractant protein-1 (MCP-1). *Immunol Today* 1990;11:97–101.
258. Villiger PM, Terkeltaub R, Lotz M. Production of monocyte chemoattractant protein-1 by inflamed synovial tissue and cultured synoviocytes. *J Immunol* 1992;149:722–727.
259. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* 1993;362:801–809.
260. Munro JM, Cotran RS. Biology of disease. The pathogenesis of atherosclerosis: Atherogenesis and inflammation. *Lab Invest* 1988;58: 249–261.
261. Cybulsky MI, Gimbrone MA Jr. Vascular endothelial cells express a monocyte adhesion molecule during atherogenesis. *Science* 1991;251: 788–791.
262. Walker LN, Reidy MA, Bowyer DE. Morphology and cell kinetics of fatty streak lesion formation in the hypercholesterolemic rabbit. *Am J Pathol* 1986;125:450–459.
263. Li H, Cybulsky MI, Gimbrone MA Jr, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arterioscler Thromb* 1993;13:197–204.
264. Cybulsky MI, Allan-Motamed M, Medoff B, Davis V, Collins T, Gimbrone MA Jr. Endothelial expression of leukocyte adhesion molecules during atherogenesis in the rabbit. *FASEB J* 1992;6:1030A.
265. Li H, Cybusky MI, Gimbrone MA Jr, Libby P. Inducible expression of vascular cell adhesion molecule-1 (VCAM-1) by vascular smooth muscle cells in vitro and within rabbit atheroma. *Am J Pathol* 1993; 143:1551–1559.
266. Richardson M, Hadcock SJ, DeReske M, Cybulsky MI. Increased expression in vivo of VCAM-1 and E-selectin by the aortic endothelium of normolipemic and hyperlipemic diabetic rabbits. *Arterioscler Thromb* 1994;14:760–769.
267. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* 1992;140:665–673.
268. Printseva OY, Peclo MM, Gown AM. Various cell types in human atherosclerotic lesions express ICAM-1: Further immunocytochemical and immunochemical studies employing monoclonal antibody 10F3. *Am J Pathol* 1992;140:889–896.
269. Wood KM, Cadogan MD, Ramshaw AL, Parums DV. The distribution of adhesion molecules in human atherosclerosis. *Histopathology* 1993;23:437–444.
270. van der Wal AC, Das PK, Tigges AJ, Becker AE. Adhesion molecules on the endothelium and mononuclear cells in human atherosclerotic lesions. *Am J Pathol* 1992;141:1427–1433.
271. Davis MJ, Gordon JL, Gearing AJH, Pigott R, Woolf N, Katz D, Kyriakopoulos A. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol* 1993;171:223–229.
272. O'Brien KD, Allen MD, McDonald TO, Chait A, Harlan JM, Fishbein D, McCarty J, Ferguson M, Hudkins K, Benjamin CD, Lobb R, Alpers CE. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. *J Clin Invest* 1993;92:945–951.
273. Gerrity RG, Goss JA, Soby L. Control of monocyte recruitment by chemotactic factor(s) in lesion-prone areas of swine aorta. *Arteriosclerosis* 1985;5:55–66.
274. Denholm EM, Lewis JC. Monocyte chemoattractants in pigeon aortic atherosclerosis. *Am J Pathol* 1987;126:464–475.
275. Boyd HC, Gown AM, Wolfbauer G, Chait A. Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am J Pathol* 1989;135:815–825.
276. Rosenfeld ME, Paklinski W, Yla-Herrtuala S, Butler S, Witztum JL. Distribution of oxidation specific lipid–protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis* 1990;10:336–349.
277. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, Gerrity R, Schwartz CJ, Fogelman AM. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci USA* 1990;87:5134–5238.
278. Rajavashisth TB, Andalibi A, Territo MC, Berliner JA, Navab M, Fogelman AM, Lusis AJ. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature* 1990;344:254–257.
279. Quinn MT, Parthasarathy S, Steinberg D. Lysophosphatidylcholine: A chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc Natl Acad Sci USA* 1988;85:2805–2809.
280. Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest* 1992;90:1138–1144.
281. Berliner JA, Territo M, Almada L, Carter A, Shafonsky E, Fogelman AM. Monocyte chemotactic factor produced by large vessel endothelial cells in vitro. *Arteriosclerosis* 1985;6:254–258.
282. Valente AJ, Fowler SR, Sprague EA, Kelley JL, Suenram CA, Schwartz CJ. Initial characterization of a peripheral blood mononuclear cell chemoattractant derived from cultured arterial smooth muscle cells. *Am J Pathol* 1984;117:409–417.
283. Navab M, Hough GP, Stevenson LW, Drinkwater DC, Laks H, Fogelman AM. Monocyte migration into the subendothelial space of a coculture of adult human aortic endothelial and smooth muscle cells. *J Clin Invest* 1988;82:1853–1863.
284. Yla-Herttuala S, Lipton BA, Rosenfeld ME, Sarkioja T, Yoshimura T, Leonard EJ, Witztum JL, Steinberg D. Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci USA* 1991;88: 5252–5256.
285. Rosenfeld ME, Yla-Herttuala S, Lipton BA, Ord VA, Witztum JL, Steinberg D. Macrophage colony-stimulating factor mRNA and protein in atherosclerotic lesion of rabbits and humans. *Am J Pathol* 1992; 140:291–300.
286. Clinton SK, Underwood R, Hayes L, Sherman ML, Kufe DW, Libby P. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. *Am J Pathol* 1992;140:301–316.
287. Averill LE, Meagher RC, Gerrity RG. Enhanced monocyte progenitor cell proliferation in bone marrow of hyperlipemic swine. *Am J Pathol* 1989;135:369–377.

288. House SD, Lipowsky HH. Leukocyte-endothelium adhesion: Microhemodynamics in the mesentary of the cat. *Microvasc Res* 1987;34: 363–379.
289. Nazziola E, House SD. Effects of hydrodynamics and leukocyte–endothelium specificity on leukocyte–endothelium interactions. *Microvasc Res* 1992;44:127–142.
290. Ku DN, Zhu C. The mechanical environment of the artery. In: Sumpio BE, ed. *Hemodynamic Forces and Vascular Cell Biology*. Austin: RG Landes Company; 1993:1–23.
291. Fujimoto T, Stroud E, Whatley RE, Prescott SM, Muszbek L, Laposata M, McEver RP. P-selectin is acylated with palmitic acid and stearic acid at cysteine 766 through a thioester linkage. *J Biol Chem* 1993;268:11394–11400.
292. Barclay AN, Birkeland ML, Brown MH, et al. *The Leucocyte Antigen Facts Book*. London: Academic Press; 1993.
293. Sutherland DR, Marsh JCW, Davidson J, Baker MA, Keating A, Mellors A. Differential sensitivity of CD34 epitopes to cleavage by *Pasteurella haemolytica* glycoprotease: Implications for purification of CD34-positive progenitor cells. *Exp Hematol* 1992;20:590–599.
294. Larson RS, Corbi AL, Berman L, Springer TA. Primary structure of the LFA-1 alpha subunit: An integrin with an embedded domain defining a protein superfamily. *J Cell Biol* 1989;108:703–712.
295. Kishimoto TK, O'Connor K, Lee A, Roberts TM, Springer TA. Cloning of the beta subunit of the leukocyte adhesion proteins: Homology to an extracellular matrix receptor defines a novel supergene family. *Cell* 1987;48:681–690.
296. Takada Y, Elices MJ, Crouse C, Hemler ME. The primary structure of α-4 subunit of VLA-4: Homology to other integrins and possible cell-cell adhesion function. *EMBO J* 1989;8:1361–1368.
297. Loftus JC, O'Toole TE, Plow EF, Glass A, Frelinger AL III, Ginsberg MH. A β_3 integrin mutation abolishes ligand binding and alters divalent cation-dependent conformation. *Science* 1990;249:915–918.
298. Carrell NA, Fitzgerald LA, Steiner B, Erickson HP, Phillips DR. Structure of human platelet membrane glycoproteins IIb and IIIa as determined by electron microscopy. *J Biol Chem* 1985;260: 1743–1749.
299. Nermut MV, Green NM, Eason P, Yamada SS, Yamada KM. Electron microscopy and structural model of human fibronectin receptor. *EMBO J* 1988;7:4093–4099.
300. Staunton DE, Dustin ML, Erickson HP, Springer TA. The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. *Cell* 1990;61:243–254.
301. Kirchhausen T, Staunton DE, Springer TA. Location of the domains of ICAM-1 by immunolabeling and single-molecule electron microscopy. *J Leukocyte Biol* 1993;53:342–346.
302. Simmons D, Makgoba MW, Seed B. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. *Nature* 1988;331:624–627.
303. Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA. Primary structure of intercellular adhesion molecule 1 (ICAM-1) demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell* 1988;52:925–933.
304. Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R. Direct cloning of vascular cell adhesion molecule 1 (VCAM-1), a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 1989;59:1203–1211.
305. Polte T, Newman W, Gopal TV. Full length vascular cell adhesion molecule 1 (VCAM-1). *Nucleic Acids Res* 1990;18:5901.
306. Schall TJ. Biology of the RANTES/SIS cytokine family. *Cytokine* 1991;3:165–183.
307. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. RANTES and macrophage inflammatory protein 1α induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;176:1489–1495.
308. Alam R. Forsythe PA, Stafford S, Lett-Brown MA, Grant JA. Macrophage inflammatory protein-1α activates basophils and mast cells. *J Exp Med* 1992;176:781–786.
309. Kameyoshi Y, Dörschner A, Mallet AI, Christophers E, Schröder JM. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992;176: 587–592.
310. Hynes RO. Integrins: Versatility, modulation, and signalling in cell adhesion. *Cell* 1992;69:11–25.
311. Hemler ME. VLA proteins in the integrin family: Structures, functions, and their role on leukocytes. *Annu Rev Immunol* 1990;8: 365–400.
312. Bochner BS, Luscinskas FW, Gimbrone MA Jr, Newman W, Sterbinsky SA, Derse-Anthony CP, Klunk D, Schleimer RP. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: Contributions of endothelial cell adhesion molecules. *J Exp Med* 1991;173:1553–1556.
313. Horgan KJ, Luce GEG, Tanaka Y, Schweighoffer T, Shimizu Y, Sharrow SO, Shaw S. Differential expression of VLA-α4 and VLA-β1 discriminates multiple subsets of $CD4^+CD45RO^+$ "memory" T cells. *J Immunol* 1992;149:4082–4087.
314. Kilshaw PJ, Murant SJ. Expression and regulation of $\beta_7(\beta_p)$ integrins on mouse lymphocytes: Relevance to the mucosal immune system. *Eur J Immunol* 1991;21:2591–2597.
315. Alon R, Hammer DA, Springer TA. Lifetime of the P-selectin: carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature* 1995;374:539–542.
316. Lawrence ML, Berg EL, Butcher EC, Springer TA. Rolling of lymphocytes and neutrophils on peripheral node addressin and subsequent arrest on ICAM-1 in shear flow. *Eur J Immunol* 1995 (*in press*).