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Adhesion Molecules Controlling Lymphocyte Migration

Minireview

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The movement of lymphocytes into organized lymphoid tissues, the recruitment of leukocytes into inflammatory lesions, and the formation of platelet plugs at sites of vascular injury all begin with the attachment of circulating cells to modified regions of the vessel wall. Multiple independently regulated cell–cell and cell–matrix interactions contribute, and several families of adhesion molecules, including members of the integrin and immunoglobulin supergene families, have been characterized. This review focuses on the adhesive interaction of lymphocytes and vessel wall during recirculation in an effort to place two new families of adhesion receptors in a physiologic context. The first includes the murine lymphocyte "homing receptor" Mel-14 (Lasky et al., 1989; Siegelman et al., 1989), the human endothelial leukocyte adhesion molecule ELAM-1 (Bevilacqua et al., 1989), and a potential adhesion structure of platelets and endothelial cells termed granule membrane protein or GMP-140 (Johnston et al., 1989). The second includes the Hermes/CD44 group of broadly distributed membrane antigens implicated in lymphocyte–endothelial cell adhesion in multiple tissues (Goldstein et al., 1989; Stamenkovic et al., 1989; Idzerda et al., 1989).

Lymphocyte Homing Depends on Tissue-Specific Adhesive Interactions with Endothelium

Studies of migratory activity in situ indicate that lymphocytes continuously recirculate from blood, through widely distributed organized lymphoid tissues, into the lymphatics, and back to the blood—demonstrating, to a degree, regional specificities (reviewed in Yednock and Rosen, 1989). In particular, peripheral lymph nodes and mucosal-associated lymphoid tissues constitute separate but overlapping recirculatory pathways. A histologically distinctive postcapillary venule termed the high endothelial venule (HEV) serves as the portal of entry for circulating cells into organized lymphoid tissues along both pathways. The concept of "lymphocyte homing"—specifically, that lymphocytes, unlike granulocytes and monocytes, are targeted for specific organs—grew out of these observations. As documented below, the organ-selective attachment of circulating cells to the HEVs of target organs both initiates recirculation and governs specificity.

Initial Characterization of Homing Receptors and Vascular Addressins

The Stamper–Woodruff assay, a technique for quantitating adhesive interactions between viable lymphocytes and HEVs in frozen sections of target organs, permits dissection of homing activity at the molecular level. In rodent model systems, this assay detects organ-selective adhesive interactions and predicts migratory activity in vivo. Discrete adhesion molecules (termed homing receptors) mediate the binding of lymphoid cells to the HEVs of peripheral lymph nodes (PNHEVs) and the intestinal Peyer's patches (PPHEVs). Blockade with specific monoclonal antibodies (MAbs) prevents the migration of circulating cells into target organs in vivo, confirming the physiologic relevance of the adhesive interactions.

In the rat, factors isolated from thoracic duct lymph, termed $HEBF_{ln}$ and $HEBF_{pp}$, bind to HEVs and block lymphocyte attachment to PNHEVs or PPHEVs exclusively (reviewed in Woodruff et al., 1987). In contrast, MAbs raised to these factors selectively block attachment by interacting with lymphocyte antigens. MAb A.11 blocks attachment to PNHEVs and precipitates glycoproteins of 135, 60 and 40 kd, whereas MAb 1.B2, an inhibitor of binding to PPHEVs, precipitates a single 80 kd species. Thus discrete structures on normal rat lymphocytes mediate the interactions with PNHEVs and PPHEVs.

In the murine system, MAb Mel-14 blocks the interactions, both in vitro and in vivo, of lymphoid cells with the HEVs of lymph nodes exclusively (reviewed in Berg et al., 1989). Expression of the Mel-14 epitope correlates with binding and recirculatory efficiency in adult thymocytes (low), interleukin-2–dependent T cell clones (low), stimulated B cells isolated from germinal centers (low), unfractionated nodal lymphocytes (high), and murine lymphomas (low–very high). The Mel-14 epitope of lymphocytes resides on a highly glycosylated 85–95 kd glycoprotein with an unusually low isoelectric point (4.0–4.5). Finally, expression of the Mel-14 epitope on all leukocytes coupled with antibody-induced blockade of leukocyte accumulation in a cutaneous model of acute inflammation suggests that this "homing receptor" may contribute broadly to leukocyte–endothelial cell interactions.

In contrast, MAb LPAM-1 effectively blocks binding of murine lymphoid cells to PPHEVs exclusively (Holzmann et al., 1989). The epitope resides on the high molecular weight chain of a heterodimer consisting of noncovalently associated 160 and 130 kd subunits. The 160 kd subunit cross-reacts with a monospecific rabbit anti-human VLA-4 α chain reagent, linking the LPAM-1 receptor to the VLA/ECMR integrin family and raising the posssibility that its endogenous ligand resides in the extracellular matrix. Alternatively, the novel β chain identified in the LPAM-1 antigen, termed β_p, may distinguish a new subfamily specialized for intercellular adhesion, analogous to the CD11a-c/CD18 group. The precise relationship of the LPAM-1 receptor to other members of the VLA/ECMR subfamily of integrins and its role in mediating selective migration in vivo remain to be determined.

The prediction that tissue-selective adhesion structures associate with HEVs has recently been confirmed. Two structurally distinct antigens, termed vascular addressins, are linked to homing in the nodal and mucosal recirculatory pathways (reviewed in Berg et al., 1989). MAbs MECA-79 and MECA-367, specific for the nodal and mucosal addressins, respectively, block interactions with the appropriate target endothelium and show a reciprocal

pattern of expression in the adult mouse: the MECA-79 antigen predominates in PNHEVs and the MECA-367 antigen predominates in PPHEVs. In contrast, both are expressed on the HEVs of mesenteric nodes. This pattern mirrors the binding characteristics of tissue-selective lymphomas that interact with mesenteric HEVs at reduced levels whether their primary target is PNHEVs or PPHEVs. These molecules therefore are prime candidates for the endogenous ligands of the Mel-14 and LPAM-1 receptors. Finally, the expression of the addressins on endothelium outside the traditional nodal and mucosal recirculatory pathways suggests that they function as inducible adhesion molecules in a variety of tissues.

Traditionally, the term "homing receptor" is applied to lymphocyte structures conferring tissue-selective migratory activity. However, these molecules are not the exclusive determinants of migration. In the murine system, anti-LFA-1 MAbs partially block (~40%) the interactions of lymphocytes with both PNHEVs and PPHEVs (Hamann et al., 1988). MAb Mel-14, in contrast, inhibits activity in vitro and in vivo by >80%. Expression of the Mel-14 antigen on lymphomas bearing markedly elevated levels of LFA-1 significantly reduces the inhibitory effect of anti-LFA-1 MAbs on binding to PNHEVs. MAb Mel-14 alone blocks attachment (>90%), regardless of the relative densities of the Mel-14 and LFA-1 antigens. Thus LFA-1 augments organ-selective interactions mediated by homing receptors. In addition, the up-regulation of LFA-1 and its ligands (e.g., ICAM-1) during activation of human lymphocytes and endothelium, respectively, suggests that these molecules may enhance the migration of circulating activated lymphocytes and foster extravasation into inflammatory lesions (Dustin and Springer, 1988). Thus the concerted action of tissue-specific and multipurpose adhesion structures regulates lymphocyte trafficking through organized lymphoid tissues. The validity of this paradigm in inflammatory lesions remains to be established. However, the development of HEV-like vessels at sites of chronic inflammation suggests mechanistic links to the recirculatory process.

Characterization of Potential HEV Adhesion Molecules on Human Lymphocytes

The Hermes/CD44 group of membrane antigens has been implicated in binding to HEVs in a variety of tissues (reviewed in Berg et al., 1989). Four principle lines of evidence support this hypothesis. First, FACS enrichment of the Hermes-1 epitope on the CEM cell line correlates with increased affinity for PNHEVs. Second, purified Hermes-1 antigen absorbs PNHEV blocking activity from rat anti-human whole lymphocyte antiserum. Third, MAb Hermes-3 blocks binding of lymphoid cells to the HEVs of mucosal lymphoid organs. Finally, mouse anti-human polyclonal Hermes antiserum blocks attachment of lymphoid cells to the HEVs of lymph nodes, mucosal lymphoid organs, and rheumatoid synovium. The absence of significant biochemical differences in the Hermes antigens isolated from cell lines binding exclusively to either lymph node or mucosal HEVs, coupled with the broad inhibitory specificity of the polyclonal antiserum, suggests that the Hermes antigens function as multipurpose adhesion receptors. Alternatively, Hermes antigens may coregulate and complex with organ-selective adhesion receptors in the cell membrane. Inhibition by Hermes-specific reagents might thus reflect steric hindrance of the principal attachment site.

An immunologically and structurally related molecule isolated from a human fibrosarcoma line, termed CR/ECMRIII, interacts with immobilized type I and type VI collagen (Carter and Wayner, 1988; Gallatin et al., 1989). Furthermore, both CR/ECMRIII and murine Pgp1, the leading candidate for the murine homolog of the Hermes/CD44 group, interact with the cytoskeleton. Thus members of this family may link adhesive interactions at the cell surface with the cytoskeleton. A more generalized role in cellular adhesion has been proposed in light of the broad expression of Hermes/CD44 epitopes on hematopoietic, mesenchymal, and epithelial cell lines.

A second potential adhesion molecule on human lymphocytes reacts with MAb 3.A.7 (Woodruff et al., 1987). This MAb blocks the binding of human lymphocytes to PNHEVs and precipitates a single 80 kd protein from surface-labeled cells. The structural relatedness of 3.A.7 to the murine Mel-14 antigen or the human Hermes/CD44 antigens cannot be determined from currently available data. This question assumes greater importance in light of the sequence data showing that the Mel-14 and Hermes/CD44 antigens are not homologs. Thus MAb 3.A.7 may join the growing legion of CD44-specific reagents or constitute the only currently available monoclonal probe for the human homolog of the Mel-14 antigen.

A Lectin-like Structure Mediates Lymphocyte Binding to the PNHEVs of Rodents and Man

In the rat, mouse, and human, functionally similar calcium-dependent phosphomannosyl binding sites on intact cells have been linked to recognition of PNHEVs (Stoolman et al., 1987; reviewed in Yednock and Rosen, 1989). In all three species, mannose-6-phosphate (M6P), the structurally related monosaccharide fructose-1-phosphate, the fucose sulfate–rich heteropolysaccharide fucoidin, and the M6P-rich phosphomannan PPME efficiently block attachment to PNHEVs. The monosaccharides inhibit maximally (>80%) at 5–10 mM, while the polysaccharides show equal inhibition at 0.1–10 nM (depending on the species). A fluorescent probe for phosphomannan receptors, constructed by derivatizing fluorescent microspheres with PPME (PPME-beads), binds to lymphoid cells in direct proportion to the cells' affinity for PNHEVs. The attachment of lymphocytes to both PNHEVs and PPME-beads is calcium dependent, trypsin sensitive, and blocked by the same group of carbohydrates. Thus PPME-beads detect a lectin-like receptor on lymphocytes mediating attachment to PNHEVs.

In the murine system this site is either contained in or intimately associated with the Mel-14 antigen. Lectin activity, cell-surface Mel-14 antigen, and affinity for PNHEVs are expressed at similar levels in thymocytes, recirculating lymphocytes, and a variety of lymphomas. Moreover, enriching the S49 lymphoma line for surface lectin activity increases expression of the Mel-14 epitope in parallel (correlation coefficient near unity). Finally, MAb Mel-14 inhibits the attachment of PPME-beads to lymphocytes in a

Compendium of Adhesion Structures

Function	Molecule	Size (kd)	Distribution	Ligand(s)
Lymphocyte Adhesion				
PNHEVs and MNHVs	A.11 antigen	40, 60, 135	rat lymphs	unknown
	3.A.7 antigen	80	human lymphs	unknown
	Mel-14 antigen[a]	90, 180	murine lymphs, PMNs, and malignant lymphoid lines	?sialyloligosaccharide on HEV
	Ca-dependent lectins[a,b]	?90, 180	lymphs and malignant lymphoid lines in rodents and humans	M6P, F1P, PPME, fucoidin, ?sialyloligosaccharide on HEV
	MECA-79 antigen	92	murine PNHEVs and MNHEVs	?Mel-14 receptor
PPHEVs and MNHEVs	1.B2	80	rat lymphs	unknown
	LPAM-1 (VLA4)[c]	160/130	murine lymphs and malignant lines	?ECM, ?MECA-367 antigen
	MECA-367 antigen	58–66	murine PPHEVs and MNHEVs	?LPAM-1
HEVs of multiple tissues	Hermes antigens (CD44, Pgp1, CR/ECMRIII, Hutch1)	80–95, 160, 180–200	hematopoietic, epithelial, mesenchymal cells and related lines in humans, nonhuman primates, and mouse	?ECM
	LFA-1 (CD11a/18)[c]	180/95	all murine and human leukocytes, related lines	ICAM-1, other HUVE ligand(s)
	ICAM-1[d]	90	widely distributed, frequently upregulated with activation (endothelium, keratinocytes)	LFA-1
Leukocyte Adhesion	ELAM-1[a]	115, 97	endothelium of immune/inflammatory lesions; cytokine-treated HUVE	unknown
	LFA-1 (CD11a/18)[c]	180/95	all murine and human leukocytes, related lines	ICAM-1, other HUVE ligand(s)
	CR3 (CD11b/18, Mac1, Mo1, OKM1, Leu15)[c]	155–165/95	PMNs, monocytes, macrophages, LGLs	C3bi, ?endothelial ligands
	p150,95 (CD11c/18)[c]	150/95	PMNs, monocytes, macrophages, CTL lines	C3bi, ?endothelial ligands
Platelet Adhesion	GMP-140[a]	140	alpha granules of platelets and Weibel–Pallade bodies of endothelium; rapidly mobilized to plasma membrane on stimulation	unknown
	GP I complex	150	platelet plasma membrane	vWF
	GP IIb, IIIa[c]	130/23/105	platelet plasma membrane	RGD sequence in fibrinogen, fibronectin, and vWF

[a] New LEC-CAM family (for lectin, EGF, and complement homology domains; see text).
[b] Characterized on intact cells only; probably equivalent to the Mel-14 antigen (mouse) and its structural homologs in rat and human.
[c] Integrin family.
[d] Immunoglobulin supergene family.
PNHEVs, MNHEVs, and PPHEVs = high endothelial venules of peripheral lymph nodes, mesenteric lymph nodes, and Peyer's patches; M6P = mannose-6-phosphate; F1P = fructose-1-phosphate; HUVE = human umbilical vein endothelium; LGLs = large granular lymphocytes; CTLs = cytolytic T cells; vWF = von Willebrand factor.

dose-dependent fashion whereas MAbs directed at a variety of other lymphocyte antigens have no effect. In all species and cell lines tested to date, calcium-dependent lectin activity at the cell surface correlates directly with affinity for PNHEVs. These functional studies predict that structurally conserved carbohydrate binding domains mediate attachment to PNHEVs in both rodents and man. As detailed below, the sequence of the murine Mel-14 antigen provides support for this hypothesis.

The endogenous ligand for the lectin has not been identified; however, terminal sialic acid residues appear critical since desialylation of murine HEVs blocks attachment to lymph nodes but not to Peyer's patches (reviewed in Yednock and Rosen, 1989). At present there is no direct evidence that M6P residues contribute to the binding site on the HEVs. Thus the anionic carbohydrate inhibitors characterized to date may mimic the charge distribution of the endogenous ligand. However, the potency and specificity of these inhibitors suggest that they resemble the endogenous structure in critical respects. One attractive hypothesis, prompted by the inhibitory activity of neuraminidase and the known structural diversity of sialic acids, is that an unusual form of sialic acid constitutes the endogenous receptor.

The Mel-14 Antigen Contains a Carbohydrate Binding Domain and Is Structurally Unrelated to the Hermes/CD44 Antigen Group

The primary sequence of Mel-14 reveals a novel structure composed of an N-terminal lectin-like domain contiguous with a single epidermal growth factor (EGF)-like repeat and two identical repeats homologous to a variety of complement binding proteins (Lasky et al., 1989; Siegelman

et al., 1989). A hydrophobic transmembrane domain and a short cytoplasmic tail with one potential phosphorylation site round out the structure. The presence of a prominent amino-terminal sequence homologous to a variety of C-type animal lectins supports the hypothesis that the carbohydrate recognition domain mediates binding to PNHEVs. In particular, the strict calcium dependence of carbohydrate recognition by sequences displaying the "Drickamer" motif (Drickamer, 1988), as in the Mel-14 antigen, mirrors precisely the cation requirements of lymphocyte interactions with both PNHEVs and PPME-beads. In contrast, two homologous complement binding molecules, C2 and protein B, associate with their ligands (C4b and C3b, respectively) in a magnesium-dependent fashion (Reid et al., 1986). Magnesium cannot substitute for calcium in functional studies of the homing receptor on intact cells. Thus the amino-terminal sequence must be considered the leading candidate for the HEV and PPME recognition domains. However, confirmation awaits structure–function studies of each extracellular domain and characterization of the endogenous ligand.

The demonstration of a similar structure for ELAM-1 (Bevilacqua et al., 1989) and GMP-140 (Johnston et al., 1989) suggests that carbohydrate-dependent adhesion may contribute to a broad range of recognition events involving hematopoietic cells. ELAM-1 mediates the attachment of neutrophils to cytokine-activated endothelium in vitro. It appears in situ on endothelium in immune/inflammatory lesions, consistent with a role in the recruitment of circulating leukocytes. The function of GMP-140, a major constituent of cytoplasmic granules in platelets and endothelium, is not established; however, its structural ties to documented adhesion molecules enhances the likelihood that it fulfills a similar function. The ligation of lectin, EGF, and complement binding domains in this group of documented and potential adhesion molecules suggests the acronym LEC-CAM (for Lectin, EGF, Complement–Cellular Adhesion Molecule) for the family as a whole.

Independent sequencing of cDNAs encoding the Hermes/CD44 antigen in several different lymphoid lines reveals a novel transmembrane molecule structurally unrelated to the Mel-14 antigen (Goldstein et al., 1989; Stamenkovic et al., 1989; Idzerda et al., 1989). A potentially significant N-terminal homology (~30%) with cartilage link proteins may provide a structural basis for interactions with proteoglycans and collagen, although an actual contribution of extracellular matrix components to Hermes-sensitive adhesive interactions has not been established. The presence of Hermes-encoding mRNAs of the same size in a variety of hematopoietic cells expressing the 80–90 kd form suggests that the antigens share a common core protein sequence. However, the slightly larger mRNAs in epithelial cell lines expressing a 160 kd form may reflect the presence of sequence heterogeneity within the extended CD44 antigen family (Stamenkovic et al., 1989). The antigenic polymorphism reported for this group may thus result from sequence heterogeneity or cell-specific posttranslational modifications of a common core peptide. Overall, the structural data are consistent with Hermes functioning as a non-tissue-selective HEV adhesion molecule in the Stamper–Woodruff assay. Confirmation of this hypothesis and delineation of the antigen's function on nonlymphoid cells remain challenging tasks for the future.

Summary

Two newly characterized structural families of adhesion molecules, in concert with known members of the integrin and immunoglobulin supergene families, mediate the interaction of circulating lymphoid cells with the vessel wall. The Hermes/CD44 antigen family participates in attachment to multiple vascular beds and consists of a common polypeptide core showing amino-terminal homology to cartilage link proteins. In contrast, the node-specific homing receptor Mel-14 consists of substructures homologous to calcium-dependent lectins, EGF, and complement binding proteins. The sequence of Mel-14 provides structural support for the hypothesis that lectin–carbohydrate interactions mediate physiologically significant adhesion events in the course of lymphocyte recirculation. The discovery of a similar structure in ELAM-1 and GMP-140 extends the reach of this family to other leukocyte and platelet interactions with the vessel wall.

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