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Selectins: Interpreters of Cell-Specific Carbohydrate Information During Inflammation

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Although a bewildering array of cell surface carbohydrate structures have been described, the physiological relevance of any of these complex molecules has often eluded biologists. A family of cell surface glycoproteins, the "selectins," has a characteristic ability to use some of these carbohydrate structures in adhesive mechanisms that help localize leukocytes to regions of inflammation. This article will review the biology of these carbohydrate-binding adhesive proteins and discuss the potential for developing anti-inflammatory antagonists that could inhibit binding events that are selectin-mediated.

Leukocytes are the major purveyors of host defense during a pathogenic onslaught. Neutrophils provide for rapid, relatively nonspecific defense mechanisms, after which a more long-lived, antigen-specific response is determined by macrophage-monocytes and B and T lymphocytes. These circulating cells are constantly scrutinizing the organism for potentially threatening situations, and it is this surveillance that prevents a rapid and wholesale destruction of the individual by the plethora of pathogens that are routinely encountered. Unfortunately, this highly regulated system can show an overzealousness that results in an attack on the host itself. The resultant tissue damage can range from mild psoriasis to multi-organ failure, asthma, or arthritis. An understanding of the various processes that mediate normal inflammatory responses may lead to novel drugs that could be effective in the treatment of diseases induced by abnormal inflammation.

One of the most important aspects of the inflammatory process involves cell adhesion events (*1–5*). Leukocytes must combine a high degree of vascular mobility with the ability to specifically adhere in a temporally relevant manner to endothelial sites that are adjacent to tissues destined to be invaded by the appropriate type of inflammatory cell. This type of endothelial adherence and tissue extravasation is encountered on a routine basis in the lymph nodes, where lymphocytes of diverse antigenic specificities constantly pass through so that they may encounter sequestered antigens appropriately presented by resident antigen-presenting cells (*6*). This category of lymphocyte–endothelial cell adherence is part of a chronic, normal inflammatory event that is critical for the rapid analysis of potentially deleterious antigens that have been encountered in the periphery.

The importance of the adhesive interactions between neutrophils and the vascular endothelium is underscored by a rare syndrome termed the leukocyte adhesion deficiency, or LAD, syndrome (*7*). Individuals suffering from this genetic disease have an abnormal degree of life-threatening bacterial infections because their leukocytes cannot adhere properly to endothelial cells. This lack of adhesion to the endothelium therefore results in a deficiency in the ability of neutrophils to extravasate to tissues that are threatened by bacterial invasion. Lymphocyte recognition of the vasculature and neutrophil extravasation toward infected tissues are two examples of the critical involvement of cell adhesive events in the immune-surveillance of leukocytes. Adhesive mechanisms similar to those utilized during these normal inflammatory responses are probably used during pathogenic inflammatory episodes as well.

Although lectin, or carbohydrate recognition, domains of proteins have been shown to be involved in a number of biological events (*8*), the relationship between carbohydrate-mediated cell adhesion and leukocyte inflammation was not always clear. The discovery of the selectins, a family of three cell surface glycoproteins that contain lectin domains that mediate regional inflammatory responses by recognition of cell-specific carbohydrates, has unified these divergent fields and has given rise to some observations that may ultimately prove to be clinically significant.

Leukocyte Trafficking

Early work by Gowans and colleagues (*6*) gave the initial indications that tissue-specific adhesive interactions could influence regional leukocyte trafficking. These investigators showed that lymphocyte populations that were derived from regional lymphoid sites, when reinjected into an animal, tended to migrate, or "home," back to the sites from which they were initially derived. One interpretation of these data was that tissue-specific adhesive interactions between lymphocytes and the endothelium directed these cells to different lymphoid sites (*9*). These findings were expanded upon when an in vitro system derived from frozen tissue sections was produced that enabled an examination of the adhesive interactions between lymphocytes and the endothelium in various lymphoid sites (*10*).

These in vitro assays supported previous in vivo data on tissue-specific mechanisms of lymphocyte homing and allowed for the production of monoclonal antibodies (MAbs) to lymphocyte surface antigens that specifically blocked these adhesive interactions. One such MAb, termed the Mel 14 antibody, blocked the binding of lymphocytes to post-capillary high endothelial venules (HEVs) of the peripheral lymph node (PLN) and recognized a ~90- to 100-kD antigen that was confined to leukocyte cell surfaces (*11*). This MAb was inefficient at inhibiting adhesion of lymphocytes to another lymphoid tissue, the Peyer's patches, which suggested a tissue-specific distribution of the ligands recognized by the Mel 14 antigen. These data were consistent with the ~90- to 100-kD leukocyte surface antigen being a cell adhesion molecule that interacted with a ligand on the HEVs and directed lymphocytes to migrate primarily to PLNs. Because of its apparent involvement in the homing of lymphocytes to PLNs, the antigen recognized by Mel 14 was termed the "homing receptor," although subsequent publications used a diversity of other names.

A similar frozen section binding assay was

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used to examine the potential role of protein-carbohydrate interactions in the binding of lymphocytes to the HEVs of PLNs. Some anionic monomeric sugars, such as mannose-6-phosphate, could inhibit lymphocyte-HEV adhesion, but only when the sugars were added at millimolar concentrations (*12*). Subsequent work showed that polymeric anionic carbohydrates, such as fucoidin (a polymer of fucose-4-sulfate) or polyphosphomannan ester (PPME: a polymer of mannose-6-phosphate), were inhibitory at much lower concentrations (*13*). Fluorescent beads covalently coated with PPME bound to the lymphocyte cell surface in a calcium-dependent manner. This result was reminiscent of the calcium dependence of lymphocyte binding to the PLN HEVs in frozen sections (*14*).

The lymphocyte surface component that was recognized by PPME-coated beads and the homing receptor recognized by the MAb to Mel 14 were shown to be the same (*15*). In addition, treatment of either frozen PLN tissue sections (*16*) or whole animals (*17*) with enzymes that remove sialic acid from carbohydrates resulted in a loss of lymphocyte adhesion to HEV and in vivo lymphocyte trafficking to PLN, respectively. This result was consistent with the hypothesis that the recognition of the HEV ligands by the homing receptor required the carbohydrate sialic acid. The simplest interpretation of these early results was that a carbohydrate-binding protein on the surface of lymphocytes, the homing receptor, mediated adhesion to HEVs, perhaps by the recognition of a sialic acid–containing carbohydrate specifically expressed in the PLNs.

Additional work suggested that other, potentially unique, adhesion molecules might be involved with the movement of leukocytes to sites of acute inflammation or thrombosis. Studies of endothelium activated by various inflammatory stimuli, such as interleukin-1β or tumor necrosis factor, showed that an adhesion molecule was induced within hours that bound neutrophils and monocytes to the endothelial cell surface. Because of its endothelial localization and function in binding leukocytes to the endothelium, this molecule was termed the endothelial leukocyte adhesion molecule (ELAM) (*18*).

Another series of experiments demonstrated that both platelets and endothelial cells contained an adhesion molecule that could be induced within minutes by activation of the cells with thrombotic stimuli (*19*). This molecule, like ELAM, was involved with neutrophil and monocyte adhesion, but, unlike ELAM, it was stored in platelet alpha granules or endothelial cell Weibel-Palade bodies. Because of its molecular size and storage properties, this molecule was termed either GMP140 (granule membrane protein of molecular weight 140 kD) or PADGEM (platelet activation–dependent granule external membrane protein). Although this early work suggested that these adhesive interactions, like those involving the binding of lymphocytes to PLN HEVs, were calcium dependent, there was no other evidence at this time to suggest that the adhesion mediated by ELAM or GMP140/PADGEM involved protein-carbohydrate interactions.

Lectin-Containing Adhesion Molecules

Before 1989, research on leukocyte-endothelial cell adhesion concentrated on the interactions between the various integrin family members on the leukocyte cell surface and their cognate immunoglobulin superfamily ligands on the endothelium (*1, 3, 4, 7*). When cDNAs that encoded the homing receptor (*20*), ELAM (*21*), and GMP140/PADGEM (*22*) were independently described, their similar protein organization suggested that another mechanism of adhesive recognition that involves carbohydrate binding may also participate in leukocyte-endothelial cell attachment. All three of these proteins had an NH_2-terminal domain that was homologous to the widely dispersed type C (calcium-dependent) lectin or carbohydrate recognition domain (summarized in Table 1) (*23*). In the case of the homing receptor, the discovery of this domain corroborated the previous body of data that suggested that the calcium-dependent adhesion mediated by this protein is due to the recognition of a carbohydrate expressed by PLN HEV. The homing receptor was a prototype for a carbohydrate-binding adhesion molecule and stimulated the search for similar types of carbohydrate recognition by ELAM and GMP140/PADGEM (below). The lectin domains were juxtaposed to conserved epidermal growth factor (EGF)–like motifs. These motifs were followed by a glycoprotein-specific number of repeated sequences that were similar to those in various complement-binding proteins. The overall sequence similarities in the lectin and EGF-like domains of the three glycoproteins were relatively high (~65%), whereas the complement binding–like motifs were less conserved.

Analysis of genomic clones encoding the three adhesive glycoproteins revealed an excellent correspondence between the domain structure of the glycoproteins and the exon-intron structure of the genes (*24*). These latter results, together with the tight clustering of these genes on a syntenic region of murine and human chromosome 1 near a site encoding other proteins containing complement binding–like motifs, suggested that these three glycoproteins arose by exon shuffling and gene duplication. The related structural and functional aspects of these three adhesive glycoproteins led a nomenclature group comprised of various individuals working in the field to rename them the selectins: L-selectin (the homing receptor), E-selectin (ELAM) and P-selectin (GMP140/PADGEM) (*25*).

Selectin Ligands

Although compelling evidence existed for the role of a carbohydrate ligand in the L-selectin–mediated adhesion of lymphocytes to PLN HEVs, there was little evidence for sugar recognition in cell binding that was mediated by E- and P-selectin. This situation changed rapidly when a number of groups, using a diversity of techniques that measured either the gain or loss of selectin-mediated cell adhesion under static conditions (below), simultaneously reported the carbohydrate nature of the cell surface ligands for E- and P-selectin.

By transfection, E-selectin was demonstrated to mediate adhesion that was induced by the expression of a specific fucosyl transferase in mammalian cells (*26*). The expression of this enzyme resulted in the de novo expression of a sialic (*N*-acetylneuraminic) acid– and fucose-containing cell surface poly-*N*-acetyllactosamine, and it was presumed that this induced carbohydrate was a ligand for E-selectin. Antibodies to a myeloid-specific cell surface carbohydrate, termed sialyl Lewis^x, could block E-selectin–mediated adhesion of neutrophils (*27*). In addition, purified sialyl Lewis^x glycolipid, in the form of liposomes, could also block E-selectin–mediated adhesion of neutrophils. Sialyl Lewis^x was a sialylated, fucosylated tetrasaccharide with the structure *N*-acetyl-D-neuraminic acid-α(2 → 3)-D-galactose-β(1 → 4)-[D-fucose-α(1 → 3)]-*N*-acetyl-D-glucosamine (Fig. 1). Isolation and mass spectral analysis of a glycolipid from neutrophils that mediated binding of COS7 cells transfected with E-selectin also supported the supposition that a sialyl Lewis^x–like compound was involved with E-selectin–mediated adhesion (*28*). Carbohydrates that lacked the appropriate sialic acid linkage, the fucose linkage, or both, were poor ligands for E-selectin.

Similar results for P-selectin–mediated adhesion were obtained using MAbs to carbohydrate and carbohydrates derived initially from human milk (*29*). These data showed that a compound related to sialyl Lewis^x, termed Lewis^x, could inhibit P-selectin–mediated adhesion, but only at relatively high concentrations. Subsequent work demonstrated that, like E- and L-selectin, P-selectin required a sialic acid residue for high-affinity binding (*30*). In one

Table 1. Selectin structure and function.

Structure*	Name	Location	Expression	Adherent Cell Types	Proposed Function
L E C C	L-selectin	Leukocytes (constitutive)	Decreases upon cell activation	PLN† endothelium Endothelium adjacent to inflammatory sites (rolling)	Lymphocyte recirculation through PLN Neutrophil (+ other leukocyte?) inflammation
L E C C C C C C	E-selectin	Endothelium (transcriptionally activated)	Increases upon inflammatory activation (IL-1, TNF, LPS) (~ hours)	Monocytes Neutrophils (rolling) T cell subsets (cutaneous?)	Leukocyte inflammation
L E C C C C C C C C C	P-selectin	Platelets (α-granules) Endothelium (Weibel-Palade bodies)	Increases upon thrombin activation, Histamine, Substance P, Peroxide (~ min)	Monocytes Neutrophils (rolling) T cell subsets (cutaneous?)	Leukocyte inflammation

* L, Lectin; E, Epidermal growth factor–like; C, Complement-binding protein–like † PLN, Peripheral lymph node

assay system sialyl Lewis^x glycolipid could function as a ligand for P-selectin (*31*). In summary, it seemed clear that cell surface carbohydrates, and specifically either sialyl Lewis^x or carbohydrates related to this structure could, under in vitro conditions, function as ligands for both E- and P-selectin.

Although the cumulative data suggest that a carbohydrate, sialyl Lewis^x, could function as a ligand for E- and P-selectin, it is clear that the adhesive interactions mediated by these two glycoproteins may be more complex. Another nonmyeloid specific polysaccharide that is related to sialyl Lewis^x is sialyl Lewis^a, *N*-acetyl-D-neuraminic acid-α(2 → 3)-D-galactose-β(1 → 3)-[D-fucose-α(1 → 4)]-*N*-acetyl-D-glucosamine, which has clear ligand activity for E-selectin. Two-dimensional NMR (nuclear magnetic resonance) analysis of both the sialyl Lewis^x and sialyl Lewis^a carbohydrates show that they have many conformational similarities (Fig. 1) (*32*). A sulfated glycolipid, sulfatide (galactose-4-sulfate ceramide), can function as a ligand for P-selectin (*33*). However, since the calcium dependence of this interaction was not described, its physiological relevance remains to be established. In addition, although either fucosyl transferase–transfected or mutant Chinese hamster ovary (CHO) cells that overexpress sialyl Lewis^x can adhere to E- and P-selectin, this adhesion may be somewhat artifactual. At least part of this potential artifact may be due to the high expression of the carbohydrate ligand by these cells (*34*). Thus, although P-selectin binding to neutrophils is a high-affinity, saturable binding event, the binding of soluble P-selectin to sialyl Lewis^x expressing CHO cells is low affinity and nonsaturable (*35*). These apparent low-affinity interactions were further accentuated by the various cell adhesion assays that were done under static conditions rather than under the conditions of fluid flow that are more representative of those found in the vasculature (see below).

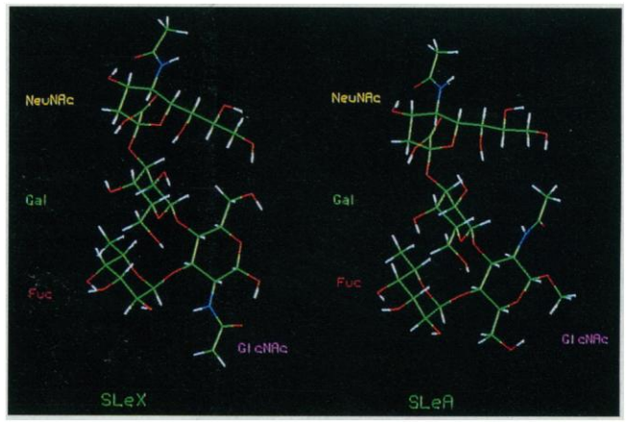

Fig. 1. Energetically preferred conformations of the selectin carbohydrate ligands sialyl Lewis^x (sLe^x) and sialyl Lewis^a (sLe^a). In both structures, the sialic acid (NeuNAc), galactose (Gal), and fucose (Fuc) residues that have been shown to be critical for ligand binding maintain similar spatial orientations. *N*-acetylglucosamine (GlcNAc) residue, however, undergoes a nearly 180 degree flip. [Photograph courtesy of N. Rao]

One possible explanation for the low-affinity results is that a protein component may be involved with the higher avidity presentation of a specific carbohydrate ligand to the selectin lectin domains; protease experiments in which cell-binding assays were used have demonstrated that P-selectin appears to bind to a carbohydrate contained on a protease-sensitive substrate (*36*). A candidate glycoprotein that may present carbohydrate ligands to P-selectin has recently been reported (*36*).

In addition, both E- and P-selectin–mediated adhesion of neutrophils is, at least in part, directed by sialyl Lewis^x presented by L-selectin on the neutrophil surface (*37*). These results are complicated, however, by the finding that E-selectin–mediated binding to neutrophils appears to be completely protease resistant (*36*), whereas neutrophil L-selectin appears to be exquisitely sensitive to proteolysis (*38*).

Finally, L-selectin binds to sialyl Lewis^x in static binding assays, although the avidity of this binding, again, appears to be quite low (*39*). Thus, although the results that show the involvement of the carbohydrate sialyl Lewis^x in E-, P-, and L-selectin–mediated binding are compelling in that they suggest that similar types of sialylated, fucosylated carbohydrate ligands may be recognized by the selectins, it appears that this carbohydrate recognition may be analogous to integrin binding to the arginine-glycine-aspartate (RGD) peptide sequence:

an important but incomplete representation of the naturally occurring ligand.

Although the carbohydrate nature of the endothelial ligand recognized by L-selectin provided strong impetus to search for carbohydrate ligands for E- and P-selectin, it has been much more difficult to analyze the structure of the naturally occurring sugar recognized by the leukocyte selectin (L-selectin). This is due to the lack of a cell line that makes large quantities of the ligand for L-selectin. In addition, the only previously available reagent that recognized a potential L-selectin ligand was a MAb, termed MECA 79, that blocked the adhesion of lymphocytes to the HEVs of PLNs but appeared to bind to a carbohydrate epitope contained on a variety of endothelial glycoproteins that were termed PLN vascular addressins (*40*).

A highly specific reagent for analyzing L-selectin ligands was generated by combining the extracellular domain of murine L-selectin with the hinge, CH2, and CH3 domains of the human immunoglobulin G1 (IgG1) (*41*). This so-called IgG chimera enabled the characterization of the L-selectin ligands. For example, histochemical studies with the chimera demonstrated that it could specifically recognize an HEV ligand for L-selectin that was expressed in PLNs but not in Peyer's patches (*41*). In agreement with previous work using lymphocyte binding to frozen PLN sections, this histochemical recognition was dependent upon sialic acid, because treatment of tissue sections with sialidase resulted in a loss of binding of the chimera to the endothelium (*41*). These results suggested that the chimera recognized a carbohydrate ligand expressed on the HEV, although the exact biochemical nature of the ligand was unknown.

In order to characterize the molecular nature of the endothelial ligand recognized by the L-selectin IgG chimera, Imai and colleagues capitalized on a previous study that suggested that the HEVs of PLNs incorporated high amounts of inorganic sulfate (*42*). Organ cultures of murine PLN were labeled with inorganic [^{35}S]sulfate, and the labeled proteins were "affinity-precipitated" with the L-selectin IgG chimera. Two glycoproteins, a predominant one of ~50 kD and a minor one of ~90 kD, incorporated sulfate and interacted with L-selectin. This interaction was tissue specific (PLN and mesenteric lymph nodes) and was carbohydrate dependent. In addition, these sulfated glycoproteins were recognized by the MECA 79 MAb, in agreement with their apparent roles as endothelial ligands for L-selectin (*40*). Finally, although both of these glycoproteins appeared to contain a large amount of carbohydrate, the carbohydrate linkages were resistant to *N*-glycanase, consistent with the possibility that the bulk of the carbohydrate was O-linked to serine and threonine residues.

A cDNA clone encoding the 50-kD L-selectin ligand was isolated (*43*), and it encoded a protein high in serine and threonine content (~30%). These residues were clustered into two domains that were, presumably, highly O-glycosylated, because comparing the native molecular size of the ligand with the deduced molecular weight from the cDNA sequence suggested that ~70% of the molecular weight of the glycoprotein was carbohydrate. The mRNA encoding this protein was expressed predominantly in mesenteric lymph node and PLN HEVs and in lungs, and immunohistochemistry using antibodies to peptides showed that the 50-kD ligand was expressed on the lumenal surface of PLN HEVs.

This cDNA appeared to encode a scaffold-like structure that presented clustered, O-linked carbohydrate ligands to the L-selectin lectin domain in a tissue-specific manner. The clustering of O-linked carbohydrates is reminiscent of similar structures found in mucins, which are highly extended, highly O-glycosylated rod-like proteins (*44*). The 50-kD L-selectin ligand represents a novel type of mucin-like cell adhesion molecule that acts to present carbohydrate ligands to the L-selectin lectin domain, with the clustering of the carbohydrate ligands on this potentially rigid structure acting to enhance the avidity of the interaction. Because of its role as a cell adhesion molecule that is glycosylation-dependent, the 50-kD ligand was renamed GlyCAM-1 (glycosylation-dependent cell adhesion molecule–1).

Although an increasing inventory of information about the carbohydrate and glycoprotein ligands for selectins has begun to accumulate, little data exists regarding the detailed manner by which the selectins interact with such ligands. A mutagenesis approach was used to investigate the regions of the E-selectin lectin domain that might be involved with the recognition of sialyl Lewis^x (*45*). The mapping of the binding sites for a panel of blocking and nonblocking anti–E-selectin MAbs was combined with the ability of mutated E-selectins to recognize immobilized sialyl Lewis^x glycolipid. A number of mutations in the lectin domain that affected both blocking and nonblocking MAbs as well as sialyl Lewis^x recognition were then superimposed onto a model of the E-selectin lectin motif generated from the x-ray structure of a related type C lectin, the mannose-binding protein (*45*). A face of the E-selectin lectin domain appeared to interact with the carbohydrate ligand, and a number of the amino acid side chains involved with this interaction were positively charged. Since sialic acid, a negatively charged carbohydrate, is involved in cell adhesion mediated by all three selectins, electrostatic interactions may contribute to binding. These mutagenesis results also support previous data that demonstrated that negatively charged carbohydrate monomers and polymers could inhibit selectin-mediated adhesion.

Selectin Function and Leukocyte Rolling

Much of the information about the adhesive roles of the selectins was derived from various in vitro cell-binding assays, except the evidence regarding the contribution of L-selectin to various leukocyte migration pathways in vivo. As mentioned above, in vivo treatment of animals with sialidase demonstrated that sialic acid was required for lymphocyte trafficking to PLNs, consistent with an in vivo role for L-selectin in this process (*16, 17*). A more direct demonstration was inhibition of the migration of lymphocytes to these organs in vivo by Mel 14 MAb (anti–murine L-selectin) (*46*). This MAb also inhibited the accumulation of neutrophils at sites of acute inflammation, suggesting that L-selectin may participate in neutrophil recruitment as well as lymphocyte migration (*46*). One caveat of these experiments, however, was the possibility that the binding of the Mel 14 MAb to the neutrophil surface induced a neutrophil activation event that indirectly inhibited the migration of these cells to the inflammatory site. In order to address this possibility, the ability of a soluble L-selectin–IgG chimera (above) to inhibit leukocyte accumulation in vivo was tested (*47*). This soluble adhesion molecule inhibited accumulation of lymphocytes to PLN as well as neutrophils to an acutely inflamed peritoneal site, presumably by competing with the L-selectin vascular endothelial ligand. Thus, L-selectin is involved with both PLN migration and acute inflammation.

At this time, a number of investigators were beginning to analyze the potential adhesive role of the selectins under conditions that more accurately mimicked those found in the vasculature. Leukocytes must adhere to the endothelium under conditions of high shear that are induced by the rheologic environment of the blood stream. Intravital microscopy studies, originally done over 100 years ago, demonstrated that leukocytes do not adhere tightly at the initiation of inflammation, but roll along the vessels at a rate that is approximately 100 times slower than the rate of blood flow (*48*). Because of this, several groups investigated leukocyte-endothelial cell interactions under shear conditions that were sim-

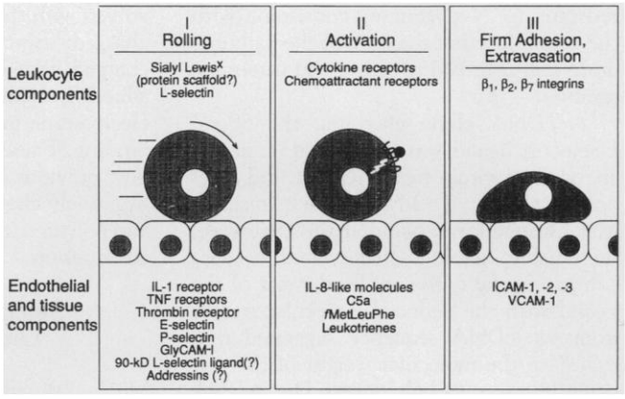

Fig. 2. Combinatorial aspects of leukocyte inflammation. Shown are the three major steps that lead to leukocyte inflammation and some of the various adhesion molecules, chemotactic factors, and cell surface receptors that are involved with the process. Step I is the initial low-affinity rolling interaction that is mediated by the selectins. Step II is the neutrophil activation event that is mediated by the concentration gradients of various chemotactic factors. C5A, chemotactic fragment of complement protein C5. Step III is the high-affinity adhesion, leukocyte shape change, and extravasation event that is mediated by the binding of various leukocyte integrins to their cognate endothelial ligands. Similar multistep models of inflammation have been previously presented (*51*). VCAM-1, vascular cell adhesion molecule–1.

ilar to those found in the post-capillary venules in vivo. Two groups used intravital microscopy of mesenteric blood vessels to demonstrate that either a soluble L-selectin–IgG chimera or monoclonal or polyclonal antibodies to L-selectin could significantly block the rolling of leukocytes (*48*). Because some of the reagents that block endothelial rolling also block inflammation, it would appear that the rolling mediated by L-selectin is a prerequisite for inflammation in vivo (*47*).

Two different in vitro approaches were taken to investigate the contributions of selectins to vascular adhesion during blood flow. In one, activated endothelial cells were used under conditions of shear to demonstrate that L-selectin mediates the initial binding of leukocytes to the endothelium (*49*). This initial, low-affinity event is presumably followed by a higher affinity binding mediated by the leukocyte integrins (*1*, *3*, *4*, *7*).

A different approach was taken by Lawrence and Springer who showed that isolated, immobilized P-selectin could mediate the rolling of leukocytes in laminar flow chambers when shear rates approached those found in post-capillary venules (*50*). This rolling, although done in vitro, appears to mimic that seen during intravital microscopy. When the cells were tested for rolling on the intercellular adhesion molecule–1 (ICAM-1), a ligand for the leukocyte β_2 integrins, they could only adhere in the absence of shear. However, if both P-selectin and ICAM-1 were immobilized together, than the leukocytes would roll until a neutrophil activator, formyl-methionyl leucyl proline (*f*MetLeuPhe), was added, at which time the neutrophils would stick tightly, presumably to ICAM-1, and undergo the shape change characteristic of neutrophil diapedesis. All of these data were consistent with a multistep, combinatorial process whereby selectins mediated the initial, low-affinity interactions with the vascular endothelium, after which a given leukocyte activator would mediate the induction of a higher affinity interaction determined by the leukocyte integrins (*51*). In this way, diverse selectins, leukocyte activation molecules, and integrins could be utilized in a combinatorial matrix to induce different leukocyte subpopulations to migrate to a given type of tissue during diverse inflammatory episodes (summarized in Fig. 2).

Antagonists of Selectin Function

The most compelling examples that indicate the potential for selectin antagonists in blocking the deleterious effects of inappropriate leukocyte inflammation come from a limited number of animal studies. As mentioned above, both MAbs to L-selectin and a soluble L-selectin–IgG chimera inhibit peritoneal inflammation, although the potential therapeutic benefit of this intervention was not investigated. A more interesting approach has been the use of MAbs to E-selectin to inhibited neutrophil-mediated damage to the vascular endothelium during an acute inflammation of the lung (*52*). This latter model is similar to the type of lung injury seen during the adult respiratory distress syndrome, and the results suggest that inhibition of E-selectin–mediated adhesion may be beneficial in such cases. Finally, there are other situations where selectin-mediated events may participate, including the movement of neutrophils to various sites during reperfusion injury and the accumulation of lymphocytes in the skin (*53*). Expression of E-selectin in skin blood vessels is particularly interesting, since this adhesion molecule may be involved in the accumulation of skin-homing memory T cells that could participate in such inflammatory skin diseases as psoraisis and contact dermatitis (*53*).

The nature of the drugs that would be used to inhibit selectin-mediated inflammation remains to be determined. Thus far, MAbs to selectins and a soluble receptor have been shown to be effective. It is possible that small molecules derived from the known structures of selectin carbohydrate ligands will prove to be the ultimate inhibitors (*54*). These molecules have a number of advantages, including probable lower manufacturing costs, potential oral and transdermal availability, and lack of antibody induction. It remains to be seen if the likely problems of low affinity and short half life can be surmounted. Suffice it to say that the discovery of the selectins and their ligands has opened up a new field in inflammation biology that will stimulate a diversity of investigations in basic research and drug development.

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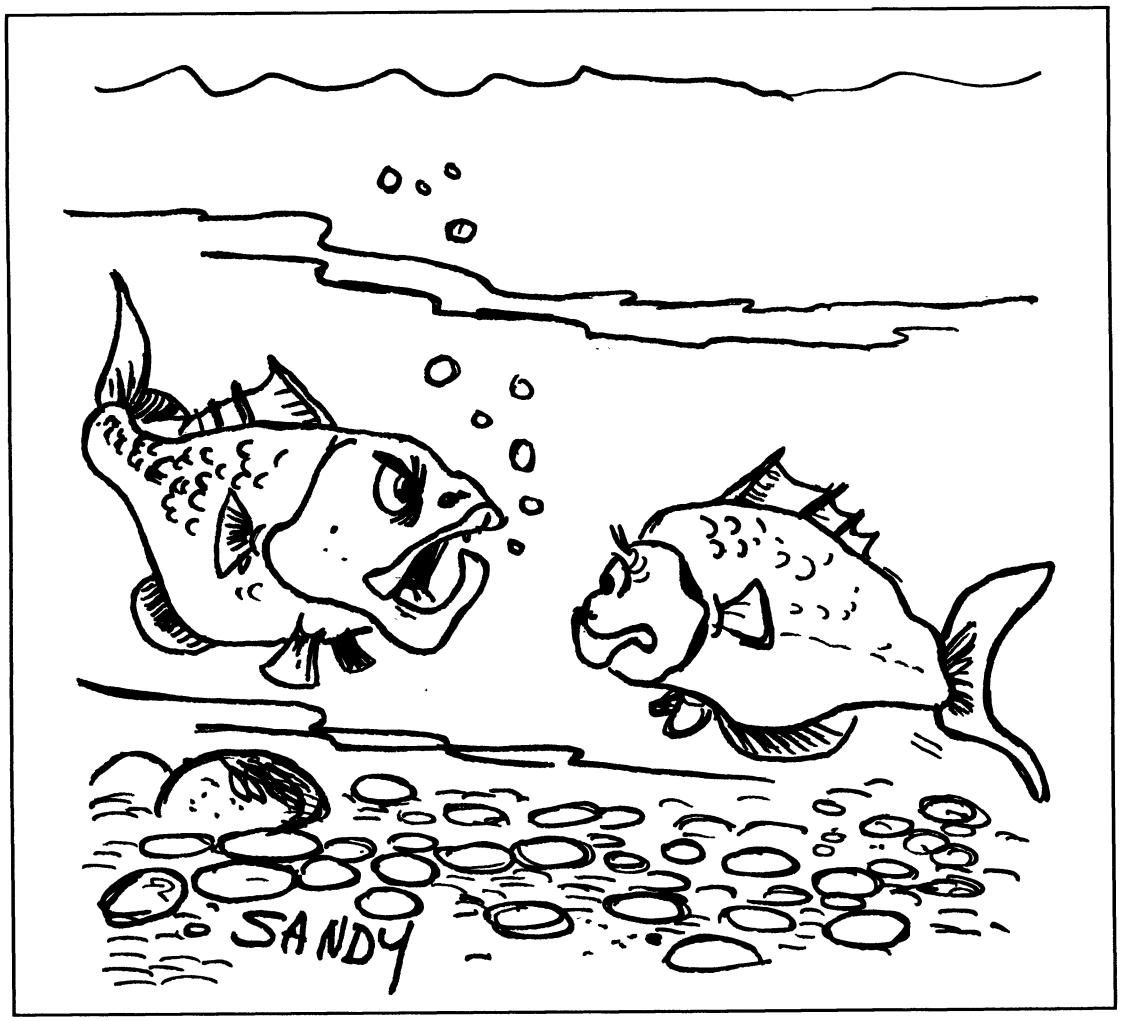

"I swim an ocean, fight my way 600 miles upstream past 300 starved grizzly bears, get pecked by a water ouzel, bash my nose on a boulder, and now* you *say you've got a headache?!"