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Review

Selectin ligands

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ABSTRACT **The selectins initiate many critical interactions among blood cells. The volume of information and diversity of opinions on the nature of the biologically relevant ligands for selectins is remarkable. This review analyzes the matter and suggests the hypothesis that at least some of the specificity may involve recognition of "clustered saccharide patches."**

Five years ago, the cloning of three vascular proteins resulted in identification of the selectin family of cell adhesion molecules (1–9). A shared N-terminal carbohydrate-recognition domain homologous to other Ca^{2+}-dependent (C-type) mammalian lectins (10) strengthened predictions from functional studies (for review, see ref. 1) that the cognate ligands for these receptors would be cell-surface carbohydrates (Fig. 1). Several groups then reported that previously known mammalian oligosaccharides bearing Fuc and sialic acid (Sia) are recognized in a Ca^{2+}-dependent manner by the selectins (11–24). Evidence for the function of selectins in leukocyte trafficking, thrombosis and inflammation, as well as the possibility for therapeutic intervention in reperfusion injury, inflammation, allergy, autoimmunity, and cancer (for review, see refs. 1–9) attracted additional investigators (>500 articles in the last 5 yr).* More recently, specific macromolecular ligands for the selectins have been reported (25–38). Molecular cloning of the polypeptide backbones of some of these molecules indicates that ligand formation is indeed glycosylation-dependent (28, 33, 34, 37, 39, 40). Some studies suggest that small sialylated, fucosylated oligosaccharides such as sialyl-Lewis^x (SLe^x) and sialyl-Lewis^a (SLe^a) (11, 12, 20, 22–24, 41–44) and/or their sulfated equivalents (45–47) are the ligands, whereas others indicate that complex-clustered saccharide motifs on specific glycoconjugates are required for biologically relevant recognition (28, 31). Some have even reported selectin ligands that lack Sia or Fuc (36, 43, 48–51). Because of recent excellent reviews on other aspects of selectin biology (3–9), this discussion will focus exclusively upon the nature of the selectin ligands.

Selectin Ligands: Historical Background. Early evidence for Ca^{2+}-dependent carbohydrate recognition by L-selectin came from the inhibitory effects of phosphorylated monosaccharides and a phosphorylated yeast mannan on the interaction of lymphocytes with high-endothelial venules (HEV) in lymph nodes (1). These studies were inspired by work on a different class of mammalian lectins, the mannose 6-phosphate receptors (52). However, sialidase treatment of lymph node sections abolished lymphocyte–HEV interaction, indicating a critical role for Sia (53). Subsequently, the selective inhibitory properties of various phosphorylated and sulfated saccharides (1) confirmed a lectin-like interaction. It is now evident that the selectin ligands are not themselves phosphorylated (nor always sulfated) and that the inhibitors worked either because of high concentrations or high charge density. In parallel, others (3) described monoclonal antibodies (mAbs) that recognized specific "addressins" involved in lymphocyte trafficking. The peripheral node addressin recognized by mAb MECA-79 (54) is a family of glycoproteins (gps), some of which interact with L-selectin in a Sia-dependent manner (25). Likewise, the mAb HECA-452 recognizes Sia-dependent epitopes of a proposed cutaneous lymphocyte antigen, which mediates recognition of skin-seeking lymphocytes by E-selectin (27). Others defined the ligands operationally, showing loss of selectin-mediated binding upon sialidase or protease treatment of the target cells (15, 18, 55–57). Taken together, these data indicated that specific carbohydrate ligands are recognized by selectins.

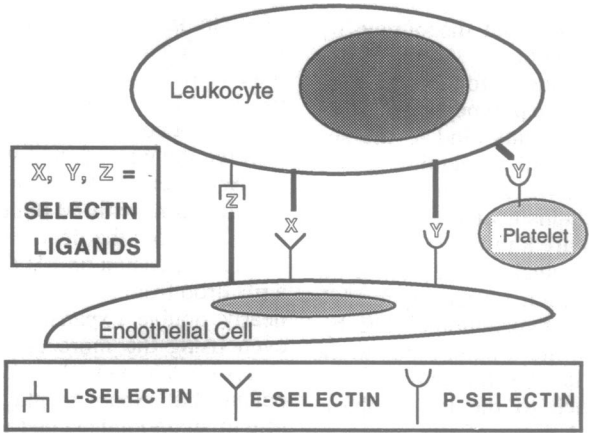

FIG. 1. Location and topology of the selectin family of cell adhesion molecules and their carbohydrate ligands. Expression of the selectins and their ligands may be constitutive or induced, depending upon the cell type, the tissue, and the biological circumstance. The selectins can also be shed from cell surfaces by proteolysis.

Many Diverse Carbohydrates Are Recognized by Selectins. A plethora of simple and complex carbohydrates have been reported as recognized by the selectins, on the basis of either direct binding experiments or inhibitory properties (see Table 1). Most, but not all, carry sialylated, sulfated, and/or fucosylated sequences normally found at the nonreducing termini of N-linked or O-linked oligosaccharides, or on glycosphingolipids. Such sequences are presented in Table 2 and contrasted with related sequences recognized by two other mammalian blood cell adhesion molecules, CD22 and sialoadhesin. The common feature of most of those recognized by selectins is a lactosamine backbone of either type 1 (Galβ1-3GlcNAc) or type 2 (Galβ1-4GlcNAc). When present, Sia is usually in α2-3 linkage, Fuc is in either α1-3 or α1-4 linkage, and the location of the Fuc residue relative to the Sia residue (determined by fucosyltransferase and cell type) is important (43, 58–60) (see Table 2). Conformational analyses suggest that a specific face of the SLe^x tetrasaccha-

Abbreviations: SLe^x, sialyl-Lewis^x; SLe^a, sialyl-Lewis^a; HEV, high-endothelial venule(s); mAbs, monoclonal antibodies; Sia, sialic acid; gp, glycoprotein.

*Because of space constraints the bibliography presented is necessarily incomplete.

ride involved in recognition is shared with the isomer SLe^a (22, 24, 61). Significant differences in binding can be generated by modifications of the GlcNAc or Sia N-acetyl groups (24, 62) or specific OH groups on the Fuc or Gal residues (63). In contrast, truncation of the Sia side chain has little effect upon recognition (24, 64).

Such data suggest a high specificity for the interaction with tetrasaccharides such as SLe^x. However, sulfate esters or uronic acids can substitute for Sia residues, and some neutral fucosylated chains can be recognized, at high densities or under specific experimental conditions (13, 20, 43, 65). Also, some sequences that lack Fuc residues are recognized (see Table 2), and P-selectin may recognize Sia residues in α2-6 linkage (63, 66). Thus, there now appears to be a wide and diverse range of candidate oligosaccharide ligands. However, selective inhibition of N- or O-linked glycosylation (38, 66–68) suggests that the biologically relevant ligands may not be carried on all glycoconjugate classes. A further difficulty is that if all three selectins recognized the same carbohydrate structure (e.g., SLe^x), the situation could be impractical because selectin and ligand might appear on the same cell (see Fig. 1). To explain these confusing data, one must carefully consider the difference between what *can* bind to a selectin in an *in vitro* assay and what *does* actually bind *in vivo*.

Importance of Assay Conditions Used to Identify Potential Ligands. *In vitro*, certain assay conditions can show selectin binding that may not be biologically relevant. For example, the use of TLC to detect potential ligands has the advantage that natural or artificial glycolipids can be separated into distinct molecular species, all of which can be probed simultaneously, with a visual readout. However, a potential disadvantage is that the oligosaccharides might be presented in an unnaturally high density, giving avid binding that might not occur if the same molecules were presented as minor components of a natural lipid bilayer. Likewise, the use of highly multivalent arrays of either selectins (e.g., as immunocomplexes) or of potential oligosaccharide ligands (e.g., as neoglycoproteins) can give a positive result that may not be relevant to the natural situation. The same is true of the use of transfected cell lines expressing very high densities of selectins. Also, static cell adhesion assays with long interaction times can show *in vitro* binding that may not be relevant in the dynamic flow situations encountered *in vivo*. In contrast, assays that use soluble molecules and/or stringent washing (e.g., direct binding of monomeric selectins, immunoprecipitation, affinity chromatography) are more likely to reveal biologically relevant binding of specific molecules. Likewise, cell interaction studies incorporating biologically relevant shear forces (e.g., in flow chambers or on rocker platforms) are more likely to reveal specific binding.

When Is Recognition by Selectins Biologically Relevant? Selectin ligands must have both the opportunity (be located at the right place at the right time), as well as adequate affinity or avidity (bind strongly enough to mediate specific biological recognition under natural circumstances). The selectins may not normally have the opportunity *in vivo* to recognize all structures listed in Table 1. Thus, some candidate oligosaccharides (e.g., SLe^a) have not been found on any of the relevant vascular cells (although type 1 chains could generate pathological ligands in carcinomas). Regarding binding strength, most studies of monomeric ligands (Table 1) report IC_{50} values (rough indicators of binding constants) in the μM–mM range (20, 51, 62, 63, 69, 70). One reported exception (71) might be explained by a 60*g* force used to induce initial cell binding. In contrast, the ability to use stringent techniques such as probing of blots, flow cytometry analysis, precipitation, and affinity chromatography (26, 29–33, 35, 36, 38, 40, 69, 72, 73) indicates that interactions with certain ligands involve much tighter binding. Indeed, in the few direct-binding studies

Table 1. General classes of molecules reported to be recognized by selectins

Class of molecules	Sources	Recognized by			Comments
		E-selectin	P-selectin	L-selectin	
Sialylated fucosylated lactosamine oligosaccharides	Widespread on N- and O-linked oligosaccharides, and on glycolipids	++	++	+	See Table 2; free oligosaccharides inhibit at high concentrations
Sulfated fucosylated lactosamine oligosaccharides	Tumor mucins, some normal mucins	+	++	++	See Table 2; free oligosaccharides inhibit at high concentrations
3-Sulfated glucuronosyl lipids	Brain glycolipids; some gps?	−	+	++	See Table 2; probably low-affinity ligands
Sulfatide	Various sources	−	++	+	See Table 2; not Ca^{2+} dependent?
Heparin (commercial)	Pig gut or bovine lung	−	++/−	++/−	Batch-to-batch variation in inhibitory effects?
Heparan sulfate proteoglycans	Cultured calf and human endothelial cells	−	++++	++++	See Table 3
Chondroitin sulfate	Various sources	−	+/−	+/−	Weak inhibition at high concentrations
Polyphosphomannan	Phosphorylated yeast polysaccharide	−	−	++	Also ligand for mannose 6-phosphate receptors
Fucoidan	Sulfated fucosylated seaweed polysaccharide	−	++	++	Sulfate and Fuc required for inhibitory action
Dextran sulfate	Semi-synthetic sulfated polysaccharide	−	++	++	Sulfate required for inhibitory action
Sialylated fucosylated mucin-type gps	Human and murine neutrophils, HL-60 cells	++	++++		See Table 3
Sialylated, sulfated, fucosylated mucin-type gps	Mouse lymph nodes	++		++++	See Table 3
Sialoglycoproteins, type unspecified	Human, bovine, and murine leukocytes	++++	++++		See Table 3

Recognition (based on binding or inhibitory capacity) by the selectins is indicated on an arbitrary scale of ++++ (best) to none (−).

Table 2. Some anionic oligosaccharide sequences recognized by mammalian lectins

Common name	Structure	Recognized by				
		E-selectin	P-selectin	L-selectin	CD22	Sialoadhesin
Sialyl Tn	Siaα2–6GalNAcα1-O-Ser/Thr				−	−
Sialyl T	Siaα2-3Galβ1-3GalNAcα1-R1/β1-R2					++
6′ Sialyl-LN	Siaα2-6Galβ1-4GlcNAcβ1-R3	−	+/−	−	++	−
3′ Sialyl-LN	Siaα2-3Galβ1-4GlcNAcβ1-R3	−	−	−	−	−
SLe^x	Siaα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-R3	++	++	+		
VIM-2 (CD65)	Siaα2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-R3	+/−	+	?+		
Sialyl dimeric $Lewis^x$	Siaα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)-GlcNAcβ1-R3	++	++	?+		
SLe^a	Siaα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-R3	++	++	+		
3′ Sulfo-$Lewis^x$	SO_4-3Galβ1-4(Fucα1-3)GlcNAcβ1-R3	+	++	++		
3′ Sulfo-$Lewis^a$	SO_4-3Galβ1-3(Fucα1-4)GlcNAcβ1-R3	+	++	++		
Sulfatide	SO_4-3Galβ1-′1-ceramide	−	+	++		
3′ Sulfo-LN	SO_4-3Galβ1-4GlcNAcβ1-R3					
SGNL (HNK-1)	SO_4-3GlcAβ1-4Galβ1-4GlcNAcβ1-R3			+		
GNL	GlcAβ1-4Galβ1-4GlcNAcβ1-R3			+		

Recognition by the individual selectins is indicated on an arbitrary scale of ++ (best) to none (−). R1, O-linked oligosaccharide; R2, ceramide-linked oligosaccharide; R3, O-linked, N-linked, or ceramide-linked oligosaccharide; GlcA, glucuronic acid.

with soluble selectins, affinities in the low nanomolar range are reported (18, 48, 73). Although the data set is incomplete, it seems to predict that biologically relevant recognition involves higher order structures that can generate a sufficient affinity (or avidity) to function *in vivo*.

Sialylated Fucosylated Lactosamines Are Necessary Components of Selectin Ligands in Humans. In a human genetic disorder called leukocyte adhesion deficiency type II (74), a general failure of fucosylation reduces expression of all fucosylated lactosamines and is associated with markedly diminished selectin-mediated binding both *in vitro* and *in vivo* (74, 75). While the patients have other unrelated abnormalities probably due to the Fuc deficiency, this experiment of nature indicates that fucosylated lactosamines are critical components of many biologically relevant selectin ligands in humans. However, the clinical abnormality is not complete, and some leukocyte trafficking can still occur in these patients, possibly due to alternate ligands that lack Fuc (36, 49–51).

Lack of Conservation of Selectin Ligands Among Species. Recognition of ligands by selectins is preserved across a variety of mammalian species (76, 77). Thus, L-selectin from one species can recognize lymph node HEV ligands in other species (36, 64, 76, 78), and cells from one species can show selectin-dependent interactions within the vasculature of others (75, 79, 80). However, studies using mAbs indicate that sialylated fucosylated lactosamines such as SLe^x are not expressed on the neutrophils of many mammals, including some rodents and nonhuman primates (81, 82). Despite this, *in vivo* blocking studies with SLe^x-based oligosaccharides (83–85) in rats indicate that their endogenous selectins can recognize these ligands. One explanation is that the nonhuman oligosaccharide ligands are masked from mAb recognition by Sia modifications or by other unknown subtleties of structure (86, 87). However, these experiments were done with an extensive mAb panel of overlapping specificities (82). A better explanation is that during evolution the binding pocket of a selectin (derived from amino acids) is less likely to have diverged than the ligands which (based on oligosaccharide sequences) are under independent selective pressures. Since sugar chains are more flexible than polypeptides, they may also be more capable of accommodating structural changes, while still preserving critical three-dimensional features for recognition. Thus, the conserved binding pockets of the selectins could cross-react with different carbohydrate ligands in different species, each of which can generate the same three-dimensional recognition structure. These observations indicate that the biologically relevant selectin ligands are unlikely to be linear, well-defined oligosaccharide sequences and may be complex structures that can be generated in more ways than one.

Mechanisms by Which Monovalent Oligosaccharide Chains Could Generate Selectin Ligands with Enhanced Affinity or Avidity. Studies of many plant and animal lectins (10, 88) indicate that monovalent carbohydrate ligands usually do not have high affinity. Similarly, most reported IC_{50} values for monovalent selectin ligands are rather high (20, 51, 62, 63, 69, 70). There are several ways in which monovalent oligosaccharide chains could generate the enhanced binding seen with biologically relevant selectin ligands (Fig. 2). Simple multivalency of both oligosaccharide and selectin by presentation on intact cell surfaces could enhance avidity (Fig. 2*A*). Oligosaccharide multivalency could be more efficiently generated by a polypeptide backbone (Fig. 2*A*). This has indeed been suggested for some high-affinity selectin ligands that are mucins (28, 31, 33, 34) which have many oligosaccharides, closely spaced to one another. However, the IC_{50} of chemically synthesized dimeric SLe^x for E-selectin showed only a 5-fold improvement over the monomeric form (70). Further, P- and L-selectin do not bind to some cell types that express considerable amounts of SLe^x, and myeloid cell recognition by P-selectin is destroyed by the enzyme *O*-sialoglycoprotease (31, 73, 89), even though the vast majority of surface SLe^x remains intact (31). Thus, simple ligand multivalency seems insufficient to explain biologically relevant selectin binding. Another possibility is multivalent aggregation of the selectin (Fig. 2*B*). However, high-affinity binding of soluble monomeric P-selectin to cell surfaces (73) indicates that this is not essential. Also, there is no published evidence for naturally occurring multimeric selectins. Ligand multivalency provided by cell surfaces or specific polypeptides could also be recognized by multiple binding sites within a single selectin lectin domain (Fig. 2*C*). However, this is negated by studies of the lectin domains of the selectins by epitope mapping, mutagenesis, and homology criteria (90–93), which indicate binding sites for carbohydrates that are quite small; this has been recently confirmed for E-selectin by x-ray crystallography (94). Fig. 3 shows that there is barely enough room in the E-selectin lectin-binding domain to accommodate one copy of a SLe^x tetrasaccharide, let alone many closely spaced units. Thus, simple ligand or selectin multivalency does not appear sufficient to explain high-affinity recognition. Another possibility is a combination of both a basic oligosaccharide sequence and an adjacent peptide-recognition site

(Fig. 2*D*). Some studies involving mutagenesis, domain swapping, or deletion of the other protein domains of selectins seem to support this (95–97). However, the persistence of high-affinity recognition after extensive denaturation or proteolytic digestion of some biologically relevant selectin ligands (28, 29, 31, 36, 69) is against this. These observations also make it unlikely that the peptide carrier forces a monovalent oligosaccharide into an unusual conformation favored for recognition (Fig. 2*E*). By exclusion, the remaining possibility is that high-affinity binding involves recognition of a "clustered saccharide patch" (31) created by a peptide carrying multiple oligosaccharides (Fig. 2 *F* and *G*).

Free oligosaccharides in solution or at single attachment sites have significant freedom of mobility and show limited interactions with associated proteins (98). However, oligosaccharides packed closely together (particularly in mucins) can form rigid rod-like structures (99, 100) that must have less mobility and yet are relatively unaffected by denaturation of the polypeptide backbone. Such clustering of *common* oligosaccharides could present *uncommon* "clustered saccharide patches" generated by multiple oligosaccharides that are closely spaced enough to restrict their motion (31). The patch recognized by a selectin could be generated in two possible ways. In the first case (Fig. 2*F*), a common oligosaccharide with low affinity for the selectin (e.g., SLe^x) might be forced by other surrounding sugar chains into an unusual conformation favored for high-affinity recognition. This could also be aided or stabilized by peptide interactions with the adjacent sugar chains. The adjacent sugars might also induce a favorable conformational change in the selectin lectin domain itself. In the second case (Fig. 2*G*), the binding site would be generated by combinations of side chains and groups from multiple oligosaccharides—i.e., a "discontiguous saccharide epitope." Thus, the patch recognized by the selectin might include, for example, a hydroxyl group from one sugar chain, an acetyl group from another sugar chain, and a carboxyl or sulfate group from a third chain. In both cases, the polypeptide need not be part of the selectin recognition site. However, it would be crucial for presenting the sugar chains in the correct arrangement. Thus, high-affinity recognition would be lost if the arrangement is disrupted, and the individual sugar chains may not have detectable affinity for the selectin. This might explain why most reported high-affinity selectin ligands are heavily glycosylated macromolecules, and why free oligosaccharides released from such ligands do not bind with recognizable affinity to the same selectin (36, 69). Such recognition would be distinct from improved binding of certain lectins to multiantennary N-linked oligosaccharides or to glycopeptides with widely spaced O-linked oligosaccharides (88). This results from correct spacing of terminal sugar residues, each of which are still recognized as distinct components of individual chains. The hypothesis also predicts that structural analyses of O-linked oligosaccharides released from a specific selectin ligand might not uncover unique sequences required for recognition. Therefore, it may be necessary to completely elucidate both the polypeptide chain sequence and the oligosaccharide structures, as well as their specific sites of attachment on the protein. Ultimately, one must reconstruct the ligand and define the position of each of its components in three-dimensional space.

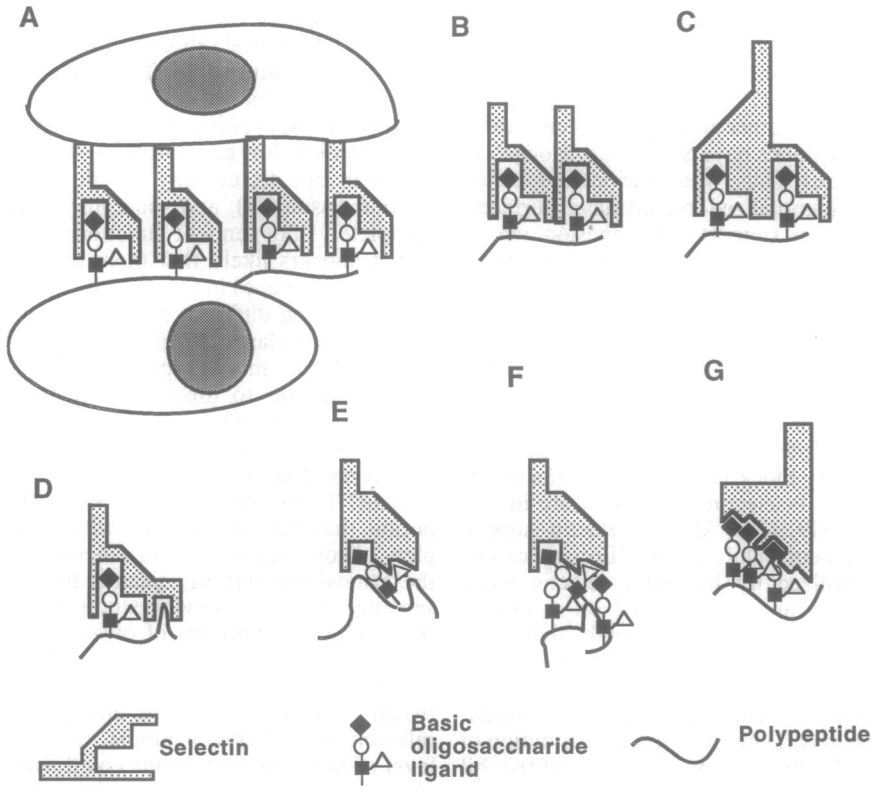

FIG. 2. Models that could explain improved binding of selectins to their natural ligands relative to the basic oligosaccharide ligands they can recognize. As discussed in the text, possibilities *A–E* cannot explain many observations concerning selectin binding. *F* and *G* present the concept of "clustered saccharide patches" that could reconcile these data.

There is, in fact, much prior evidence for recognition of "clustered saccharide patches." The enzyme *O*-sialoglycoprotease (101), which has been of particular value in identifying high-affinity selectin ligands, will only recognize and cleave mucins with large numbers of closely spaced O-linked oligosaccharides (31). Many mAbs that detect specific glycoproteins have "sialidase-sensitive" or "Sia-dependent" epitopes—e.g., cancer mucins, CD24, CD45, and CD43 (see ref. 31 for citations). With some heavily glycosylated O-linked gps, almost all available mAbs require glycosylation for specific recognition. Many such proteins with sialidase-sensitive epitopes are either mucins with clustered O-linked oligosaccharides or have large numbers of N-linked oligosaccharides. However, when the sialyl-oligosaccharides on such proteins are examined, they are not unusual in structure compared with those from other proteins of the same cells. In a well-documented example, the recognition of a common sialylated oligosaccharide sequence by different mAbs differed depending upon the type of glycoconjugate attached (102). Other examples include the reactivity of a mucin-specific lectin from *Sambucus sieboldiana* (103), recognition of clustered O-linked oligosaccharides on glycophorin A by the invasion receptor of *Plasmodium falciparum* (104), the specific binding of rotaviruses to intact mucins (105), and recognition of the sperm receptor on the mucin-type zona pellucida glycoprotein 3 of the mouse egg (106). In such instances, unique clustering of relatively common oligosaccharides can explain the specificity of recognition.

Hypothesis: A Clustered Saccharide Patch Could Be Shared by Seemingly Disparate Glycoconjugates. Selectins can also recognize glycosaminoglycan chains in a Ca^{2+}-dependent manner (23, 36, 48, 51, 107). Glycosaminoglycans have repeating disaccharide structures that do

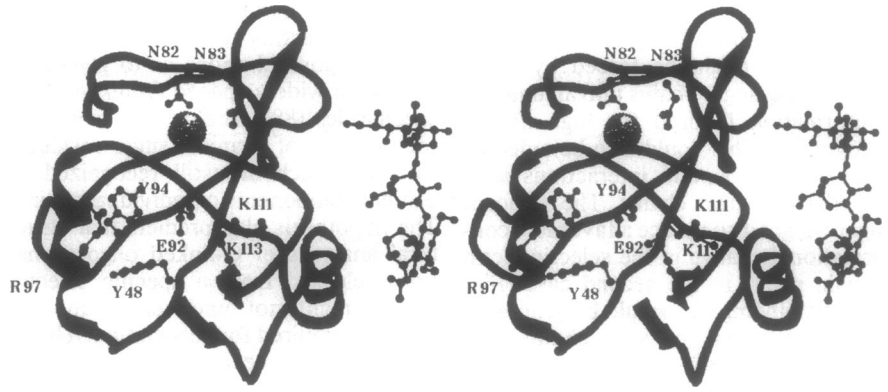

FIG. 3. Size comparison of the E-selectin lectin domain and SLe^x (courtesy of B. Graves, based on ref. 94). Stereo diagram of a "top" view of the E-selectin lectin domain, alongside an SLe^x tetrasaccharide. The protein is depicted as a ribbon and tube diagram, with explicit atoms shown for the side chains of the eight amino acid residues known to affect carbohydrate binding and for the calcium ion (with a van der Waals dot surface around it).

not typically contain Sia or Fuc (108) but have variable numbers of sulfate esters and uronic acids. Competition experiments and Ca^{2+} dependence suggest that the binding site is the same or overlapping with that for sialylated fucosylated oligosaccharides. How could two such disparate structures be recognized by the same binding site? One explanation is that a "clustered saccharide patch" recognized by a selectin can be generated by different types of glycoconjugates. Thus, the patch generated by a cluster of O-linked oligosaccharides on a mucin type glycoprotein might be mimicked by a proper arrangement of carboxyl, hydroxyl, and sulfate groups on a heparan sulfate glycosaminoglycan chain. In the latter case, the chain must generate the recognition site itself, because release from the polypeptide backbone does not cause loss of binding (36). This hypothesis could also explain why relatively high-affinity binding of selectins is seen when glycosphingolipids are immobilized at high density on plates or on TLC plates (43, 46). Here, the clustered saccharide patch might be artificially recreated on the immobile surface because of a high density not normally found on cell surfaces. However, because glycosphingolipids can coaggregate in patches on cell surfaces (109), this could also be a natural mechanism for generating specific selectin recognition.

Selectin Ligands That Are Probably Biologically Relevant. From the foregoing, most, if not all of the biologically relevant selectin ligands appear to be complex macromolecules. Table 3 lists such candidate ligands chosen based on expression in the right cell type and demonstrated ability to bind with high affinity to selectins in a Ca^{2+}- and carbohydrate-dependent way. The definition of "high affinity" is somewhat arbitrary and based upon ligand binding surviving extensive washing during procedures such as direct binding to cells, affinity chromatography, or detection by blotting. Most, but not all, of these ligands are mucin-type gps. Less well-characterized ligands not listed in the table include multiple sulfated gps found in a rat-HEV derived cell line Ax (30), 230-kDa and 130-kDa P-selectin ligands reported on murine myeloid cells and HL-60 cells (38), a sialidase-resistant ligand found on central nervous system myelin (110), sialidase-sensitive ligands on activated endothelial cells (55–57), and a variety of carcinoma-associated ligands (44, 111–113). Also not included are various neutrophil gps bearing SLe^x that are recognized by E- and/or P-selectin *in vitro*, including LFA-1 (114, 115), nonspecific cross-reacting antigens CD66/CD67 (116), LAMP-1 (117), and L-selectin itself (118). Like most other neutrophil proteins, these polypeptides have SLe^x-bearing oligosaccharides. Thus, when purified in quantity and immobilized on artificial surfaces, they can support cell adhesion via E- and/or P-selectin. However, their role in presenting high-affinity, biologically relevant ligands in the natural situation is unclear. A particularly confusing case is that of the L-selectin molecule on neutrophils, which has been suggested as a major presenter of ligands for P- and E-selectin (118). Indeed, anti-L-selectin antibodies partially block binding in systems where E- and P-selectin recognition are important (119, 120). Also, L-selectin isolated from neutrophils carries SLe^x and can be recognized by E- and P-selectin when purified and immobilized on a surface (118). However, neutrophils that have shed L-selectin continue to bind well (18), as do many cells (e.g., HL-60) that lack L-selectin. Thus, an alternative explanation may lie in the unusual distribution of L-selectin on the tips of neutrophil pseudopodia (118). Perhaps the true ligands for E- and/or P-selectin are also located in the same favored site for interactions; if so, anti-L-selectin antibodies may act by locally hindering the recognition of these other ligands. It is also difficult to know whether to include specific glycosphingolipids among the biologically relevant ligands. Molecules such as sulfatide show binding (47, 49) to some selectins, but this is not always Ca^{2+}-dependent, and a large number of unrelated adhesion proteins are known to bind to this small sulfated glycolipid (121). SLe^x-bearing glycosphingolipids can clearly support E-selectin-based binding when purified and immobilized on artificial surfaces. However, whether they can attain similar densities on cell surfaces is unknown.

Nomenclature Difficulties. The nomenclature for the low-affinity monomeric oligosaccharide structures recognized by selectins is straightforward and is simply a description of their structures (Table 2). There is also no problem with the nomenclature of ligands defined by mAbs recognizing multiple and/or unknown species with unusual carbohydrate epitopes—e.g., peripheral node addressin (MECA-79) (25), cutaneous lymphocyte antigen (HECA-452) (27), and SLe^x-var (2F3) (86). These antibodies were important in understanding selectin-mediated recognition—e.g., MECA-79 reacts with cells that bear L-selectin ligands (25, 34) and with ligands from murine lymph nodes (26, 69). However, they may also react with related carbohydrate epitopes on other molecules that are not ligands for the selectins. Regarding the discrete biological ligands for the selectins (Table 3), there are several difficulties with current nomenclature. (*i*) The different names and terms do not relate well to each other. (*ii*) A polypeptide that acts as a scaffolding for generating a selectin ligand in one tissue may not do so in another tissue (39), and some may have completely independent functions (34). (*iii*) It appears likely that there are significant differences in functionally active ligands among different animal species. (*iv*) Different classes of glycoconjugates (N-linked gps, mucin-type gps, proteoglycans) appear to function as ligands. The issue of nomenclature for these ligands deserves more attention.

Is Weak Binding Also Biologically Important? This discussion has favored the notion that macromolecules with complex oligosaccharide arrangements are the critical high-affinity ligands for the selectins. However, *in vitro* studies show that high-copy numbers of oligosaccharides such as SLe^x on cell surfaces can give weak, but detectable, binding between cells. Is such binding biologically relevant *in vivo*? For example, the high level of SLe^x on neutrophils could actually be responsible for the *initial* recognition of a neutrophil by E- or P-selectin on an endothelial cell. This could be necessary, but not sufficient, to initiate rolling, serving as the first recognition

Table 3. Natural selectin ligands likely to be of biological relevance

Original name	Source	Polypeptide name	Natural ligand for	Recognition requires			Comments	Refs.
				Sia	Fuc	SO_4		
sgp50 HEV L-selectin ligand	Mouse peripheral lymph node	GlyCAM-1	L-selectin	+	+?	+	Primarily secreted—a natural antiadhesin? Susceptible to *O*-sialoglycoprotease	26, 28
120-kDa myeloid P-selectin ligand	Human neutrophil, HL-60 cells	PSGL-1	P-selectin	+	+		Cell surface homodimer—minor component of total surface gps. Susceptible to *O*-sialoglycoprotease. Murine homologue?	29, 31, 37
150-kDa myeloid E-selectin ligand	Mouse bone marrow cells (80% neutrophils)	Polypeptide unknown	E-selectin	+			N-linked oligosaccharides required for binding. Resistant to *O*-sialoglycoprotease. Human homologue?	32, 38
sgp90 sulfated L-selectin ligand	Mouse peripheral lymph node	CD34	L-selectin	+		+	Polypeptide also present in nonlymphoid endothelium—not a ligand in these locations	33
MAdCAM-1	Mouse mesenteric lymph node	MAdCAM-1	L-selectin	+			Polypeptide also present in Peyers patch HEV—not a major ligand in this location because glycosylation is not appropriate	34
250-kDa E-selectin ligand	Bovine γ/δ lymphocytes	Polypeptide unknown	E-selectin	+			Isolated by affinity chromatography	35
Heparan sulfate	Bovine and human endothelial cells	Core protein not required	L-selectin	−		+	Isolated by affinity chromatography from cultured cells—subcellular location and function in intact tissues not proven	36
160-kDa myeloid P-selectin ligand	Mouse bone marrow cells (80% neutrophils)	Polypeptide unknown	P-selectin	+			Susceptible to *O*-sialoglycoprotease N-linked oligosaccharides also required for binding? Homologue in human HL-60 cells?	32, 38

sgp, Sulfated gp; PSGL-1, P-selectin gp ligand 1; MAdCAM, mucosal addressin cell adhesion molecule; GlyCAM-1, glycosylation-dependent cell adhesion molecule 1.

step that permits subsequent interactions with the higher-affinity (but lower copy number) macromolecular ligands on the same cell. Such recognition could also be important pathologically in carcinoma cells, which have high levels of SLe^x and/or SLe^a on their cell surfaces (44, 111–113).

Are Some "Ligands" Actually "Antiadhesins"? In at least two instances a portion of the ligand synthesized by cells *in vitro* is found in the culture medium—i.e., glycosylation-dependent cell adhesion molecule (28) and heparan sulfate proteoglycans (36) (K. Norgard-Sumnicht and A.V., unpublished work). These soluble ligands could act as inhibitors of selectin binding rather than as adhesion molecules. Whether their potential antiadhesive function is biologically relevant remains to be seen.

Are the Ligands Shared Between Different Selectins and Between Different Leukocyte Types? To date, most attention has been directed toward the recognition of high-endothelial venule ligands by L-selectin on lymphocytes and of neutrophil ligands by endothelial E- and P-selectin. However, monocytes and eosinophils possess ligands for E-selectin, which are functional in interaction with immobilized selectins or activated endothelium *in vitro* (56, 122–124), and distinct subsets of lymphocytes carry ligands for P- or E-selectin (27, 72, 87, 125, 126). The nature of these ligands, their structural relationship to the neutrophil ligands, and their importance in the *in vivo* situation remain uncertain. If the ligands are shared among different leukocytes, the differential trafficking of cell types might be explained by the multistep combinatorial nature of leukocyte extravasation (127).

Most studies have focused upon the interaction of a single selectin with a single ligand from a particular cell type. However, the overlap in recognition of small oligosaccharides by the selectins (20, 41, 42, 51, 62) predicts that overlaps may also exist with regard to the macromolecular ligands. While some overlaps have been seen (38, 66, 73, 91, 128), interactions with a particular ligand are not comparably strong between the selectins. Detailed comparisons of the recognition of specific ligands by the three selectins are needed.

Therapeutic Inhibition of Selectins *in Vivo* by Ligand Analogs—Problems and Prospects. The development of selectin ligand analogs that can interfere *in vivo* has obvious therapeutic potential in a variety of pathological processes (3–9). The use of sugar analogs or related structures is attractive also because of their relative lack of immune reactivity, as well as their ease of handling for therapeutic use. The small SLe^x-based therapeutics currently entering clinical trials (J. Paulson, personal communication) have the potential to partially block the functions of all three selectins. Studies of the complex macromolecular ligands listed in Table 3 could lead to design of specific analogs that discriminate between selectins and permit selective blockade in specific disease states. Additional considerations include a better half-life in the circulation and enough affinity to use small amounts for sustained blockade of selectin function *in vivo*.

Future Directions. There are many other unexplained mysteries and interesting questions regarding selectin ligands that deserve investigation. The fact that selectin ligands survive treatments such as heating, ionic detergents, protease treatment, and organic extraction (26, 28, 29, 31, 36, 69) suggests that primary recognition involves carbohydrates alone. However, other domains of the selectins could be recognizing other as-yet-unidentified polypeptides via protein–protein interactions. These could be important in facilitating or modulating some recognition events mediated by the selectins. The epidermal growth factor-like and complement-regulatory-like domains of the selectins are candidates for such interactions. The "variant" forms of

SLe^x on blood cell surfaces detected by specific mAbs such as HECA-452 (22) and 2F3 (86) remain unexplained. Another mystery is the fact that concentrations of small oligosaccharides that seem inadequate to block selectin function *in vitro* are surprisingly successful in abrogating some selectin-mediated pathological reactions in the intact animal (83, 84, 129). They may function by an alternative mechanism *in vivo*—e.g., by inducing down-regulation of selectin expression on cell surfaces (130, 131). Regarding the high-affinity cell-specific macromolecular ligands, it is possible that they have other biological functions mediated by their ligation—e.g., the binding of P-selectin to neutrophils causes biological responses (132). Another area of confusion is the description of "partially Ca^{2+}-dependent" ligands, seen particularly in the interactions of P- and L-selectin with sulfated ligands. Thus, addition of excess EDTA to chelate Ca^{2+} sometimes may not completely reverse a selectin-based binding. This may occur despite the fact that the primary binding requires Ca^{2+}. One explanation is that ligand-dependent activation or an "induced-fit" might occur, such that Ca^{2+} is required for initial binding but not for subsequent maintenance of the complex. Alternatively, a Ca^{2+} could form a very tight complex with the ligand after initial binding, which might then be very difficult to remove without prolonged EDTA exposure. Regardless, these and other data (47, 49, 93, 97) suggest that P- and L-selectin may have a sulfate-recognition site whose function is not strictly Ca^{2+}-dependent. Finally, most studies on the role of selectins in neutrophil traffic have focused upon pathological situations. It is of interest to know if the selectins are involved in normal turnover and/or "margination" of neutrophils, which have very short half-lives in circulation.

Summary and Conclusions. Much evidence suggests that the biologically relevant ligands for the selectins are diverse and complex macromolecules that share in common certain types of anionic carbohydrate structures. While details regarding these molecules begin to emerge, it remains to be seen which ligands are important for specific biological processes involving the selectins. High-affinity recognition of seemingly disparate oligosaccharides may involve the formation of similar "clustered saccharide patches." If so, fully understanding selectin–ligand interactions will require defining the precise three-dimensional structures of both partners. Such knowledge could also permit the design of a more specific and efficacious therapeutic ligand analog to be used for intervention in the many disease processes involving selectins.

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