Minimal Sulfated Carbohydrates for Recognition by L-selectin and the MECA-79 Antibody*

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Sulfated forms of sialyl-LeX containing Gal-6-SO4 or GlcNAc-6-SO4 have been implicated as potential recognition determinants on high endothelial venule ligands for L-selectin. The optimal configuration of sulfate esters on the N-acetyllactosamine (Galβ1→4GlcNAc) core of sulfosialyl-LeX, however, remains unsettled. Using a panel of sulfated lactose (Galβ1→4Glc) neoglycolipids as substrates in direct binding assays, we found that 6′,6-disulfolactose was the preferred structure for L-selectin, although significant binding to 6′ and 6-sulfolactose was observed as well. Binding was EDTA-sensitive and blocked by L-selectin-specific monoclonal antibodies. Surprisingly, 6′,6-disulfolactose was poorly recognized by MECA-79, a carbohydrate- and sulfate-dependent monoclonal antibody that binds competitively to L-selectin ligands. Instead, MECA-79 bound preferentially to 6-sulfolactose. The difference in preferred substrates between L-selectin and MECA-79 may explain the variable activity of MECA-79 as an inhibitor of lymphocyte adhesion to high endothelial venules in lymphoid organs. Our results suggest that both Gal-6-SO4 and GlcNAc-6-SO4 may contribute to L-selectin recognition, either as components of sulfosialyl-LeX capping groups or in internal structures. By contrast, only GlcNAc-6-SO4 appears to contribute to MECA-79 binding.

L-selectin, the “leukocyte selectin,” mediates the tethering and rolling of lymphocytes along high endothelial venules (HEVs) in peripheral lymph nodes, a prerequisite for extravasation of the lymphocytes (1–3). By virtue of a calcium-type lectin domain at its amino terminus, L-selectin functions as a calcium-dependent, carbohydrate-binding receptor that recognizes a set of discrete counter-receptors (generally termed ligands) displayed on the luminal aspect of HEVs (reviewed in Refs. 4 and 5). Several HEV-expressed, L-selectin-reactive ligands (all of which possess mucin-like domains) have been identified as potential physiological ligands for L-selectin (reviewed in Ref. 6). These include GlyCAM-1 (7), CD34 (8, 9), and podocalyxin (10).

A large body of evidence has established that the optimal interaction between L-selectin and these HEV-expressed ligands requires sialylation, fucosylation, and carbohydrate sulfation (11–17). In an attempt to rationalize these requirements in terms of carbohydrate structures, a detailed analysis of the O-linked oligosaccharide side chains of GlyCAM-1 was conducted (18–20). Sulfation analysis of acid-hydrolyzed glycans revealed monosulfated monosaccharides and disaccharides (N-acetyllactosamine) with equivalent levels of Gal-6-SO4 and GlcNAc-6-SO4. Analysis of the simplest oligosaccharide side chains identified equivalent levels of two novel sulfated isomers of sialyl-LeX (sLeX), 6′-sulfo-sLeX, containing Gal-6-SO4, and 6-sulfo-sLeX, containing GlcNAc-6-SO4 (see Fig. 1), as capping groups of core-2 branched oligosaccharides. The finding of a sLeX motif was significant because this tetrasaccharide binds to all three selectins (reviewed in Refs. 21–25), although weakly.

Following the identification of the 6′- and 6-sulfo forms of sLeX within GlyCAM-1, a number of studies have been directed at examining the binding of these and analogous structures to L-selectin (reviewed in Ref. 26). There is general agreement that sulfation at C-6 of GlcNAc, in the context of 6-sulfo-sLeX, enhances L-selectin binding relative to sLeX (27–29). Similar findings have been obtained using 3′-sulfo-LeX and 3′-sulfo-LeX as mimetics of sLeX (28, 30–32). Furthermore, Kannagi and coworkers (33, 34) have described 6-sulfo-sLeX-reactive antibodies that stain HEVs and inhibit L-selectin binding to HEVs. With respect to the contribution of Gal-6-SO4, several groups have reported that this modification of sLeX or of sLeX mimetics either enhances or does not affect L-selectin binding (29, 30, 35–37). In marked contrast, Feizi and co-workers found that this modification eliminates binding to L-selectin (28, 38).

Previously, in an attempt to understand the contribution of the two sulfate esters in question to L-selectin binding, we synthesized a limited series of sulfated lactose derivatives (39). As inferred from the ability of these compounds to compete the binding of an L-selectin/IgG chimera to GlyCAM-1, 6′,6-disulfocolactose (containing sulfate esters in positions analogous to those in 6′,6-disulfo-sLeX) exhibited significant affinity for L-selectin. In the present study, we have synthesized a more extensive series of mono- and disulfated lactose derivatives with various combinations of sulfate esters on Gal and Glc. These compounds were derivatized with lipid tails, creating neoglycolipids that could be coated onto wells of microtiter plates. Thus, direct binding studies could be done with L-selectin chimeras and L-selectin bearing lymphocytes, allowing specificity controls to verify that the observed binding was functionally relevant. These studies have led to the definition of the minimal sulfated structures that support recognition by...
L-selectin. In addition, we employed these compounds to investigate the epitope corresponding to MECA-79, a widely used monoclonal antibody (mAb) that recognizes HEV-expressed ligands for L-selectin in human and other species (6, 40). The MECA-79 epitope has been shown to be sulfation-dependent, but the structural context for this modification has heretofore not been defined (14–16). In the present study, we find that L-selectin favors the disulfated lactose derivative with modifications at both C-6 of Gal and C-6 of Glc, whereas MECA-79 favors the monosulfated lactose derivative bearing Glc-6-SO4.

**EXPERIMENTAL PROCEDURES**

**General**—Ninety-six-well polyvinyl chloride microtiter plates (Falcon 3912) were from Becton Dickinson, and 96-well polystyrene plates (Immulon II) were from Dynatech Laboratories, Inc. (Alexandria, VA). The siyali-Le6 analog neoglycolipid (αLe6), \(N^3(O(3-O(-2-aceic acid)-\beta-D-galactopyranosyly)-(1-4)-O-(\alpha-L-fucopyranosyl)-(1-3)-O(\beta-D-glucopyranosyl)-1-acetamido-6-(10,12-pentacontadynamide)-3-thioxane,\) was a gift of Dr. Jon Nagy. Sulfated lactose neoglycolipids were synthesized and verified as described (3). Cholesterol, phosphatidylcholine, bovine serum albumin (BSA), ELISA, and p-nitrophenyl phosphate (phosphatase substrate) were from Sigma. BPEC/PMAM was from Calbiochem. Allyl diisopropylethylamine, dimethylformamide, and steryl chloride were from Aldrich.

**Sialylactose Neoglycolipid**—The sialylactose neoglycolipid was synthesized from 1-\(\beta\)-O-allyllactose by first forming an N-allyl glycoside (42) and then coupling the glycosylamine to steroyl chloride. A solution of 0.65 g (1.0 mmol) of sialyllactose (NeuAc\((4\rightarrow2)\) and then coupling the glycosylamine to steroyl chloride. A solution of 0.65 g (1.0 mmol) of sialyllactose (NeuAc\((4\rightarrow2)\)) was suspended in 40% aqueous acetonitrile, frozen, and lyophilized to yield \(C_{44}H_{78}N_{2}O_{19}\) was 938.52, and the measured mass was 961.6 \([M + Na]^+\). The neoglycolipids were resuspended in \(400 \mu g/ml\) cholesterol and 2.5 \(\mu g/ml\) phosphatidylcholine. Serial dilutions were coated onto polyvinyl chloride microtiter plates at 40 \(\mu g/well\), and the solvent was evaporated at 37°C. It is assumed in enzyme-linked immunosorbent assays of this type that the input concentration of lipid accurately predicts the coating concentration. The finding that the rank orders of reactivity of the neoglycolipids for L-selectin, E-selectin, and MECA-79 are very distinct (see “Results”) indicates that the differences are not just due to variable amounts of lipid coated. The coated wells were washed once with distilled water and then blocked with 3% BSA in PBS for 2 h.

**Minimal Recognition Determinants for L-selectin and MECA-79**

**Cell Adhesion Assay**—L-selectin-expressing Jurkat T-cells were obtained from the laboratory of Dr. Arthur Weiss and maintained in RPMI 1640 medium supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 2 \(\mu g/ml\) glutamine, and 5% heat-inactivated fetal calf serum (HyClone Laboratories, Logan, UT). Neoglycolipids were diluted to 200 \(\mu g/ml\) in 5\% ethanol, 5 \(\mu g/ml\) cholesterol, and 2.5 \(\mu g/ml\) phosphatidylcholine and coated onto microtiter plates at 40 \(\mu g/well\) (8 nmol/well).

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2 R. E. Bruehl, S. D. Rosen, and C. R. Bertozzi, submitted for publication.
After drying, the wells were blocked with 3% BSA in PBS for 2 h. Jurkat cells were centrifuged and resuspended to $5 \times 10^6$ ml in 0.1% BSA in PBS. The fluorescent dye BCECF/AM was added at a dilution of 1:1000 from a 2 mM stock solution in dimethyl sulfoxide and incubated with the cells for 20 min in the dark. The labeled cells were centrifuged, resuspended to $2 \times 10^6$ ml in 0.1% BSA in PBS, and transferred to the neoglycolipid-coated plate at 100 $\mu$l/well. After 30 min, the plate was washed twice with PBS, and 0.1% BSA in PBS was added at 100 $\mu$l/well. Arbitrary fluorescence intensity units were recorded at 485 nm excitation and 530 nm emission on a CytoFluor II fluorescence multiwell plate reader (PerSeptive Biosystems, Foster City, CA). To demonstrate L-selectin-dependent binding, some cells were incubated with 10 mM EDTA or with DREG-56 or a class-matched control antibody at 10 $\mu$g/ml for 20 min prior to incubation with the immobilized neoglycolipids. All reactions, washes, and centrifugation steps were performed at ambient temperature.

**Statistics**—Significant differences among means were determined by one-way analysis of variance. When significant differences were detected, multiple pairwise comparisons were performed using a Tukey test (SigmaStat statistical software, SPSS Inc., Chicago, IL). Unless stated otherwise, the data shown are from representative experiments and are the average of duplicate wells after subtraction of background signal (carrier lipids only), with error bars denoting the range in signal.

## RESULTS

**Binding of Recombinant L-selectin to Sulfated Lactose Neoglycolipids**—To investigate the contribution of sulfate esters in defined positions to L-selectin binding, serial dilutions of a panel of sulfated lactose neoglycolipids with 25-atom single chain hydrocarbon tails (Fig. 1) were immobilized on microtiter plate wells and assayed for their ability to support the binding of L-selectin/IgM chimeras. For both the human and murine L-selectin chimeras, the best substrate for binding was 6',6'-disulfolactose ($\text{SO}_4\text{-6}\text{Gal}\beta\text{1-4(SO}_4\text{-6Glc})$, but significant binding was also observed with the 6'-sulfolactose ($\text{SO}_4\text{-6Gal}\beta\text{1-4Glc})$ and 6-sulfolactose ($\text{Gal}\beta\text{1-4(SO}_4\text{-6Glc})$ derivatives (Fig. 2, A and B). At a coating concentration of 5 nmol/well, 6',6'-disulfolactose generated a signal 2.4-fold greater than 6-sulfolactose ($p < 0.001$) and 2.8–3.6-fold greater than 6'- and 3'-sulfolactose ($\text{SO}_4\text{-3Gal}\beta\text{1-4Glc}$) ($p < 0.001$). Differences in reactivity between 6',6'-disulfolactose and the other sulfated derivatives and between all of the sulfated derivatives and lactose or carrier lipids only (data not shown) were statistically significant ($p < 0.001$). These results are consistent with previous data in which similar compounds were used as soluble inhibitors of L-selectin binding to GlyCAM-1 (39). There were no significant differences in the reactivity profiles between the human and murine chimeras; however, the murine chimera consistently generated higher signals than the human chimera at comparable concentrations. There was no detectable binding of either chimera to non-sulfated lactose or sialyllactose (data not shown).

Gal-3-$\text{SO}_4$ has not been detected in acid hydrolysates of GlyCAM-1 (18). However, a number of studies have established binding of L-selectin to Gal-3-$\text{SO}_4$-containing structures, including sulfatides, 3'-sulfo- Le$a$ ($\text{SO}_4\text{-3Gal}\beta\text{1-4(Fucol-1-4GlcNAc})$, and 3'-sulfo-LeX ($\text{SO}_4\text{-3Gal}\beta\text{1-4(Fucol-1-3GlcNAc})$ (28, 30–32, 47, 48). Structures of this kind could be important determinants in the L-selectin-mediated association of leukocytes with tumor cells (49–51). As shown in Fig. 2 (A and B), L-selectin binding to 3'-sulfolactose was relatively weak compared with that to 6'- and 6-sulfolactose.

To address the possibility that the greater reactivity of L-selectin for 6',6'-disulfolactose relative to the monosulfated derivatives was due to an increase in negative charge rather than to a specific configuration of sulfate esters, L-selectin binding to 3',6'-disulfolactose ($\text{SO}_4\text{-3(SO}_4\text{-6Gal}\beta\text{1-4Glc})$ and 3',6'-disulfolactose ($\text{SO}_4\text{-3Gal}\beta\text{1-4(SO}_4\text{-6Glc})$) was determined (Fig. 3). The addition of a sulfated ester to C-3' of either 6'- or 6-sulfolactose did not yield binding greater than that of the parent monosulfated lactose derivative.

To further characterize the binding of L-selectin to the sulfated lactose neoglycolipids, we determined the effects of a
function-blocking mAb (DREG-56) (52) and divalent cation chelation on the binding of the human L-selectin chimera. As shown in Fig. 4, the binding of L-selectin to all of the sulfated lactose derivatives was strongly inhibited by DREG-56 or by 10 mM EDTA. Similarly, mouse L-selectin binding to these sulfated compounds was inhibited by a function-blocking mAb (MEL-14) or by EDTA (data not shown). The binding of wheat germ agglutinin and ricin toxin agglutinin to lactose or 6,6′-disulfolactose, respectively, was not inhibited by 20 mM EDTA, indicating that EDTA does not strip the neoglycolipid substrate from the microtiter well (data not shown).

We investigated the divalent cation dependence of L-selectin binding to the most active sulfated lactose neoglycolipids in detail by measuring chimera binding as a function of EDTA concentration. As shown in Fig. 5, L-selectin binding to 6′-sulfolactose, 6-sulfolactose, and 6,6′-disulfolactose exhibited the identical divalent cation dependence as observed for its binding to GlyCAM-1. 50% inhibition of binding was achieved at 0.9 mM EDTA, which corresponds to a calcium concentration of 80 μM. This value is in accord with previous estimates of the amount of calcium needed for L-selectin function (66).

Binding of Jurkat T-cells—To investigate the binding of L-selectin in a cellular context, we examined the adhesion of an L-selectin-expressing T-cell line (Jurkat) to the same panel of sulfated lactose neoglycolipids. As was the case for the L-selectin chimeras, the Jurkat cells bound best to 6′,6′-disulfolactose (Fig. 6), and differences in reactivity between 6′,6′-disulfolactose and the other sulfated derivatives (3′,6′- and 3′,6′-disulfolactose) were statistically significant (p < 0.001). 6′,6′-disulfolactose; 6′,6′-disulfolactose; 3′,6′-disulfolactose; 6′,6′-disulfolactose.

Binding of MECA-79—To determine whether there was overlap between the MECA-79 epitope and the L-selectin recognition determinant, we assayed MECA-79 binding to the sulfated lactose neoglycolipids (Fig. 7). The strongest binding was observed with 6-sulfolactose, whereas much less binding was observed with 6′- and 3′-sulfolactose. Surprisingly, the weakest signal among the sulfated compounds was seen with 6′,6′-disulfolactose. MECA-79 binding, however, was much weaker relative to the L-selectin/IgM chimeras, as evidenced by a significantly longer substrate conversion time. The control rat IgM was not reactive with any of the compounds tested (data not shown).

Binding of E-selectin—E-selectin has previously been shown to bind to sulfated derivatives of LeX (Galβ1→4) (Fucα1→3)GlcNAc or LeY (Galβ1→3) (Fucα1→4) GlcNAc (30, 38, 53, 54). In view of the binding of L-selectin to sulfated lactose derivatives, we wanted to examine the interaction of E-selectin with the same compounds in parallel assays. The preferred structure for the E-selectin/IgM chimera was the non-sulfated sLeX analog, sLeX (Fig. 8A). This compound consists of a LeX-like trisaccharide with Glc substituting for GlcNAc and with a 3′-O-acetic acid substituting for the 3′-sialic acid of sLeX (Fig. 1C). The L-selectin chimera did not...
Minimal Recognition Determinants for L-selectin and MECA-79

DISCUSSION

Interest in the Gal-6-SO₄ and GlcNAc-6-SO₄ modifications within L-selectin ligands was originally prompted by our analysis of the acid hydrolysis products of GlyCAM-1 (18). Reconstitution experiments performed with recently cloned Gal- and GlcNAc-6-O-sulfotransferases have justified this interest by confirming that both of these modifications (i.e. Gal-6-SO₄ and GlcNAc-6-SO₄) can contribute to L-selectin ligand activity (59–61). Interestingly, transfection of Chinese hamster ovary cells with a combination of GlcNAc- and Gal-6-O-sulfotransferase cDNAs imparted much greater L-selectin ligand activity than either sulfotransferase alone, indicating a synergistic contribution from the two kinds of sulfation. The present study was directed at examining the contribution of Gal-6-SO₄ and GlcNAc-6-SO₄ to L-selectin ligand activity in isolation from the influence of sialylation and fucosylation. Guided by the analysis of the simplest chains of GlyCAM-1 (18–20), the minimal structures that we synthesized were based on the N-acetyllactosamine core (Galβ1→4GlcNAc) of sulfo-sLeX. Although the C-2 N-acetate of GlcNAc in N-acetyllactosamine is missing in the sulfated lactose (Galβ1→4Glc) derivatives studied here, this alteration has been shown to have a minimal effect on L-selectin binding (62).

The best substrate for the recombinant L-selectin/IgM chimera was 6′,6'-disulfolactose, which supported binding significantly better than the next most adhesive substrate, 6-sulfolactose (containing Glc-6-SO₄) over a range of neoglycolipid concentrations. Binding to other sulfated structures containing Gal-3-SO₄ (3′,6′- and 3′,6′,6'-disulfolactose) was distinctly less than that to 6′,6'-disulfolactose, demonstrating that the position of sulfate esters, rather than the overall charge, is responsible for efficacy of this compound. A similar pattern of binding was seen with L-selectin-expressing Jurkat T-cells, with the 6′,6'-disulfolactose neoglycolipid clearly superior to the other compounds. These parallels strongly validate the use of the

![FIG. 6. Binding of Jurkat cells to sulfated lactose neoglycolipids.](image)

![FIG. 7. Binding of MECA-79 to sulfated lactose neoglycolipids.](image)

![FIG. 8. Comparison of E- and L-selectin binding to sulfated lactose and sLeX analog neoglycolipids.](image)
L-selectin/IgM chimera as a probe for L-selectin binding.

A significant advantage of the direct binding studies over competition studies is that important specificity controls could be performed. Thus, we were able to establish that the binding of the L-selectin/IgM chimeras to the sulfated neoglycolipids (as well as Jurkat cell binding to 6'-6-disulfolactose) could be effectively inhibited by function-blocking antibodies or by divalent cation chelation. EDTA titration demonstrated that the divalent cation requirement for L-selectin binding to the preferred sulfated lactose derivatives (6',6-disulfolactose, 6'-sulfolactose, and 6-sulfolactose) was identical to that of native GlyCAM-1. These results strongly argue that these compounds are engaged by a site in the L-selectin calcium-type lectin domain that is critical for recognition of physiological ligands. Further supporting evidence for this conclusion derives from our previous observation that soluble sulfated lactose derivatives (with 6',6-disulfolactose as the best inhibitor) can compete the binding of L-selectin to GlyCAM-1 (39). The fact that specific sulfated lactose derivatives, lacking fucose and sialic acid, can bind to relevant sites in L-selectin again emphasizes the importance of sulfate esters with the appropriate spatial orientation as recognition elements. The observation that L-selectin bound better to the sulfated lactose neoglycolipids than to the non-sulfated sLeX analog is consistent with our previous report that 6',6-disulfolactose is a superior inhibitor of L-selectin binding to GlyCAM-1 than sLeX (39).

Further work will be necessary to understand the relationship between the minimal binding structures defined above and actual recognition determinants of HEV-expressed ligands for L-selectin. As reviewed above, a variety of studies have established that 6-sulfo-sLeX (NeuAcα2→3Galβ1→4(Fucα1→3)(SO4)6(GlcNAc) is an important recognition determinant (27, 34, 38, 59–61, 63). Contained within this structure is 6-sulfonate-α-acetylgalactosamine, which, from the data presented herein, can contribute an significant degree of L-selectin binding by itself. Given that L-selectin functions in the dynamic processes of tethering and rolling of lymphocytes, a complete analysis of 6-sulfo-sLeX must examine the contribution of each modification (including sulfation) to both kinetic constants as well as to the overall equilibrium constant. A previous study has shown that sulfated LeX derivatives can exhibit very different inhibitory activities against L-selectin depending upon whether equilibrium assays or flow chamber assays are used (37).

The structural context for the contribution of Gal-6-SO4 is less certain at the present time. As reviewed above, there is significant controversy as to whether sulfation at C-6 of Gal augments the affinity of sLeX or 6-sulfo-sLeX for L-selectin. Furthermore, antibody staining studies by Kannagi and colleagues (34) have failed to detect the presence of 6'-sulfo-sLeX (NeuAcα2→3SO4)(6Galβ1→4(Fucα1→3)(SO4)6(GlcNAc) or 6'-disulfolactose (NeuAcα2→3SO4)(6Galβ1→4(Fucα1→3)(SO4)6(GlcNAc) determinants on HEVs in human lymphoid organs. The present study demonstrates that 6'-sulfolactose and especially 6',6-disulfolactose can support L-selectin binding without sialylation or fucosylation. One possibility is that Gal-6-SO4 may contribute to L-selectin binding in the context of 6'-sulfo- or 6',6-disulfolactose, lacking fucose and/or sialic acid. Such structures might exist as glycan capping groups or be present internally in extended oligosaccharide side chains. In this context, it should be stressed that native GlyCAM-1 possesses extended and multisulfated chains, the structures of which have not been solved (20).

The finding that L-selectin binding to the sulfated lactose derivatives was highly sensitive to divalent cation chelation by EDTA is somewhat surprising. Structural analysis of an engineered form of E-selectin complexed with sLeX revealed a role for calcium in coordinating the C-2 and C-3 hydroxyls of fucose, in addition to four amino acids in the lectin domain (64). Furthermore, calcium-independent L-selectin binding has been demonstrated for a number of non-fucosylated anionic molecules, e.g. sulfatide, lipid A, lipopolysaccharide, and cardiolipin (48, 65, 67). Thus, it was expected that binding to the sulfated lactose neoglycolipids, which lack fucose, would be calcium-independent. It is possible that in addition to coordinating vicinal hydroxyls on fucose, calcium is important for maintaining a properly folded lectin domain for optimal engagement of carbohydrate or specific sulfate esters. Indeed, this is the case with other calcium-type lectins (68, 69). Further study of this issue is warranted.

The MECA-79 antibody has been an invaluable probe for L-selectin ligands on HEVs of normal lymphoid organs and on activated endothelium at sites of chronic inflammation (6). Sulfation, but not fucosylation or sialylation, is required for this epitope, but detailed structural information has been lacking (15, 17). Recent experiments using a cloned sulfotransferase have revealed an essential contribution of GlcNAc-6-SO4 for MECA-79 binding, but again did not illuminate a structural context for this modification (60). The experiments conducted here showed that 6-sulfolactose supported MECA-79 binding considerably better than the other sulfated derivatives. This result is consistent with the demonstrated requirement for GlcNAc-6-SO4, but the inactivity of the 6',6-disulfated derivative was unexpected. In light of our results, one possibility is that the function-blocking activity of this antibody is achieved by neutralizing recognition determinants such as 6-sulfo-sLeX and 6-sulfo-N-acetyllactosamine. The reduced affinity of MECA-79 for 6'-sulfolactose and 6',6-disulfolactose may signify that this antibody cannot directly neutralize the contribution of Gal-6-SO4 to ligand activity. These considerations may explain the variable ability of MECA-79 to block lymphocyte attachment to HEVs at different anatomical sites (40, 70, 71).

The studies reported here advance our understanding of the minimal sulfated structures that may be involved in L-selectin binding. A key question for future work will be to define the full recognition determinants of its endothelial ligands. There is considerable indirect evidence that the structures of these determinants differ as a function of the anatomical location of the secondary lymphoid organ (34, 72). Varying contributions from Gal-6-SO4 and GlcNAc-6-SO4 may underlie this apparent diversity of recognition elements. The need for further information also applies to the inducible L-selectin ligands on non-lymphoid endothelium, which are implicated in the inflammatory trafficking of leukocytes (41, 73).

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