Identification of L-selectin Binding Heparan Sulfates Attached to Collagen Type XVIII

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L-selectin is a C-type lectin expressed on leukocytes that is involved in both lymphocyte homing to the lymph node and leukocyte extravasation during inflammation. Known L-selectin ligands include sulfated Lewis-type carbohydrates, glycolipids, and proteoglycans. Previously, we have shown that in situ detection of different types of L-selectin ligands is highly dependent on the tissue fixation protocol used. Here we use this knowledge to specifically examine the expression of L-selectin binding proteoglycans in normal mouse tissues. We show that L-selectin binding chondroitin/dermatan sulfate proteoglycans are present in cartilage, whereas L-selectin binding heparan sulfate proteoglycans are present in spleen and kidney. Furthermore, we show that L-selectin only binds a subset of renal heparan sulfates, attached to a collagen type XVIII protein backbone and predominantly present in medullary tubular and vascular basement membranes. As L-selectin does not bind other renal heparan sulfate proteoglycans such as perlecain, agrin, and syndecan-4, and not all collagen type XVIII expressed in the kidney binds L-selectin, this indicates that there is a specific L-selectin binding domain on heparan sulfate glycosaminoglycan chains. Using an in vitro L-selectin binding assay, we studied the contribution of N-sulfation, O-sulfation, C5-epimerization, unsubstituted glucosamine residues, and chain length in L-selectin binding to heparan sulfate/heparin glycosaminoglycan chains. Based on our results and the accepted model of heparan sulfate domain organization, we propose a model for the interaction of L-selectin with heparan sulfate glycosaminoglycan chains. Interestingly, this opens the possibility of active regulation of L-selectin binding to heparan sulfate proteoglycans, e.g., under inflammatory conditions.

A well known function for L-selectin, a C-type lectin expressed on leukocytes, is its key role in the homing of lymphocytes to lymph nodes. This process involves the binding of L-selectin to its ligands expressed on high endothelial venules (HEVs), which induces rolling of the lymphocytes and results in extravasation. L-selectin ligands on HEVs are well characterized and known to be mucin-like glycoproteins containing sulfated, sialylated, and fucosylated Lewis-related carbohydrate structures, which are expressed by the high endothelial cells (1, 2). In addition to the lymph node, L-selectin ligands have been described to be expressed on endothelium under inflammatory conditions, including Crohn disease, asthma, heart and kidney acute allograft rejection, and a number of skin diseases (1, 2). In vitro studies show that, in addition to the well known Lewis-related glycoproteins, L-selectin can also bind to other types of molecules, including glycolipids and proteoglycans (3–5).

Proteoglycans are glycoconjugates that consist of a core protein to which linear carbohydrate chains (glycosaminoglycans, GAGs) are linked. The composition of these GAGs is specific for the different types of proteoglycans, which are chondroitin sulfate proteoglycans (CSPGs), dermatan sulfate proteoglycans (DSPGs), heparan sulfate proteoglycans (HSPGs), and keratan sulfate proteoglycans (KSPGs) (6). Heparin, which is an over-sulfated heparan sulfate, is produced by mast cells and stored in intracellular granules. A number of HSPGs, including perlecain, agrin, and collagen type XVIII, are important structural components of basement membranes in different tissues (7, 8). In addition, HSPGs are expressed on the cell surface, where they are involved in cell-cell and cell-matrix interactions. Furthermore, depending on their sulfation pattern, HSPGs are able to bind and present many different proteins, including chemokines and growth factors (reviewed in Refs. 9 and 10).

Previously, it has been shown that L-selectin can bind to HSPGs and CSPGs isolated either from renal tissue or from a cell line (11–13). Although the identified proteoglycan ligands for L-selectin (collagen type XVIII and versican, respectively) are typically basement membrane or extracellular matrix components, in situ binding of L-selectin in these studies mainly detected intracellular ligands. Recently, we were able to show that in situ detection of different types of L-selectin ligands is highly dependent on the tissue section fixation protocol used (14) and that this is a likely explanation for this apparent discrepancy. In this study, we used this knowledge to specifically examine the expression of L-selectin binding proteoglycans in different normal mouse tissues. We showed that L-selectin binding proteoglycans are present in a number of different tissues, including the kidney. Using different proteoglycan knock-out mice, we identified the renal L-selectin binding HSPG and showed that a specific L-selectin binding domain on the HS GAG chain is involved. Finally, we identified a number of structural properties of HS/heparin chains that...
termine L-selectin binding. Combining these results with the well accepted model for HS domain organization enabled us to propose a model for L-selectin interaction with HS chains, under both normal and inflammatory conditions.

**EXPERIMENTAL PROCEDURES**

**Fusion Proteins and Enzymes—**L-selectin-IgM, P-selectin-IgM, and E-selectin-IgM fusion proteins, consisting of the extracellular domain of human L-selectin, P-selectin, or E-selectin linked to human IgM Fe-tail, were secreted into OptiMEM (Invitrogen) by COS-7 cells after transfection with the corresponding plasmids (15, 16). Fusion protein containing medium was concentrated 25× using Amicon Ultra centrifugal filter devices (molecular weight cut-off 30,000; Millipore Corp.). L-selectin-IgG fusion protein, consisting of the extracellular domain of L-selectin linked to human IgG Fe-tail, was produced as described (17).

Sialidase ([*Streptococcus sp.*], EC 3.2.1.18), heparinase ([*Flavobacterium heparinum*, EC 4.2.2.7], and heparitinase I ([*F. heparinum*, EC 4.2.2.8]) were obtained from Seikagaku Corp. Chondroitinase ABC ([*Proteus vulgaris*, EC 4.2.2.4]) was obtained from Sigma.

**Animals and Tissues—**Adult C57/B6 mice were bred in the laboratory animal facilities at the Vrije Universiteit Faculty of Medicine, Amsterdam, the Netherlands. Wistar rats were obtained from Harlan Cebi NL, Houten, The Netherlands. DREG-56 ([*Cobitis derma*], and MECA-32 ([*Cobitis derma*], were purchased from Pharmingen. As secondary antibodies, goat anti-rabbit IgG-Alexa Fluor 488 and goat anti-mouse IgG-Alexa Fluor 594 were used (Molecular Probes). In all experiments, controls for background staining were included by omission of primary antibodies and finally N-Deacetylation was performed by hydrazinolysis as described previously (26). N-Acetylation of polysaccharides was performed by treatment with acetic anhydride as described by Danishefsky and Steiner (27). Completely (N- and O-) deacetylated heparin was prepared according to Backstrom et al. (28) and recrystallized as described above. N′+O′-sulfated K5 was produced by N-deacetylation (hydrazinolysis), subsequent O-desulfation with trifluoroacetic acid, and finally N-deacetylation of O-sulfated K5. O-desulfated heparin was produced by N′-sulfation (sodium trioxide-trimethylamine) followed by N-acetylation of completely desulfated heparin. Unfractionated heparin was cleaved by deamination with HNO₂ at pH 3.9, which results in selective cleavage of glucosamine residues (23). Fraxiparin (molecular weight = ~4500) was purchased from Sanofi Winthrop, Massaglio, Switzerland; Fragmin (molecular weight = ~5900) was purchased from Pharmacia & Upjohn, and Enoxaparin (molecular weight = ~4500) was purchased from Rhone-Poulenc Rorer, Paris, France.

**RESULTS**

**L-selectin Ligands in Lymph Nodes—**We were previously able to show that commonly used fixation protocols for frozen tissue sections can greatly influence the in situ detection of L-selectin ligands (14). As we are interested in the expression and function of L-selectin binding proteoglycans, we set out to examine a panel of normal mouse tissues for the expression of L-selectin binding proteoglycans using formaldehyde-fixed frozen tissue sections, which enables visualization of L-selectin binding proteoglycans and glycoproteins.

First of all, and as expected, L-selectin ligands were found on the HEVs in normal mouse lymph nodes. In addition, very strong granular staining of mast cells was observed (Fig. 1A). Double staining with an anti-IgE antibody, detecting mouse IgE bound to the mast cell IgE-receptor, confirmed the identity of these cells (data not shown).

Next, we used different carbohydrate-degrading enzymes to determine the types of L-selectin ligands expressed. Pretreatment of the tissue sections with sialidase, which specifically removes sialic acid from a carbohydrate backbone, resulted in significantly decreased L-selectin-IgM-staining of HEVs, showing that binding of L-selectin to its ligands on HEVs is mainly sialic acid-dependent (Fig. 1, A and B). Staining of mast cells present in the lymph node tissue sections was unaffected by pretreatment with sialidase (Fig. 1B). However, pretreatment with heparinase, which specifically cleaves heparin, resulted in complete abrogation of L-selectin staining of mast cells (Fig. 1, C and D), showing that the L-selectin ligand present in mast cell granules is heparin. Binding of the L-selectin-IgM chimera to both HEVs and mast cells could be blocked upon the addition of L-selectin-blocking antibody DREG-56 or the calcium chelator EGTA (data not shown), indicating that the same carbohydrate-degrading enzyme was used in both experiments.
drate binding site on L-selectin is involved in binding to these ligands. As controls, E-selectin-IgM and P-selectin-IgM chimeric proteins were used to determine specificity for L-selectin. E-selectin-IgM did bind to HEVs, as described previously (31), but not to mast cells (data not shown). P-selectin-IgM did not bind to HEVs but did bind to mast cells (not shown). Together, these results indicated that the L-selectin-IgM chimera is a sensitive and specific probe to show the presence of different types of L-selectin ligands in tissues and that enzymatic treatment of the tissue sections can be used to unravel the identities of these ligands.

Proteoglycans as Ligands for L-selectin—Next, we examined a panel of mouse tissues for the expression of L-selectin binding proteoglycans (Table I). In a number of tissues, including the small intestine and salivary gland, L-selectin ligands were detected, but binding was neither reduced by heparitinase I nor reduced by chondroitinase ABC, showing that these ligands are not proteoglycans. However, in a number of tissues, L-selectin binding proteoglycans were found. In cartilage, L-selectin binding was observed surrounding chondrocytes (Fig. 2A) and was shown to be chondroitinase ABC-sensitive (Fig. 2B). Heparitinase I pretreatment of the tissue sections had no significant effect on L-selectin binding (data not shown). This showed that L-selectin ligands found in cartilage are CS/DSPGs.

Examination of the expression of L-selectin ligands in normal mouse spleen revealed a specific binding of the L-selectin-IgM chimera to vascular basement membranes in the spleen. Staining was located in the red pulp just outside the marginal zone (Fig. 2C) and was directly adjacent to endothelial cells (identified using the MECA-32 antibody directed against mouse endothelium; not shown). Furthermore, binding of L-selectin-IgM to these vascular basement membranes was shown to be sensitive to heparitinase I (Fig. 2D), whereas chondroitinase ABC pretreatment had no effect (data not shown), which shows that these ligands are heparan sulfate proteoglycans.

In situ detection of L-selectin ligands in the mouse kidney revealed a very prominent staining of tubular basement membranes in the medulla (Fig. 2E). In addition, staining was

Table I

| Non-proteoglycan ligands | L-selectin binding proteoglycans |
|--------------------------|----------------------------------|
| Cervical lymph nodes     | +                                |
| Peyer’s patches          | +                                |
| Mesenteric lymph nodes   | +                                |
| Salivary gland           | +                                |
| Stomach                  | +                                |
| Small intestine (duodenum, jejunum) | +    |
| Small intestine (ileum)  | +                                |
| Colon                    | –                                |
| Liver                    | –                                |
| Spleen                   | –                                |
| Kidney                   | –                                |
| Brain                    | –                                |
| Lungs                    | –                                |
| Heart                    | –                                |
| Skin                     | –                                |
| Cartilage                | –                                |
observed associated with the vasa recta vascular bundles, in Bowman’s capsule in the cortex, and in the papilla (not shown). The glomeruli were completely negative for L-selectin binding. Enzymatic digestion using heparitinase I significantly reduced staining in the papilla and completely abrogated staining in the medulla, cortex, and vasa recta vasculature, which indicates that the L-selectin ligands found are mainly HSPGs (Fig. 2F).

Treatment of kidney tissue sections with sialidase had no effect on L-selectin-IgM staining, whereas chondroitinase ABC pretreatment slightly decreased staining in the papilla only (data not shown). These observations are in complete concordance with those observed in normal rat kidney (14).

Identification of a Subset of Collagen Type XVIII as Renal L-selectin Ligand—As many of our antibodies directed against basement membrane HSPGs are generated in mice and recognize their epitopes in rat, we decided to perform the following experiments using normal rat kidneys. Various basement membrane HSPGs are present in normal mouse and rat kidney, including agrin, perlecan, and collagen type XVIII. All of these HSPGs have GAG chains to which L-selectin could potentially bind. Using an antibody directed against HS stubs (3G10) after heparitinase I treatment, all HS chains present in normal rat kidney can be visualized (Fig. 3, A and B). When comparing this staining with the L-selectin binding pattern of untreated sections (Fig. 3, C and D), it is clear that L-selectin binds only a subset of renal HS. Using antibodies directed against the protein cores of perlecan (Fig. 4A), agrin (Fig. 4B), and collagen type XVIII (Fig. 4C), it can be clearly shown that...
L-selectin binding partially colocalizes with agrin and collagen type XVIII, and to a lesser extent, with perlecan.

To further identify the L-selectin binding HSPGs, we stained kidney tissues from different knock-out mice. First of all, binding of L-selectin to kidney tissue sections of both CD44 and syndecan-4 knock-out mice was identical to that in wild type (data not shown). In Hspg2-3 mutant mice, exon 3 of the perlecan gene is deleted, resulting in the loss of attachment sites for three of four potential HS chains (21). In situ binding of the L-selectin-IgM chimera to kidneys of these mice also revealed a staining pattern identical to that of wild type (Fig. 5A). In contrast, L-selectin-IgM binding to kidney tissue sections of Col18a1-/- mice (20) was abrogated, with the exception of some residual staining in the papilla, which was heparitinase I-insensitive (not shown).

Formally, we cannot exclude the possibility that due to the absence of collagen type XVIII in both the Col18a1-/- single mutant and Hspg2-33/33 x Col18a1-/- double mutant mice, lacking both perlecan-HS chains and functional collagen type XVIII (21). In these kidneys, no binding of the L-selectin chimera was observed (data not shown), with the exception of some residual staining in the papilla, which was heparitinase I-insensitive (not shown).

Similarly, we cannot exclude the possibility that due to the absence of collagen type XVIII in both the Col18a1-/- single mutant and Hspg2-33/33 x Col18a1-/- double mutant mice, the normal distribution of other HSPGs present in renal basement membranes was also disrupted. However, heparitinase I treatment followed by 3G10 staining (detecting HS stubs) of kidney tissue sections of both Col18a1-/- single mutant (Fig. 5D) and Hspg2-33/33 x Col18a1-/- double mutant mice (not shown) revealed that HS chains were present in the medullary basement membranes. Furthermore, the 3G10 staining of Col18a1-/- single mutant kidney appeared similar to that of Hspg2-33/33 mutant kidney (Fig. 5C), to which L-selectin was able to bind.

Together, these data showed that L-selectin selectively binds to heparan sulfate GAG chains expressed on collagen type XVIII in the renal medulla and cortex. Furthermore, there are certain structures in the kidney where collagen type XVIII is expressed, such as the glomerulus, but to which the L-selectin-IgM chimera cannot bind (Fig. 4C, single red staining). This indicated that not all collagen type XVIII molecules in the kidney express the heparan sulfate GAG chains to which L-selectin can bind.

L-selectin Binds In Vitro to Heparin, CS-B, and Dextran Sulfate and Differentially to HS Isolated from Different Sources—As we have shown that L-selectin can bind to both CS/DSPGs and HSPGs in normal mouse tissue sections, we set up an in vitro L-selectin binding assay using a panel of different GAGs. HS isolated from human aorta was coated and shown to bind L-selectin (Table II). Different GAGs were added as fluid phase inhibitors, and the IC50 (concentration of inhibitor that results in a 50% reduction of binding to coated HS) was determined, which is a measure for binding efficiency of these inhibitors to L-selectin. L-selectin binding to HS could be inhibited very efficiently by heparin and dextran sulfate, and to a lesser extent, by CS-B (Table II). No binding to CS-A, CS-C, KS, hyaluronic acid, or dextran was observed. As described previously, sulfation was shown to play an important role in L-selectin binding as no binding to dextran was observed, whereas dextran sulfate bound L-selectin with very high efficiency.

As we have shown, L-selectin only binds to a specific subset of HS presented on collagen type XVIII molecules in normal mouse kidneys. Therefore, we examined whether L-selectin could bind to a number of HS preparations isolated from different sources. As shown in Table II, HS isolated from porcine intestine and from bovine intestine could slightly inhibit L-selectin binding to HS isolated from human aorta. HS isolated...
from bovine aorta or bovine kidney did not bind to L-selectin. These data further indicated that there are specific domain requirements for L-selectin binding to HS chains, and not all HS preparations meet these requirements.

**DISCUSSION**

Essentially all leukocytes express the C-type lectin L-selectin on their cell surface. The interaction between this adhesion molecule and its ligands is critical for the first step of leukocyte extravasation, both during lymphocyte homing to the lymph node and upon inflammation. Therefore, it is possible that an interaction between L-selectin and proteoglycans plays a role in leukocyte migration. We previously showed that in situ detection of L-selectin ligands in tissue sections is highly dependent on the fixation protocol used (14). We now show that L-selectin binds to certain proteoglycans in a number of tissues, most dominantly in a subset of renal basement membranes. Furthermore, we show that although HSPGs are abundantly present in the kidney, L-selectin only binds a subset of collagen type XVIII HSPGs and that binding is dependent on the presence of a specific L-selectin binding domain on the HS...
GAG chains, which we characterize to some extent. We thus found L-selectin binding to HS/heparin to be critically dependent on O-sulfation and length of the GAG chain. Furthermore, the presence of iduronate residues interferes with L-selectin binding, whereas N-unsubstituted GlcNH$_3^+$ units do not play a role. Together, these results raise some fundamental questions regarding the function and regulation of these L-selectin binding HSPGs.

Interestingly, in addition to the kidney, L-selectin binding HSPGs were also found in vascular basement membranes of only a subset of small blood vessels in the splenic red pulp. Although the process of lymphocyte homing to the lymph node has been elucidated to a large extent, lymphocyte migration in the spleen remains relatively unclear. Recently, Grayson et al. (33) described the existence of small vessels through which trafficking of T-cells from the blood to the splenic white pulp would occur. However, it has also been described that L-selectin is not involved in this process (33, 34). Therefore, the role of L-selectin ligands located in the splenic red pulp remains to be elucidated.

Although collagen type XVIII has previously been described to bind L-selectin (12), we showed here, using kidney tissue from different knock-out mice, that L-selectin selectively binds to HS attached to a collagen type XVIII protein backbone, although many more HSPGs are present. Furthermore, there are regions in the kidney, such as the glomerulus, where collagen type XVIII is clearly expressed but to which L-selectin cannot bind. Based on these results, it can be concluded that there is a specific L-selectin binding domain on HS that is expressed only on a subset of collagen type XVIII HSPGs.

In addition to L-selectin binding HSPGs located mainly in the renal medulla, L-selectin binding CS/DSPGs were present only in the papilla as chondroitinase ABC pretreatment decreased binding of the L-selectin-IgM chimera in this region of normal mouse kidney tissue sections. In kidney tissue from Col18a1$^{-/-}$ single mutant and Hspg2$^{2/-/2}$×Col18a1$^{-/-}$ double mutant mice, binding of the chimera in the renal papilla remained, and this binding was heparitinase I-insensitive (not shown). Versican, a CSPG previously described to be a renal L-selectin ligand (11, 35), is a very likely candidate for the L-selectin binding CS/DSPG we observe in the renal papilla. Although versican was described to be abundantly present in the renal papilla, no binding of an L-selectin chimeric protein to this region was observed by these investigators (11, 35). However, in our opinion, the lack of binding of the L-selectin chimeric protein in these studies may be due to the fixation protocol used.

Upon concluding that an L-selectin binding domain on HS GAG chains exists, we set out to further characterize this domain. Using different chemically modified heparinoids and bacterial non-sulfated HS/heparin-like polysaccharide K5, we performed in vitro L-selectin binding assays. Based on the results, we were able to determine a number of structural characteristics of HS/heparin GAG chains that influence L-selectin binding. First of all, we were interested in a possible role of unsubstituted GlcN residues in L-selectin binding to HS GAG chains, which had been suggested in an earlier report (3). This raised our particular interest as we have previously shown the binding of anti-HS monoclonal antibody JM-403 to be critically dependent on the presence of these units (32). Within the kidney, however, this JM-403 antibody stains the glomerular basement membranes in a dominant fashion, whereas L-selectin completely fails to bind this structure. Furthermore, N-acetylation of HS (isolated from human aorta)
results in a complete loss of JM-403 binding (32), whereas we showed here that L-selectin binding to HS/heparin is essentially unaltered by N-acetylation. Taken together, we can conclude that unsubstituted GlcN residues do not play a role in L-selectin binding. However, the possibility that these GlcNH₂-groups somehow can influence the biosynthesis of L-selectin binding sites on HSPGs remains.

Sulfation clearly plays an important role in determining L-selectin ligand activity, as shown by the fact that non-sulfated dextrins do not bind L-selectin, whereas sulfated dextrins bind L-selectin with very high efficiency. Concerning the contribution of HS sulfation for L-selectin binding, our results indicated that N-sulfation is not critical since both O-sulfated K5 and DS completely lack N-sulfates, but do bind L-selectin. Although N-desulfation of heparin did reduce L-selectin binding, this may also be due to some loss of O-sulfates occurring during the N-desulfation procedure (36). O-sulfation of HS/heparin and bacterial K5 polysaccharide was critical for L-selectin binding as all O-sulfated derivatives did bind L-selectin, whereas all non-O-sulfated polysaccharides did not. Although we did not further pursue the details of O-sulfation requirements, others have shown 6-O-sulfation to be a critical determinant (37).

However, not only degrees of sulfation and linkage of sulfate-groups are important for L-selectin binding. The presence of idurionate residues seemed to inhibit L-selectin binding, limiting the binding site on HS/heparin for L-selectin to regions with high 6-O-sulfation and a high GlcA content.

Combining these results with the well accepted model of HS domain organization (Fig. 6) (24, 38), we can conclude that the L-selectin binding domain is located in the NA/NS domain of HS (consisting of alternating N-acetylated and N-sulfated residues). In this domain, both N- and (6-)O-sulfation are present, combined with relatively few idurionate residues as compared with the highly N+O-sulfated NS domain, in which essentially all GlcA residues have been converted to IdoA residues. However, L-selectin binding may also occur in the NA domain of HS, where no N-sulfation or IdoA residues are present, but occasionally 6-O-sulfated GlcN residues could mediate L-selectin binding. Interestingly, any HS chain is composed of a number of alternating NS, NA/NS, and NA domains. We now suggest that multiple domains on each HS/heparin chain are involved in L-selectin binding. This is supported by the fact that nitrous acid deamination (pH 3.9) products of heparin and O-sulfated bacterial K5 hexasaccharides, as well as low molecular weight heparins, are less capable of L-selectin binding than the corresponding larger fragments. It is known that L-selectin molecules are concentrated at the tips of the microvilli of leukocytes (39), which further supports the hypothesis of multivalent binding of L-selectin molecules to HS/heparin. Together, these specific requirements may well explain the observed differences in L-selectin binding activity between HSPGs isolated from different sources and the fact that although HSPGs are abundantly present in many tissues, L-selectin only binds to a specific subset of HSPGs.

In addition to L-selectin, many other proteins are able to bind HS, and their binding also appears to be dependent on HS structure (40). This opens the possibility of active regulation of specific protein binding capacity of HSPGs. As there are many steps involved in HSPG biosynthesis, including level of protein backbone expression, HS chain length, and modification, it is well possible that under some conditions, HSPG biosynthesis is slightly altered, which could highly influence binding of certain proteins to these HSPGs. Of special interest for the formation of an L-selectin binding domain on HS is the 6-O-sulfotransferase HS6ST, of which three isoforms have been described in mice and two alternatively spliced isoforms have been described in human (41, 42). These enzymes are differentially expressed in many different tissues, including kidney, liver, and spleen, and may thereby be involved in the highly tissue-specific L-selectin binding to HS. Another possible level of regulation of L-selectin binding to HS is the expression of sulfatase on the cell surface, which are shown to specifically cleave 6-O-sulfates from heparin (43).

The biological relevance of an interaction between L-selectin and HSPGs present in basement membranes has not been proven to this point. It is known that during acute renal allograft rejection, leukocytes leave the blood, migrate toward the tubular basement membranes, and invade the tubular epithelium, resulting in renal tubulitis (44). In this setting, leukocytes expressing L-selectin may encounter the L-selectin binding HSPGs in tubular basement membranes, which may possibly lead to adhesion, retention, and/or activation of the cells. Furthermore, it is known that chemokines also bind to, and are presented on, HS GAG chains. Therefore, it is possible
that a combined signal of simultaneous L-selectin binding to HS and chemokine recognition is necessary for appropriate cell activation in an inflammatory response. In addition, although under non-inflammatory conditions only a specific subset of collagen type XVIII HSPGs present in tubular basement membranes was shown to bind L-selectin, the presence of a specific L-selectin binding domain on HS opens the possibility that upon inflammation, HSPGs convert into L-selectin binding HS and chemokine recognition is necessary for appropriate cell activation in an inflammatory response. In addition, although under non-inflammatory conditions only a specific subset of collagen type XVIII HSPGs present in tubular basement membranes was shown to bind L-selectin, the presence of a specific L-selectin binding domain on HS opens the possibility that upon inflammation, HSPGs convert into L-selectin binding HS and chemokine recognition is necessary for appropriate cell activation in an inflammatory response. In addition, although under non-inflammatory conditions only a specific subset of collagen type XVIII HSPGs present in tubular basement membranes was shown to bind L-selectin, the presence of a specific L-selectin binding domain on HS opens the possibility that upon inflammation, HSPGs convert into L-selectin binding HS and chemokine recognition is necessary for appropriate cell activation in an inflammatory response.

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REFERENCES
1. Rosen, S. D. (1999) Am. J. Pathol. 155, 1013–1019
2. Rosen, S. D. (2004) Annu. Rev. Immunol. 22, 129–156
3. Norgard-Summicht, K. E., and Varki, A. (1995) J. Biol. Chem. 270, 12012–12024
4. Koenig, A., Norgard-Sumnicht, K. E., and Varki, A. (1998) J. Clin. Invest. 101, 877–889
5. Varki, A. (1997) J. Clin. Invest. 99, 158–162
6. Prydz, K., and Dalen, K. T. (2000) J. Cell Science 113, 193–205
7. Joze, R. V. (1998) Annu. Rev. Biochem. 67, 609–652
8. Erickson, A. C., and Couchman, J. R. (2000) J. Histochem. Cytochem. 48, 1291–1306
9. Bernfeld, M., Götte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Linecium, J., and Zako, M. (1999) Annu. Rev. Biochem. 68, 729–777.
10. Esko, J. D., and Selleck, S. B. (2002) Annu. Rev. Biochem. 71, 435–471
11. Kawashima, H., Hirose, M., Hirose, J., Nagakubo, D., Plaa, A. H. K., and Miyasaka, M. (2000) J. Biol. Chem. 275, 35448–35456
12. Kawashima, H., Watanabe, N., Hirose, M., Sun, X., Atarashi, K., Kimura, T., Shikata, K., Matsuda, M., Ogawa, D., Heljasvaara, R., Rehn, M., Pihlajaniemi, T., and Miyasaka, M. (2003) J. Biol. Chem. 278, 13069–13076
13. Li, Y., Kawashima, H., Watanabe, N., and Miyasaka, M. (1999) FEBS Lett. 444, 201–205
14. Celie, J. W. A. M., Beelen, R. H. J., and van den Born, J. (2005) J. Immunol. Methods, 298, 155–159
15. Bistrop, A., Bhakta, S., Lee, J. K., Below, Y. Y., Gunn, M. D., Zuo, F., Huang, C., Kannagi, R., Rosen, S. D., and Hemmerich, S. (1999) J. Cell Biology 145, 899–910
16. Spertini, O., Cordey, A. S., Monai, N., Giuffre, L., and Schapira, M. (1996) J. Cell Biol. 135, 523–531
17. Watson, S. R., Inmai, Y., Pennie, C., Geoffroy, J. S., Rosen, S. D., and Lasky, L. A. (1990) J. Cell Biology 110, 2221–2229
18. Rousshop, K. M., Sennwald, M. E., Claessen, N., Roelofs, J. J., Hoedemaeker, I., van der, R. N., Aten, J., Pals, S. T., Weening, J. J., and Florquin, S. (2004) Am. J. Pathol. 164, 674–686
19. Echtermeyer, F., Streit, M., Wilcox-Adelman, S., Saoncella, S., Denhez, F., Detmar, M., and Goetinck, P. F. (2001) J. Clin. Invest. 107, R9–R14
20. Fukai, N., Kikunosh, L., Marreros, A. G., Oh, S. P., Keene, D. R., Tamarkin, L., Niemela, M., Lives, M., Li, E., Pihlajaniemi, T., and Olsen, B. R. (2002) EMBO J. 21, 1535–1544
21. Rossi, M., Morita, H., Sormunen, R., Airenne, S., Kerevi, M., Wang, L., Fukai, N., Olsen, B. R., Tryggvason, K., and Soo, M. (2003) EMBO J. 22, 236–245
22. Hoch, W., Campanelli, J. T., Harrison, S., and Scheller, R. H. (1994) EMBO J. 13, 2814–2822
23. Iverius, P. H. (1971) Biochem. J. 124, 677–683
24. Maccarana, M., Sakura, Y., Tawada, A., Yoshiida, K., and Lindahl, U. (1996) J. Biol. Chem. 271, 17804–17810
25. Vann, W. F., Schmid, M. A., Jann, B., and Jann, K. (1981) Eur. J. Biochem. 116, 359–364
26. Shakklee, P. N., and Conrad, H. E. (1984) Biochem. J. 217, 187–197
27. Danishefsky, J., and Steinher, H. (1985) Biochim. Biophys. Acta 101, 37–45
28. Backstrom, G., Hoff, M., Lindahl, U., Feingold, D. S., Malmstrom, A., Roden, L., and Jacobsson, I. (1979) J. Biol. Chem. 254, 2975–2982
29. Rej, R. N., Ludwig-Bistrop, K. G., and Perlin, A. S. (1991) Carbohydr. Res. 210, 299–310
30. Peijler, G., Backstrom, G., Lindahl, U., Paulsson, M., Dziadek, M., Fuyi-wara, M., and Timpl, R. (1987) J. Biol. Chem. 262, 5036–5043
31. Mebius, R. E., and Watson, S. R. (1993) J. Immunol. 151, 3252–3260
32. van den Born, J., Gunnarsson, K., Bakker, M. A., Kjellin, L., Kusche-Gullberg, M., Maccarana, M., Berden, J. H., and Lindahl, U. (1995) J. Biol. Chem. 270, 31303–31309
33. Grayson, M. H., Hotchkiss, R. S., Karl, I. E., Holtzman, M. J., and Chaplin, D. D. (2003) Am. J. Physiol. 284, H2213–H2226
34. Nolte, M. A., Hamann, A., Kraal, G., and Mebius, R. E. (2002) Immunology 106, 299–307
35. Kawashima, H., Li, Y., Watanabe, N., Hirose, J., Hirose, M., and Miyasaka, M. (1999) Int. Immunol. 11, 393–405
36. Inoue, Y., and Nagasawa, K. (1976) Carbohydr. Res. 46, 87–95
37. Wang, L., Brown, J. R., Varki, A., and Esko, J. D. (2002) J. Clin. Invest. 110, 127–136
38. Lindahl, U., Kusche-Gullberg, M., and Kjellin, L. (1998) J. Biol. Chem. 273, 24079–24082
39. Vestweber, D., and Blanc, J. E. (1999) Physiol. Rev. 79, 181–213
40. Nakato, H., and Kimata, K. (2002) Biochim. Biophys. Acta 1573, 312–318
41. Habuchi, H., Tanaka, M., Habuchi, O., Yoshida, K., Suzuki, H., Ban, K., and Kimata, K. (2000) J. Biol. Chem. 275, 2659–2668
42. Habuchi, H., Miyake, G., Nogami, K., Kuroiwa, A., Matsuda, Y., Kusche-Gullberg, M., Habuchi, O., Tanaka, M., and Kimata, K. (2003) Biochem. J. 371, 131–142
43. Morimoto-Tomita, M., Uchinuma, K., Verb, Z., Hammerich, S., and Rosen, S. D. (2002) J. Biol. Chem. 277, 49175–49185
44. Robertson, H., and Kirby, J. A. (2003) Am. J. Transplant. 3, 3–10
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