The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/20913

Please be advised that this information was generated on 2019-01-31 and may be subject to change.
Presence of N-Unsubstituted Glucosamine Units in Native Heparan Sulfate Revealed by a Monoclonal Antibody*

(Received for publication, August 29, 1995)

Jacob van den Born‡‡, Kerstin Gunnarsson†, Marinka A. H. Bakker‡, Lena Kjellénl, Marion Kusche-Gullbergq, Marco Maccarana, Jo H. M. Berden, and Ulf Lindahl

From the ‡Division of Nephrology, University Hospital St. Radboud, 6500 HB Nijmegen, The Netherlands, †Department of Medical and Physiological Chemistry, University of Uppsala, S-75123 Uppsala, Sweden, and §Swedish University of Agricultural Sciences, Department of Veterinary Medical Chemistry, University of Uppsala, S-75123 Uppsala, Sweden

Immunohistochemical application of antibodies against heparan sulfate proteoglycan core protein and heparitinase-digested heparan sulfate stubs showed the presence of heparan sulfate proteoglycan in all basement membranes of the rat kidney. However, a monoclonal antibody (JM-403) against native heparan sulfate (van den Born, J., van den Heuvel, L. P. W. J., Bakker, M. A. H., Vercamp, J. H., Assmann, K. J. M., and Berden, J. H. M. (1992) Kidney Int. 41, 115–123) largely failed to stain tubular basement membranes, suggesting the presence of heparan sulfate chains lacking the specific JM-403 epitope. Heparan sulfate preparations from various sources differed markedly with regard to JM-403 binding, as demonstrated by liquid phase inhibition in enzyme-linked immunosorbent assay, the interaction decreasing with increasing sulfate contents of the polysaccharide. Mapping of the JM-403 epitope indicated that it was dominated by one or more N-unsubstituted glucosamine units(s), since treatments that destroyed or altered the structure of such units in heparan sulfate preparations (cleavage at N-unsubstituted glucosamine units with HNO₂ at pH 3.9 and O-acetylation with acetic anhydride, respectively), abolished antibody binding. Conversely, immunoreactivity could be induced in a (N-glucuronyl-1,4-N-acetyl-1-glucosaminyl-1,4) polysaccharide by the generation of N-unsubstituted glucosamine units by chemical N-deacetylation. The presence of N-unsubstituted glucosamine in a JM-403-binding heparan sulfate (preparation HS-II from human aorta) was demonstrated by an 3-fold reduction in molecular size following HNO₂ (pH 3.9) treatment. Further characterization of the epitope recognized by JM-403, based on enzyme-linked immunosorbent assay inhibition tests with chemically/enzymatically modified polysaccharides, indicated that one or more N-unsubstituted glucosamine units are invariably present, whereas 1-iduronic acid and O-sulfate residues appear to inhibit JM-403 reactivity. It is concluded that the epitope contains one or more N-unsubstituted glucosamine and 1-glucuronic acid units and is located in a region of the heparan sulfate chain composed of mixed N-sulfated and N-acetylated disaccharide units.

Proteoglycans consist of one or more glycosaminoglycan side chains covalently bound to a core protein (1, 2). The heparan sulfate proteoglycans (HSPGs) constitute a major class of proteoglycans that are found in the extracellular matrix, especially in basement membranes, and at the cell surface, associated with the cell membrane (3–5). Many biological activities of HSPGs are due to interactions between the heparan sulfate (HS) polysaccharide side chains and a variety of proteins, which include extracellular matrix molecules, enzymes, en­zyme inhibitors, growth factors and other cytokines (2, 5–7). These interactions can be specific, dependent on defined sulfation patterns within given sequences of sugar residues, as described for antithrombin (8), basic fibroblast growth factor (9, 10), and heparin cofactor II (11); others appear to be mainly based on relatively nonspecific electrostatic interactions, and involve proteins such as lipoprotein lipase (12), platelet factor 4 (13) and mast cell protease I (14) (for a general discussion, see Ref. 15).

The biosynthesis of HS involves the formation of a nonsulfated (GlcA)₃₋₄-GlcNAc-OH, precursor polysaccharide, which subsequently undergoes a series of polymer-modification reactions. These reactions start with N-deacetylation/N-sulfation of GlcNAc residues, which is followed by C-5 epimerization of GlcA to iduronic acid (IdoA) units, and finally by O-sulfation at various positions (5). The GlcA C-5 epimerization and O-sulfation reactions occur in the close vicinity of N-sulfate groups, pointing to a key role for the glucosaminyl N-deacetylase/N-sulfotransferase enzyme in determining the overall extent of modification of the HS chain. Structural analysis of HS preparations have revealed that the modifications tend to colocalize in block sequences, separated by relatively unmodified domains (16–19). The extent of biosynthetic modification, affecting the number, length, and substitution patterns of the modified domains as well as their position along the HS chain, may differ among cell types (20), alter during proliferation (21), and change as a result of cell transformation (22, 23). Structural analysis of HS is complicated by the fact that even highly purified and uniform preparations consist of mixtures of polysaccharide chains that have reached different levels of modi­fication. Monoclonal antibodies (mAbs) that specifically recognize well defined epitopes in HS could be major tools in such analysis. We recently described the production of such an an

*This study was supported by Grant 90-1040 from the Dutch Kidney Foundation and by Grants 25890 and 05250 from the Swedish Medical Research Council. The Nephrology Division in Nijmegen and the Department of Veterinary Medical Chemistry in Uppsala participate in a concerted action regarding “Alterations in extracellular matrix components in diabetic nephropathy and other glomerular diseases,” which is financially supported by the European Community within the Biomed I program BMH1-4795-1768. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed: Div. of Nephrology, University Hospital St. Radboud, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: 31-80-61-47-61; Fax: 31-80-54-00-22.

† The abbreviations used are: HSPG, heparan sulfate proteoglycan; HS, heparan sulfate; mAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; PAPS, adenosine 3’-phosphate 5’-phosphosulfate.
N-Unsubstituted D-Glucosamine Units in Heparan Sulfates

Materials and Methods

Glycosaminoglycans—HS (preparation HS-11), isolated from human aorta essentially according to Iverius (28), was provided by W. Murphy (University of Monash, Melbourne, Australia). Five HS preparations, isolated from bovine aorta, lung, intestine, and kidney (two different preparations), were provided by K. Yoshida (Seikagaku Corp., Tokyo, Japan). HS from bovine kidney (used to coat the ELISA plates) was purchased from Seikagaku. HS from bovine liver was as described previously (26). HS from pig intestine, the Escherichia coli K5 capsular polysaccharide, with the same (GlcA-GlcNAc) structure as the nonsulfated HS/heparin precursor polysaccharide (27), chemically O-sulfated K5 polysaccharide, and N-desulfated heparin were given by Dr. G. van Dedem (Organon Corp., Oss, The Netherlands). Intact heparin (stage 4) from pig intestinal mucosa was obtained from Inoxel Pharmaceutical Division (Park Forest South, IL) and purified by repeated precipitation with cetylpyridinium chloride from 1.2 M NaCl (28).

Chemical/Enzymatic Modifications of Polysaccharides—Glycosaminoglycans were cleaved by deamination with HNO3 under two sets of conditions. Reaction at pH 1.5 (10 min) was performed as described previously (29) and results in selective attack of N-sulfated GlcN units, whereas deamination at pH 3.9 (29) cleaves the polysaccharide chain at the deamination products (essentially tetrasaccharides) were recovered.

Acetylated oligosaccharides were recovered following exhaustive cleavage of the enzymes by treating with acetic anhydride (31) or 10 units of heparitinase (Seikagaku, enzyme also known as heparinase III, EC 4.2.2.8) or 10 units of heparinase I (Sigma; EC 4.2.2.7) and incubated at 37 °C for 6 h. To ensure end-point digestion, the same amount of heparitinase was added to the sample and incubated for an additional 1.5 h. The supernatants obtained after centrifugation at 13,000 rpm for 5 min were recovered.

For heparin lyase digestion, samples (1 mg) of HS were digested in 200 μl of 50 mM HEPES, pH 7.0, 1 mM CaCl2, containing 10 milliunits of heparitinase (Seikagaku) and incubated at 37 °C for 5 h. To ensure end-point digestion, the same amount of heparitinase was added to the sample and incubated for an additional 1.5 h. The supernatants obtained after centrifugation at 13,000 rpm for 5 min were recovered.

N-Acetylation of primary amino groups was performed by applying 50 mM tricine buffer, pH 7.0, containing 20 μM acetic anhydride/ml for 3 h at 37 °C for 5 h.

N-Acetylation of primary amino groups was performed by applying 50 mM tricine buffer, pH 7.0, containing 20 μM acetic anhydride/ml for 3 h at 37 °C for 5 h.

Immunochemical Procedures—Indirect immunofluorescence was performed as described previously (35) on 2-μm rat kidney cryostat sections, using biotinylated mAb JM-403 (24), which has now been partially characterized with regard to the epitope requirement. In the present report, evidence is provided that the epitope is dominated by the presence of an unsubstituted glucosamine residue(s). Moreover, immunohistochemical application of the mAb demonstrates that the N-unsubstituted GlcN units occur in situ, hence in native HS, and is not introduced as an artifact during the isolation procedure. The possible biological significance of these findings is discussed.

RESULTS

Expression of the JM-403 Epitope in Different Heparan Sulfates—Immunofluorescence with anti-HS mAb JM-403 on cryostat sections showed the presence of the cognate epitope in a variety of basement membranes, as demonstrated in Fig. 1A for the rat kidney. The glomerular basement membrane stained most intensely, along with Bowman’s capsule and the basal lamina surrounding vascular smooth muscle cells. By contrast, the tubular basement membranes were largely negative. On the other hand, all renal basement membranes were stained by an antibody against the core protein of a HS-GP derived from human glomerular basement membrane (Fig. 1B). They were likewise positive for mAb 3G10, which reacts with the residual HS stubs remaining after enzymatic cleavage by heparitinase (Fig. 1C). The resultant 3G10 epitope contains an essential, terminal, 4,5-unsaturated uronate residue and thus can serve as a general HS marker (39). These results indicate the presence of HS-GPs in all renal basement membranes, only some of which contain the JM-403 epitope. To strengthen this conclusion, we performed double-staining experiments on rat kidney cryostat sections, using biotinylated mAb JM-403 and another anti-HS mAb (JM-13), which also shows a selective basement membrane staining (37). We thus found HS positive tubular basement membranes with anti-HS mAb JM-13 (Fig. 2A), which are negative (or below the detection limit of the method) for JM-403 (Fig. 2B).

The heterogeneity of HS preparations with regard to JM-403 binding was furthermore demonstrated by testing the inhibitory capacity of eight HS preparations, isolated from different sources, in the JM-403 ELISA. The results (Table I) clearly demonstrate a great variability in antibody binding to these HS preparations. Interestingly, the sulfate content of these polysaccharides correlated with the IC50 (p < 0.0011) in the JM-403 ELISA, indicating that the JM-403 epitope is predominantly found in low sulfated (<1.0 sulfate group/disaccharide unit) HS species.

The JM-403 Epitope Is Dependent on the Presence of an N-Unsubstituted Glucosamine Residue(s)—In order to further define the JM-403 epitope, HS from human aorta (HS-II) was selectively degraded so as to allow the separation of N-sulfated and N-acetylated block structures. N-Sulfated oligosaccharides were isolated after deacetylation of GlcNAc residues using hydrazinolysis followed by exhaustive digestion at pH 3.9, which results in cleavage of the polysaccharide at the N-unsubstituted units (see “Materials and Methods”). N-Acetylated oligosaccharides were recovered following exhaust-
Fig. 1. Indirect immunofluorescence staining of rat kidney cryostat sections. A, anti-HS mAb JM-403; B, anti-HSPG-core protein antibodies; C, anti-HS-stub mAb 3G10. Magnification, × 350. For further experimental data see "Materials and Methods."

Fig. 2. Double-staining of heparan sulfate in rat kidney cryostat sections by indirect immunofluorescence. A, anti-HS mAb JM-13; B, anti-HS mAb JM-403. Arrows indicate tubular basement membranes positive for JM-13 but negative for JM-403. Magnification, × 350.

N- and N-sulfated GlcN residues would seem to be important, as indicated by the loss of antibody binding after deamination at pH 1.5.

Presence of N-Unsubstituted Glucosamine Units in Native HS—The presence of N-unsubstituted GlcN units in native HS-II was investigated by gel chromatography on Sephacryl
N-Unsubstituted D-Glucosamine Units in Heparan Sulfates

TABLE I

<table>
<thead>
<tr>
<th>HS preparation</th>
<th>Sulfate groups/disaccharide</th>
<th>IC50 (ng HS/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human aorta</td>
<td>0.60</td>
<td>300</td>
</tr>
<tr>
<td>Bovine aorta</td>
<td>0.56</td>
<td>320</td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>0.64</td>
<td>392</td>
</tr>
<tr>
<td>Bovine lung</td>
<td>0.91</td>
<td>351</td>
</tr>
<tr>
<td>Bovine intestine</td>
<td>0.98</td>
<td>1133</td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>1.01</td>
<td>1872</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>1.50</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Pig intestine</td>
<td>1.64</td>
<td>&gt;5000</td>
</tr>
</tbody>
</table>

*See Ref. 25.

*As determined by Organon.

**Effect of high-pH deamination on HS-II.** Samples of HS-II were subjected to gel chromatography on Sephacryl S-300, as described under "Materials and Methods," before (O) and after (•) treatment with HNO3 at pH 3.9. Effluent fractions were analyzed for hexuronic acid by the carbazole reaction. The elution pattern of a sample of HS-II that had been subjected to chemical N-acetylation followed by deamination at pH 3.9 was essentially unchanged compared with that of native, untreated HS-II (data not shown).

**Fig. 3.** Fluid phase inhibition of JM-403 binding to kidney heparan sulfate by chemically modified HS-II. N, and heparin preparations. Dose-response inhibition curves were generated as described under "Materials and Methods." A, native HS-II (·); N-deacetylated HS-II (O); HS-II treated with HNO3 at pH 1.5. HS-II treated with HNO3 at pH 3.9, N-deacetylated HS-II treated with HNO3 at pH 3.9, or N-acetylated HS-II (O; all samples showing lack of reactivity). B, N-deacetylated K5 polysaccharide (·); N,O-desulfated, N-deacetylated heparin (+); N-deacetylated, O-sulfated K5 polysaccharide (O); native K5 polysaccharide, native heparin, N-deacetylated heparin, N-desulfated heparin (O).

S-300, before and after exhaustive deamination of the polysaccharide at pH 3.9. A shift of the peak elution position, corresponding to a decrease in 

\[ M_r \sim \frac{45 \times 10^3}{12-15 \times 10^3}, \]

was observed (Fig. 4), suggesting the presence of, on the average, two or three N-unsubstituted GlcN units/chain. By contrast, HS-II that had been previously N-acetylated by reaction with acetic anhydride resisted deamination under these conditions. This control experiment ascertained that deaminative cleavage at pH 3.9 is indeed restricted to N-unsubstituted GlcN residues.

N-Acetylation of kidney cryostat sections with acetic anhydride abolished all staining due to mAb JM-403 (data not shown). This treatment simultaneously precluded staining of the section with fluorescamine, as expected for acetylation of primary amino groups. By contrast, the staining intensities of three other anti-HS mAbs (JM-13, 10E4, and HepSS1) as well as of the anti-HSPG core antibody B131, were only marginally affected by the treatment with acetic anhydride (data not shown). These findings indicate that unsubstituted amino groups do indeed occur in native HS, as present in tissue sections that have not been treated or fixed in any way (see Fig. 1A) and confirm our conclusion that N-unsubstituted GlcN units are essential for recognition by mAb JM-403.

Recognition of Chemically Modified Heparin and E. coli K5 Capsular Polysaccharides by mAb JM-403—Neither the non-sulfated E. coli K5 capsular polysaccharide, which has the same (GlcA-GlcNac),, structure as the unmodified biosynthetic precursor of heparin/HS (27), nor native heparin, which is extensively modified and carries ~2,5 sulfate groups/disaccharide unit, showed any binding to JM-403 (Fig. 3B). N-Decacylating the polysaccharides by hydrazinolysis induced strong antibody reactivity in the K5 polysaccharide, but not in heparin (Fig. 3B). These findings demonstrate that a structure composed of alternating GlcA and N-unsubstituted GlcN units, joined by the appropriate linkages, is sufficient for antibody recognition. Why is the N-deacetylated heparin, which is expected to contain 2–3 N-unsubstituted GlcN units/chain (41), not recognized by JM-403? Since N-desulfation of heparin also failed to induce JM-403 reactivity (Fig. 3B), it seemed likely that factors other than the availability of N-unsubstituted GlcN units would have to be considered.

N-Decetylated, N/O-desulfated ("naked") heparin differs from N-deacetylated K5 polysaccharide in one major regard, i.e., the occurrence of IdoA units. The effects of these constituents were evaluated by testing N-deacetylated K5 polysaccharide as well as naked heparin in the JM-403 inhibition ELISA.
The inhibitory activity of naked heparin was ~100-fold lower than that of the N-deacetylated K5 polysaccharide (Fig. 3B). Since the naked heparin differs from the N-deacetylated K5 polysaccharide merely by the presence of Idoa, it is inferred that such units interfere with antibody binding; the residual activity is probably associated with the limited number of GlcA residues present in the polymer (~20% of the total hexuronic acid contents (41)). Contrary to the native heparin, N-deacetylated heparin and N-desulfated heparin, the naked heparin was reactive (Fig. 3B), indicating that removal of O-sulfate groups exposed epitope structures. To confirm the negative effect of O-sulfate groups on JM-403 binding, we compared in the ELISA the inhibition induced by N-deacetylated K5 polysaccharide with that of the N-deacetylated, O-sulfated analog. This latter K5 derivative contains an average of 1.2 O-sulfate (but no N-sulfate) groups/disaccharide unit. The locations of the O-sulfate groups were not defined but would presumably primarily involve C-6 of the GlCN units, along with C-2 and/or C-3 of the GlcA units. The results in Fig. 3B clearly show that O-sulfation of N-deacetylated K5 polysaccharide strongly reduces JM-403 binding.

Taken together, these data explain the lack of JM-403 reactivity toward native, N-deacetylated, or N-desulfated heparin, and, moreover, conform to the observed decrease in antibody binding to HS preparations with increasing degree of sulfation (hence, increasing Idoa contents (42)). Furthermore, the experiments demonstrate that neither (N- or O-) sulfated groups nor N-acetyl groups are required for JM-403 binding, since N-deacetylated K5 (and naked heparin) lack such groups yet are recognized by the antibody.

**Location of JM-403 Epitopes in the HS Chain**—In order to define the location of the JM-403 epitope along the HS chain, samples of HS-II were enzymatically or chemically degraded using reagents/conditions with well defined cleavage specificity. The resultant oligosaccharides were then tested in the JM-403 ELISA (Table II). As indicated above, cleavage of HS-II at N-sulfated GlcN residues by the HNO2/pH 1.5 procedure abolished all binding to JM-403 (Fig. 3A). This finding excludes an epitope consisting of merely N-unsubstituted and N-acetylated disaccharide units. On the other hand, N-sulfate groups are not required for JM-403 binding (as shown by the N-deacetylated K5 sample; Fig. 3B). These results therefore indicate that N-sulfate groups, while not essential for reactivity, are tolerated, and, in addition, are invariably present in the HS epitope. In fact, complete loss of reactivity occurred on low pH degradation of six different HS preparations from different sources (the inhibitory samples from Table I). Digestion of HS-II by heparinase (heparinase III) also abolished all reactivity. This enzyme cleaves glucosaminidic linkages between either N-acetylated or N-sulfated GlcN residues and nonsulfated hexuronic acid units. By contrast, HS-II binding to JM-403 was found to be essentially insensitive to heparinase I, which cleaves glucosaminidic linkages between N-sulfated GlcN and 2-O-sulfated Idoa units. The epitope is thus in all probability located outside the heavily modified, heparin-like, N-sulfated block sequences of the HS chain. The most likely site for such a structure is in regions composed of mixed N-acetylated and N-sulfated disaccharide units, with a relative high GlcA content, at the boundaries between unmoldified and highly sulfated blocks of HS (Table II).

**DISCUSSION**

The present study deals with the saccharide recognition properties of anti-HS mAb JM-403, the generation and basic characteristics of which were described previously (24). Applying the antibody to immunofluorescence studies on rat kidney sections revealed that the carbohydrate epitope is not evenly distributed among the HS subspecies of this tissue. All basement membranes, as expected, were found to contain HS-PCs as evidenced using other antibodies. However, the polysaccharide present in tubular basement membranes stained poorly with JM-403, suggesting the existence of HS-isofoms lacking the corresponding epitope. It thus seemed important to characterize this epitope with regard to saccharide structure.

The results of such studies demonstrated a critical role for an N-unsubstituted GlcN unit(s) within the epitope. The occurrence of these residues in appropriate linkage to GlcA units suffices to induce strong antibody binding. However, the naturally occurring epitope(s) in native HS chains apparently have a more complex structure. The results of chemical and enzymatic degradation experiments suggest that the HS epitopes contain not only GlcA units but also N-sulfated GlcN residues, whereas Idoa units and O-sulfate groups appear to impede antibody recognition. Such structures are likely to be found in regions of the HS chains that contain mixed N-acetylated and N-sulfated sequences. It seems probable that N-unsubstituted GlcN units in a highly N- and O-sulfated, Idoa-rich surrounding, as presumably occurring also in heparin (43), will escape detection by JM-403. Indeed, we cannot exclude that the epitope represents only a minor proportion of the N-unsubstituted GlcN units in HS and that such units occur in multiple structural contents.

Considering possible mechanisms for the generation of GlcN units with unsubstituted amino groups, there are two major alternatives (disregarding the theoretical but unlikely possibility of a UDP-GlcN sugar nucleotide precursor containing an N-unsubstituted GlcN unit). The normal conversion of N-acetylated to N-sulfated GlcN units in heparin/HS biosynthesis is catalyzed by a single enzyme protein, which is capable of promoting both the N-deacetylation and the N-sulfation reactions. Two related yet distinct glucosaminyl N-deacetylasen/sulfotransferase enzymes have been described, one from rat liver (44) and the other from mouse mastocytoma (45), that differ with regard to size of mRNA transcript, amino-acid sequence, cofactor requirement, and kinetic properties (46–48). While the interrelationship between these enzymes with regard to the biosynthesis of heparin and HS is unclear, both enzymes express both catalytic activities, which normally appear to be tightly coupled. However, under the appropriate experimental conditions the two activities are readily dissociated. Thus, formation of a heparin precursor polysaccharide by incubating a mouse mastocytoma microsomal fraction with UDP-GlcA and UDP-GlcNac, but in the absence of adenosine 3'-phosphate 5'-phosphosulfate (PAPS), yields a product in which about one-third of the GlcN units are N-deacetylated and have an unsubstituted amino group (49). Indeed, recent exper-
N-Unsubstituted D-Glucosamine Units in Heparan Sulfates

A similar effect can be achieved by treating intact cells with chloride, which will block the formation of PAPS (50). It is possible that limited formation of N-unsubstituted GlcN units may occur during the normal biosynthesis of HS, by N-deacetylation of selected (?) target GlcNAc residues that is not followed by N-sulfation, or after completion of the biosynthesis, maybe in the extracellular space.

An alternative mechanism for the formation of N-unsubstituted GlcN units was considered. Enzymes that can cleave the terminal GlcN unit on the intact polysaccharide chain. While sulfamidases have been implicated in HS metabolism, these enzymes seem to act in an exo-facial fashion, at the non-reducing terminus of the chain (51). No endo-

zyme of the type required to generate the JM-403 epitope has yet been described. Notably, however, Dawes and Pepper (62) proposed that heparin and HS may be extensively desulfated without demethylation in vascular endothelial cells. Although not yet verified, these results would imply the existence of a sulfatase(s) acting on the intact polysaccharide chain. The demonstration of N-unsubstituted GlcN units in native HS preparations raises intriguing questions regarding the biological significance of these constituents. It has been reported that endothelial heparin-related molecules can bind to L-selectin. Recently, this ligand, associated with the cultured cells or secreted into the medium/extracellular matrix, was identified as HS (54). Surprisingly, these L-selectin-binding HS chains were found to be enriched in N-unsubstituted GlcN residues, suggesting a role for the free amino groups in L-selectin binding or in the control of the biosynthetic process leading to the formation of L-selectin-binding saccharide sequences (54). The L-selectin binding HS species were produced by cultured bovine endothelial and human umbilical vein endothelial cells but not by Chinese hamster ovary cells. These findings correlate with our demonstration of strong JM-403 staining of vascular basement membranes (which are produced by endothelial cells). Functionally, this might indicate that vascular basement membranes are capable of binding leukocytes, which constitutively express L-selectin on their cell surface. Other potential important biological activities of vascular HS, to be considered in this context, include anti-proliferative effect on arterial smooth muscle cells (16), inhibition of mesangial cell growth (55), and inhibition of angiogenesis (56).

We have previously shown that intravenous injection of mAb JM-403 into rats leads to instantaneous albuminuria (24). We hypothesized that this effect was due to blocking of the anionic charges of HS in the glomerular basement membranes by the antibodies. Alternatively, binding of JM-403 to epitopes containing N-unsubstituted GlcN units might interfere in a more specific manner with the interactions between HS and other basement membrane constituents, thus inducing a more leaky ultrastructure. The possible influence of the N-unsubstituted GlcN units on the interaction between HS and other extracellular matrix molecules should be investigated.

The expression of the JM-403 epitope by glomerular basement membrane HS is decreased in glomerular diseases that are characterized by mesangial matrix expansion and/or proteinuria (57). This finding could be explained by (a) masking of HS by cationic molecules or immune complexes; (b) decreased synthesis and/or increased degradation of HS; or (c) an altered structure of HS resulting in loss of the JM-403 epitope, for instance, due to loss of N-unsubstituted GlcN units and/or HS oversulfation. The latter alternative has potential implications with regard to the activities of growth factors that are modulated through interactions with HS (58–61) and, furthermore, are known to be involved in various forms of glomerulonephritis (62, 63).

REFERENCES

N-Unsubstituted D-Glucosamine Units in Heparan Sulfates