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Distribution of GBM heparan sulfate proteoglycan core protein and side chains in human glomerular diseases

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Distribution of GBM heparan sulfate proteoglycan core protein and side chains in human glomerular diseases. Using monoclonal antibodies (mAbs) recognizing either the core protein or the heparan sulfate (HS) side chain of human GBM heparan sulfate proteoglycan (HSPG), we investigated their glomerular distribution on cryostat sections of human kidney tissues. The study involved 95 biopsies comprising twelve different glomerulopathies. Four normal kidney specimens served as controls. A homogenous to linear staining of the GBM was observed in the normal kidney with anti-HSPG-core mAb (JM-72) and anti-HS mAb (JM-403). In human glomerulopathies the major alteration was a segmental or total absence of GBM staining with anti-HS mAb JM-403, which is most pronounced in lupus nephritis, membranous glomerulonephritis (GN), minimal change disease and diabetic nephropathy, whereas the HSPG-core staining by mAb JM-72 was unaltered. In addition we found HSPG-core protein in the mesangial matrix when this was increased in membranoproliferative GN Type I, Schönlein-Henoch GN, IgA nephropathy, lupus nephritis, diabetic nephropathy and in focal glomerulosclerosis. Also staining with the anti-HS mAb JM-403 became positive within the mesangium, although to a lesser extent. Furthermore, amyloid deposits in AL and AA amyloidosis clearly stained with anti-HSPG-core mAb JM-72, and to a lesser degree with anti-HS mAb JM-403. Finally, in membranous GN (stage II and III), the GBM staining with anti-HSPG-core mAb JM-72 became irregular or granular, probably related to the formation of spikes. In conclusion, major alterations were observed in the glomerular distribution of HS and HSPG-core in various human glomerulopathies. The mAbs can be useful to further delineate the significance of HSPG and HS for glomerular diseases.

The biochemical and biophysical properties of the glomerular capillary wall (GCW), which functions both as a size and a charge selective filter, are, in addition to hemodynamic and oncotic factors, important determinants of glomerular ultrafiltration [1]. The physicochemical basis for the charge-selective filtration is thought to be an electrostatic repulsion of the negative charge of albumin and other plasma proteins by the fixed negative charge of the GCW [2, 3]. Initially it was thought that sialoglycoproteins, located on the cell membranes of glomerular endothelial and epithelial cells, were mainly responsible for the negative charge of the GCW. Later on it became clear that also the anionic glycosaminoglycan heparan sulfate (HS), which is the side chain of heparan sulfate proteoglycan (HSPG), present in the glomerular basement membrane (GBM), was a major determinant of the charge-dependent permeability of the GCW [4–7]. Enzymatic digestion of HS in the GCW causes an increased passage of native ferritin and albumin into the urinary space [4, 5]. Furthermore, intrarenal or intravenous injection of cationic molecules leads to an increased GBM permeability due to the neutralization of primarily HS-associated anionic sites of the GCW [8–12]. Recently, we demonstrated the induction of an acute selective proteinuria after intravenous injection of a monoclonal antibody (mAb) against GBM HS [13].

In many types of glomerular disease, major alterations occur in the permeability of the GBM, resulting in proteinuria. For that reason several investigators have tried to relate the status of GBM HSPG to the onset or existence of proteinuria in many types of glomerulopathy [14]. Firstly, using cationic probes both in humans and in experimental animals, a reduction of GBM anionic sites was found in systemic lupus erythematosus [15–17], aminonucleoside nephropathy [18], minimal change disease [19], membranous glomerulonephritis (GN) [20–24], congenital nephrotic syndrome [25], IgA nephropathy [26, 27] and diabetic nephropathy [28–30]. Secondly, with biochemical and immunochemical methods HSPG alterations were observed in membranous GN [31], polycystic kidney disease [32] and diabetic nephropathy [33–37]. However, in many of these studies the methods used (cationic probes, ^{35}S-sulfate incorporation) are not specific for HS and controversies still exist that hamper a clear interpretation [38–44]. Finally, some reports have described changes in the immunohistochemical distribution of HSPG-core protein in several glomerulopathies [45–51]. However, an immunohistochemical analysis of the distribution of glomerular HS has not been performed so far.

Recently, we developed mAbs against the core protein of human GBM HSPG [52] and against the HS-side chain of GBM HSPG [13]. In this paper we describe the glomerular distribution of HSPG and HS in twelve different types of human glomerular diseases using these mAbs in indirect immunofluorescence (IF) on cryostat sections of kidney biopsies. One major alteration that we observed was a strong decrease or even absence of GBM staining with anti-HS mAb JM-403 in several glomerular diseases, whereas the HSPG-core staining by mAb JM-72 was unaltered. These results indicate an impaired HS

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Table 1. Characteristics of anti-human GBM HSPG-core mAb JM-72 and anti-HS mAb JM-403

Characteristic	Anti-HSPG-core mAb JM-72	Anti-HS mAb JM-403
Isotype	IgG1	IgM
Immunogen	human GBM HSPG	rat glomerular HSPG
Species specificity	human	rat, mouse, human
Binding to[a]		
Human GBM HSPG	yes	yes
Human GBM HSPG-core	yes	no
HS-side chain	no	yes
Staining of the GBM[b]	yes	yes
Staining sensitive for heparitinase[c]	no	yes

[a] Binding was studied in ELISA, inhibition ELISA and Western blot
[b] In indirect immunofluorescence and immunoelectron microscopy
[c] Pretreatment of cryostat kidney sections with heparitinase and subsequent indirect immunofluorescence

expression or accessibility in several types of human glomerular diseases.

Methods

Kidney tissue specimens

This study includes 99 renal tissue specimens. Four of them were control tissues obtained during operation or taken from cadaveric donor kidneys which were not suitable for transplantation. The remaining 95 renal biopsies originated from the Departments of Pathology of the University Hospitals of Nijmegen and Groningen, and were either obtained by percutaneous renal biopsy or at autopsy from patients suffering from various renal diseases. The tissue specimens were snap-frozen in liquid nitrogen and stored at −70°C. Classification of glomerular disease was established using routine light, immunofluorescence (IF) and if possible electron microscopy.

Antibodies

The two mAbs recognizing different epitopes of human GBM HSPG used in this study have been described previously [13, 52]. One of them (JM-72) is directed against the core protein; the other (mAb JM-403) recognizes the HS-side chain of GBM HSPG. The major characteristics of these mAbs are summarized in Table 1. Immunohistochemical detection of AA amyloidosis was done with mAb REU 86.2 against AA amyloid (Immuno Quality Products, Groningen, The Netherlands).

Inhibition ELISA

Inhibition ELISAs were performed as described previously [13]. Briefly, a constant amount of mAb (JM-72 or JM-403) was added to an increasing amount of the following inhibitors: HS (from bovine kidney; Seikagaku, Tokyo, Japan), human GBM HSPG and its core protein, isolated as described previously [53]. These mixtures were tested in ELISAs with GBM HSPG (for JM-72) or HS (for JM-403) as coated antigens. Results are expressed as % inhibition of the ELISA signal calculated as $[1 - (A450 + \text{inhibitor}/A450 - \text{inhibitor})] \times 100\%$.

Indirect IF

Initially, kidney biopsies were analyzed with JM-72 alone. Two μm cryostat sections were fixed in acetone for five minutes. Thereafter, 100 μl of JM-72 ascites, diluted 1:200 in PBS containing 1% BSA and 0.05% sodium azide (IF buffer), were applied to the sections for 30 minutes at room temperature. Incubations of the sections with non-relevant mAbs with an IgG_1 isotype served as negative controls. After washing with PBS, binding of JM-72 (anti-GBM HSPG-core) was visualized with $F(ab)_2$-fragments of FITC-labeled sheep anti-mouse IgG, heavy and light chains (Cappel-Organon Teknika, Turnhout, Belgium), 100 μl diluted 1:500 in IF buffer. After incubation for 30 minutes in the dark at room temperature, the sections were washed with PBS and embedded in Aquamount. Later on, when the anti-HS mAb JM-403 became available, renal biopsies were double-stained with anti-HS mAb JM-403 and mAb JM-72 (anti-HSPG-core). Sequential incubations were done as follows: (a) mAb JM-403 (1:200 dilution of ascites fluid in IF buffer); (b) FITC-labeled goat anti-mouse IgM, Fc-specific (Nordic, Tilburg, The Netherlands, 1:100 in IF buffer); (c) mAb JM-72 (1:200 dilution of ascites fluid in IF buffer containing 5% normal human serum); (d) TRITC-labeled goat anti-mouse IgG_1 (Nordic, 1:25 in IF buffer containing 5% normal human serum and 10 μg/ml mouse IgM to prevent any cross reactivity with mAb JM-403). The double staining experiments included the following controls: (a) omitting mAb JM-403 or JM-72 or both; (b) incubating mAb JM-403 or JM-72 with the conjugate used for the detection of the other mAb in the double staining. Sections were examined on a Leitz ortholux microscope equipped with a Ploemopak epi-illuminator.

Results

Characteristics of the anti-HSPG mAbs

The two mAbs used in this study recognize different epitopes of GBM HSPG (Table 1, Fig. 1). The mAb JM-72 is directed against an epitope on the core protein of human GBM HSPG (Fig. 1A), whereas mAb JM-403 recognizes an epitope on the HS-side chain of HSPG (Fig. 1B). Although mAb JM-403 was obtained after immunization with rat glomerular HSPG, this mAb cross reacts with the HS-side chain of human GBM HSPG in ELISA and indirect IF on human kidney sections. The glomerular staining patterns of these mAbs in normal human kidney are shown by a double staining technique in the indirect IF in Figure 2. A strong linear staining of the GBM is observed with mAb JM-72 (Fig. 2A). A homogeneous, fine granular staining was seen along the GBM with anti-HS mAb JM-403 (Fig. 2B). In normal kidney biopsies of young individuals a very faint staining of the mesangium was observed with both mAbs, but this staining increased with advanced age if paralleled by an expansion of the mesangium.

Alterations in HSPG distribution in glomerular diseases

The various types of glomerulopathies and the number of biopsies that were analyzed for their HSPG distribution are listed in Table 2. Less biopsies were available for the evaluation of JM-403 staining ($N = 74$) compared to mAb JM-72 ($N = 95$), since JM-403 was produced and characterized later, and for some biopsies, tissue was not available. The alterations in glomerular HSPG immunolocalization can be categorized as follows: (a) the most important finding was a segmental or total absence of GBM staining with anti-HS mAb JM-403 in various glomerulopathies; in addition (b) the presence of HSPG in

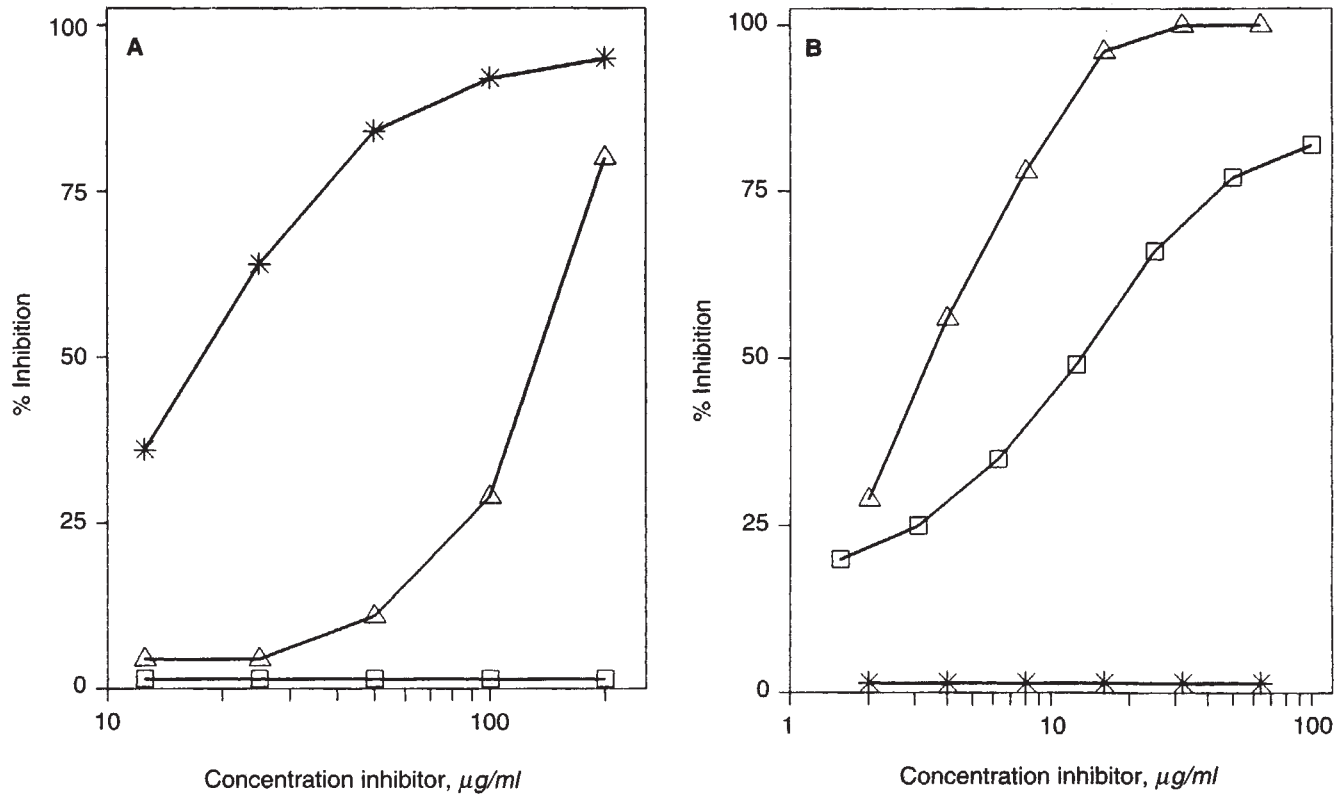

Fig. 1. *Antigen specificity of mAb JM-72 (A) and mAb JM-403 (B), demonstrated in the inhibition ELISA.* Human GBM HSPG was used as coated antigen in (**A**), HS in (**B**). A dose-dependent inhibition of mAb JM-72 (**A**) was found with intact GBM HSPG (–△–) and its core protein (–*–), but not by HS (–□–). MAb JM-403 (**B**) was inhibited in a dose-dependent manner by intact GBM HSPG (–△–) and HS (–□–), but not by the core protein of GBM HSPG (–*–).

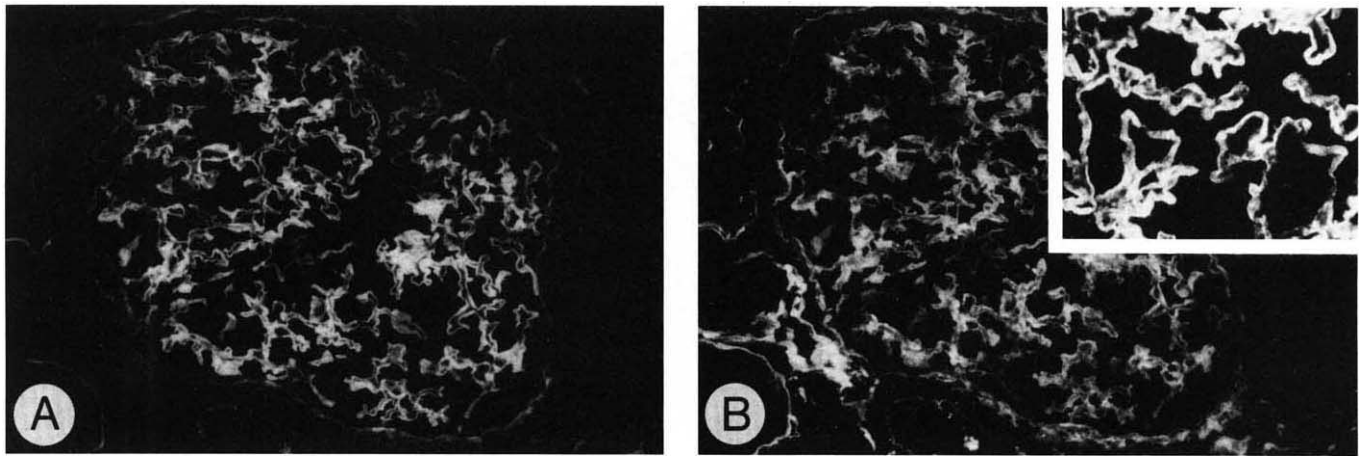

Fig. 2. *HSPG localization in the normal human kidney.* Indirect IF on normal human kidney cryostat sections with anti-HSPG-core mAb JM-72 (**A**) and anti-HS mAb JM-403 (**B**) in a double staining technique. In this control kidney there is also positive staining of the mesangium, due to age-related expansion of the mesangial matrix. Insert in (**B**) demonstrates the GBM staining of mAb JM-403 at a higher magnification (**A** and **B** × 260, insert × 450).

expanded mesangial matrix in several glomerulopathies; (c) the presence of HSPG in glomerular amyloid deposits; and (d) an irregular GBM staining in stage II and III membranous GN with anti-HSPG-core mAb JM-72.

Decreased anti-HS mAb JM-403 staining. The majority of biopsies investigated in this study with anti-HS mAb JM-403 showed a segmental or total absence of GBM staining (Table 2). This phenomenon was most pronounced in SLE nephritis, membranous GN, minimal change disease and diabetic nephropathy and was not observed in IgA nephropathy or Alport's syndrome. In all other glomerulopathies listed in Table 2, a lesser degree of reduced GBM staining with JM-403 was

Table 2. Types of glomerulopathies studied with anti-HSPG-core mAb JM-72 and anti-HS mAb JM-403 together with the GBM staining pattern of mAb JM-403

Glomerulopathy	JM-72[a,b]	JM-403[a,c]		
		Normal	Segmental	Absent
Proliferative				
Membranoproliferative GN[d] type I	6	2		1
Crescentic GN	7	2	4	
Diffuse endocapillary GN (post-infectious)	2		1	
Schönlein-Henoch purpura	4	1	1	
IgA nephropathy	4	3		
Lupus nephritis (WHO class III, IV and V)	13		1	12
Non-proliferative				
Minimal change disease	7		3	3
Focal glomerulosclerosis	8	1	3	1
Membranous GN (Stage II and III)	6		1	4
Other				
Diabetic nephropathy (nodular and diffuse)	13	2	5	3
Alport's syndrome	2	1		
Amyloidosis				
AL	7	1	4	
AA	16	3	10	1

[a] The numbers of biopsies examined with each mAb are given
[b] For JM-72 the GBM staining was normal in all biopsies studied
[c] For anti-HS mAb JM-403 biopsies were categorized according to their GBM staining pattern
[d] GN = glomerulonephritis

observed. Although the decrease or absence of anti-HS staining may be related to the nature and progression of the disease, we were not able to correlate the GBM staining pattern with anti-HS mAb JM-403 to the severity of the disease. No differences were observed between patients with focal (N = 3) or diffuse (N = 3) crescentic GN, patients with WHO class III (N = 2), class IV (N = 8) or class V (N = 3) lupus nephritis, patients with stage II (N = 4) or stage III (N = 1) membranous GN, and patients with nodular (N = 4) or diffuse (N = 6) diabetic nephropathy. We also did not observe an association between the GBM staining for HS and the degree of proteinuria. The most striking observation in this respect is that in patients with minimal lesions glomerulopathy who were in remission (N = 4), the decrease in HS staining was identical to those who were still nephrotic (N = 2). In spite of the clear decrease in JM-403 staining, all biopsies showed a normal staining of the GBM with the anti-HSPG-core mAb JM-72 as can be seen in the double staining shown in Figure 3 for lupus nephritis, minimal change disease, and diabetic nephropathy, respectively.

HSPG in expanded mesangial matrix. The HSPG-core protein could be demonstrated in mesangial areas of kidney specimens showing expansion of mesangial matrix (Table 3). This presence of HSPG in the mesangium was seen in membranoproliferative GN type I (Fig. 4A), Schönlein-Henoch GN, IgA nephropathy, lupus nephritis (Fig. 3A), diabetic nephropathy, mostly in the noduli (Fig. 3E), and in focal glomerulosclerosis (Fig. 4B). However, an increase in mesangial matrix was not observed in all biopsies of the aforementioned glomerular diseases, and in these biopsies HSPG could not be demonstrated in the mesangium (Table 3). The mesangial HS staining with mAb JM-403 was less clear than for the HSPG-core (Table 3). The intensity of the mesangial staining paralleled the intensity of the HS-staining in the GBM; if the GBM staining was decreased or negative, the mesangium showed also a decreased or negative staining despite the expansion, as illustrated for lupus nephritis in Fig. 3B and the diabetic nephropathy in Fig. 3F. However, in biopsies showing expansion of mesangial matrix in combination with an unaffected HS-staining in the GBM, HS could be demonstrated in the mesangial areas. This relation between GBM and mesangial HS staining is illustrated in Figure 4C in a biopsy with focal glomerulosclerosis, although in the majority of cases with focal glomerulosclerosis (Table 2) the HS staining of the GBM and the mesangium was reduced.

HSPG in glomerular amyloid. A brilliant staining was observed with anti-HSPG-core mAb JM-72 of the amyloid deposits, localized within the mesangial areas and sometimes also along the capillary wall in AL (Fig. 5A) as well as in AA (Fig. 5B) amyloidosis (Table 4). These forms of amyloidosis were distinguished on clinical features and/or the use of an anti-AA amyloid mAb. The GBM staining with anti-HSPG-core mAb JM-72 was unaltered in these biopsies. In 10 out of 19 biopsies with amyloidosis, staining of the amyloid deposits was seen with anti-HS mAb JM-403 (Fig. 5C), although the staining was sometimes weak. Although the numbers were small, it seemed that a positive HS staining was more prevalent in AA amyloidosis than in AL.

Membranous GN. An irregular, granular staining of the GBM was observed with anti-HSPG-core mAb JM-72 in all biopsies with stage II and III membranous GN (Fig. 6). This irregular, moth-eaten pattern was not seen in every capillary loop and was in all probability related to the formation of spikes. Since the anti-HS staining was negative in four out of five cases with membranous nephropathy (Table 2), this also suggests that the newly formed basement membrane matrix at the sites of spike formation is negative for HS.

Normal distribution of HSPG and HS

A normal glomerular staining with anti-HSPG-core mAb JM-72 (linear GBM and in general no mesangial staining) was observed in crescentic GN, minimal change disease and Alport's syndrome. No alterations in GBM staining with anti-HS mAb JM-403 were observed in IgA nephropathy and Alport's syndrome and in some kidney biopsies belonging to other glomerulopathies (Table 2).

All control experiments (**Methods**) we performed in the indirect IF and the double staining experiments were negative.

Discussion

In this study, the glomerular HSPG distribution was analyzed in human glomerulopathies by indirect IF, using mAbs specific for the HSPG-core protein and for the strongly anionic HS-side chains of HSPG, which are thought to be main determinants of the charge selective filter of the GCW. Although other reports have described the distribution of the HSPG-core protein in several glomerular diseases [45–51], the present study used an unique combination of mAbs, which permitted a comparison of the HS-staining with that for the HSPG-core protein. Since the anti-HSPG-core mAb JM-72 had a different isotype compared

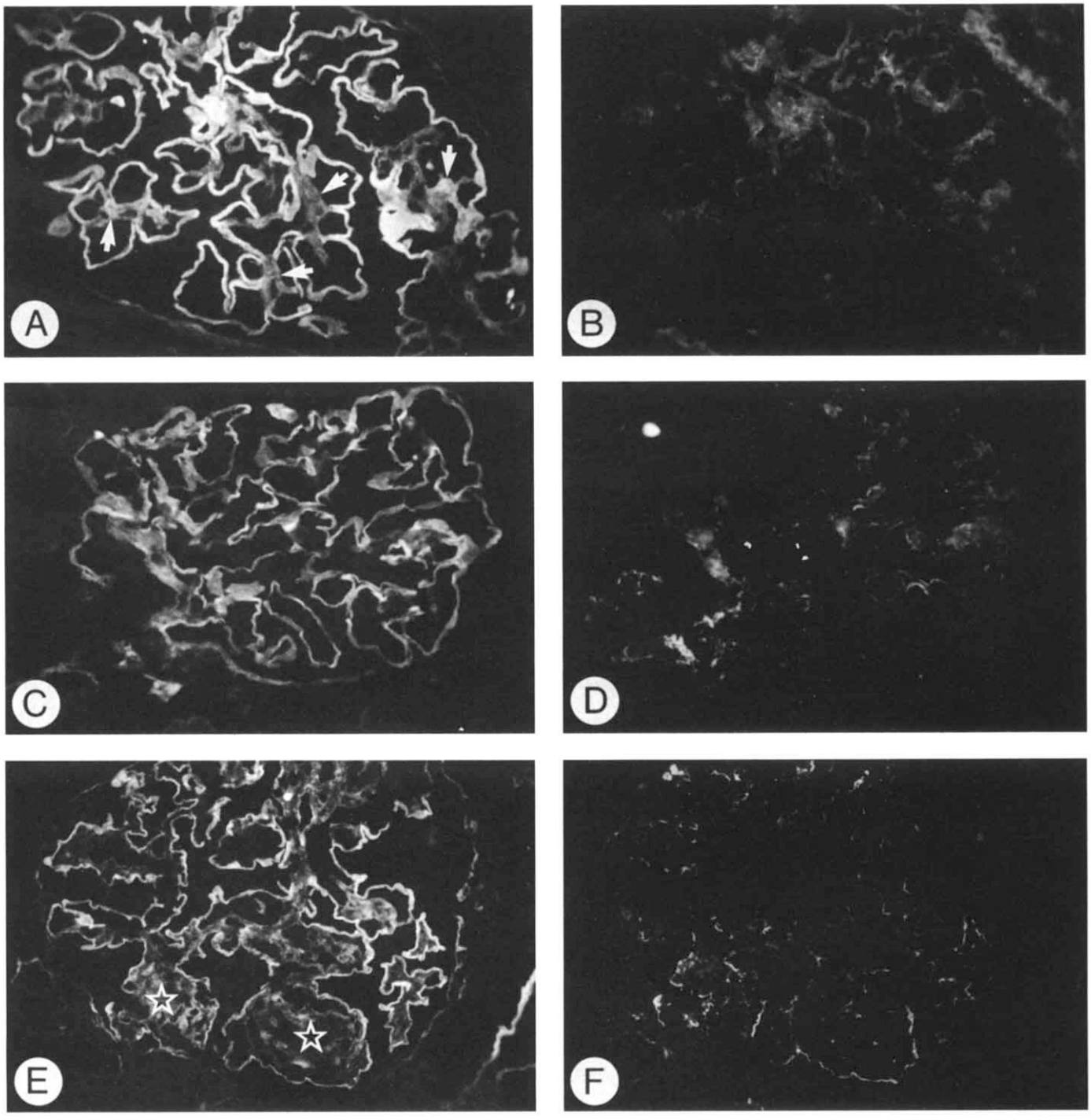

Fig. 3. *HSPG localization in some glomerulopathies.* Double staining of anti-HSPG-core mAb JM-72 (**A**, **C** and **E**) and anti-HS mAb JM-403 (**B**, **D** and **F**) in indirect IF on kidney sections of lupus nephritis (**A** and **B**), minimal change disease (**C** and **D**) and diabetic nephropathy (**E** and **F**). Note the strongly decreased anti-HS staining, accompanied with an unaltered anti-HSPG-core staining. Arrows in A and asterisks in E indicate the staining of the expanded mesangium with anti-HSPG-core mAb JM-72 (A and B × 360; C and D × 440; E and F × 220).

to anti-HS mAb JM-403 (IgG_1 vs. IgM), biopsies could be studied using a double staining technique.

The most remarkable finding was a segmental or global decrease or even absent staining of anti-HS mAb JM-403 accompanied by a normal staining for HSPG-core protein in various glomerular diseases, most notably in lupus nephritis, membranous GN, minimal change disease, and diabetic nephropathy, while it was normal in IgA nephropathy and Alport's syndrome. This decrease can be due firstly to a decreased HS expression in the GBM as a consequence of an impaired synthesis or enhanced degradation of HS. Alterations in GBM HS have been described for diabetic nephropathy [33–37] and

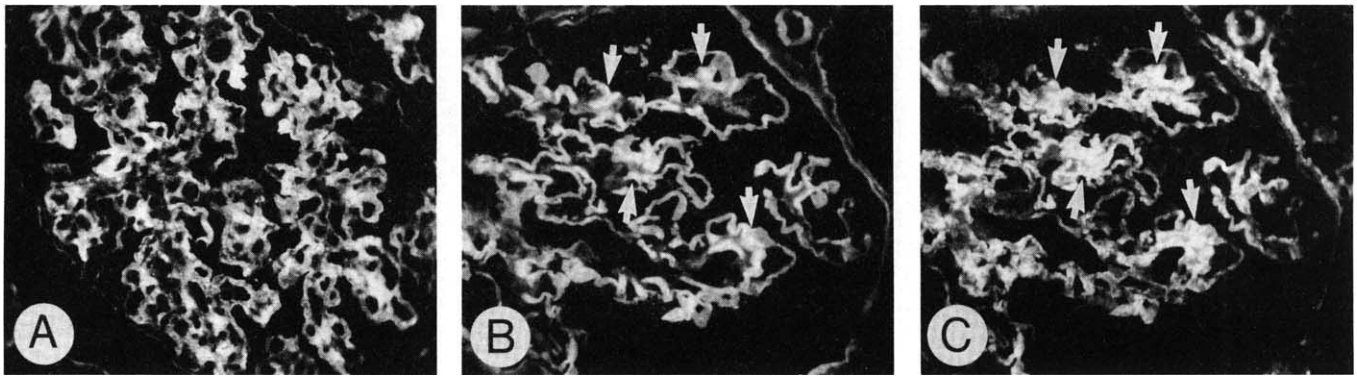

Fig. 4. *Presence of HSPG in expanded mesangial matrix.* Indirect IF with anti-HSPG-core mAb JM-72 (**A** and **B**) or anti-HS mAb JM-403 (**C**) demonstrates the presence of HSPG in mesangial matrix in membranoproliferative GN Type I (**A**), and focal glomerulosclerosis (**B** and **C**). **B** and **C** were carried out by a double staining, arrows indicate the increased mesangial matrix (A × 310; B and C × 260).

Table 3. Staining of the mesangial matrix with anti-HSPG core mAb JM-72 and anti-HS mAb JM-403

Glomerulopathy	Anti-HSPG-core mAb JM-72	Anti-HS mAb JM-403
Membranoproliferative GN type I	5/6[a]	2/3
Schönlein-Henoch GN	1/4	0/4
IgA nephropathy	2/4	1/3
Lupus nephritis	9/13	0/13
Focal glomerulosclerosis	4/8	1/5
Diabetic nephropathy	11/13	2/8

[a] Number of positive specimens/specimens studied are given

for an experimental model of membranous GN [31]. However, in all glomerulopathies showing a segmental or complete absence of JM-403 staining, anti-HSPG-core staining was normal, which indicates that the HSPG-core expression was unaltered. Secondly, the decrease of JM-403 staining might be due to a blocking effect of cationic molecules or immune complexes, that become bound to the GBM by charge-charge interactions. With cationic probes, various investigators found an impaired anionic charge of the GCW in experimental and human nephropathies [15–30]. In addition, a number of studies [15, 54–63] showed the involvement of endogenous cationic molecules in the pathogenesis of glomerular diseases. In one study the GBM binding of basic proteins was accompanied by a decrease of the anionic charge of the GBM and onset of proteinuria [60]. It is tempting to speculate that our results, demonstrating specifically a decrease or absence of GBM HS staining may be related to GBM binding of cationic proteins or immune complexes, leading to a decrease of the net negative charge of the GBM, as suggested for SLE nephritis [15–17, 54–56], minimal change disease [58, 64] and membranous GN [20–24]. The relevance of the JM-403 HS-epitope for the charge-selective properties of the GBM is suggested by our recent observation that JM-403 is able to induce an acute selective proteinuria in rats after a single intravenous injection [13]. Recent work from our laboratory [65, 66] and that of others [54] points towards a role of histones in lupus nephritis. Recently, the glomerular localization of histones was demonstrated in human SLE kidney biopsies [55]. The binding of histones to the GBM might explain our immunohistochemical findings in the SLE kidney biopsies showing a complete absence of JM-403 staining, accompanied by an unaltered JM-72 binding. Preliminary data from our laboratory show that cationic molecules like histones are indeed able to inhibit the binding of JM-403 to HS in ELISA, but not the binding of JM-72 to HSPG (unpublished data). Whether a similar mechanism is also true for other glomerular diseases remains to be elucidated and is under current investigation.

In the present study, we were not able to correlate the GBM staining pattern with anti-HS mAb JM-403 to clinical parameters of the patients, especially proteinuria. This may be due to the retrospective character of this study. However, we have preliminary data in the adriamycin model in the rat demonstrating a significant correlation between the GBM staining with mAb JM-403 and the fractional urinary excretion of albumin in course of time. It is attractive to hypothesize that an increase in urinary albumin excretion may be the result of an altered HS expression/accessibility in the GBM. An alternative explanation for the decreased HS staining of the GBM could be an altered HS metabolism as a consequence of the high levels of proteinuria. In that case, a decreased anti-HS staining would be an aspecific phenomenon related to the presence of proteinuria. An argument against the latter explanation is the fact that we found a normal HS staining in IgA nephropathy, Alport's syndrome and some biopsies from several other glomerular diseases with increased proteinuria.

A second alteration we observed in glomerular HSPG distribution deals with the presence of HSPG in areas with increased mesangial matrix production, suggesting the production of HSPG by mesangial cells in these glomerular diseases (Table 3). Normally, chondroitin sulfate proteoglycan appears to be concentrated in the mesangial matrix [67] and is the major proteoglycan produced by mesangial cells in culture [68–70]. However, in several experimental and human glomerular diseases, an expansion of the mesangium is accompanied by an altered composition of the mesangial matrix [71–75], one of them showing the presence of HSPG [71].

In amyloidosis we observed a brilliant staining of the amyloid deposits with anti-HSPG-core mAb JM-72 and in some, but not all cases with anti-HS mAb JM-403. The presence of HSPG in various forms of amyloid has been known for several years [reviewed in 76], and was recently demonstrated for neural and

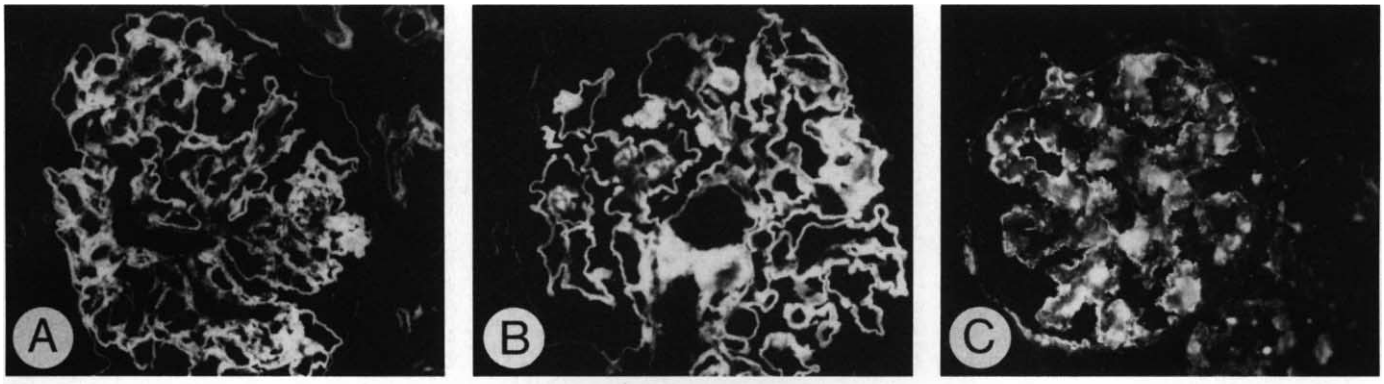

Fig. 5. *Presence of HSPG in amyloid deposits.* Indirect IF with anti-HSPG-core mAb JM-72 (**A** and **B**) or anti-HS mAb JM-403 (**C**) demonstrating the presence of HSPG in AL (**A**) and AA (**B** and **C**) amyloidosis (A × 190; B × 290; C × 230).

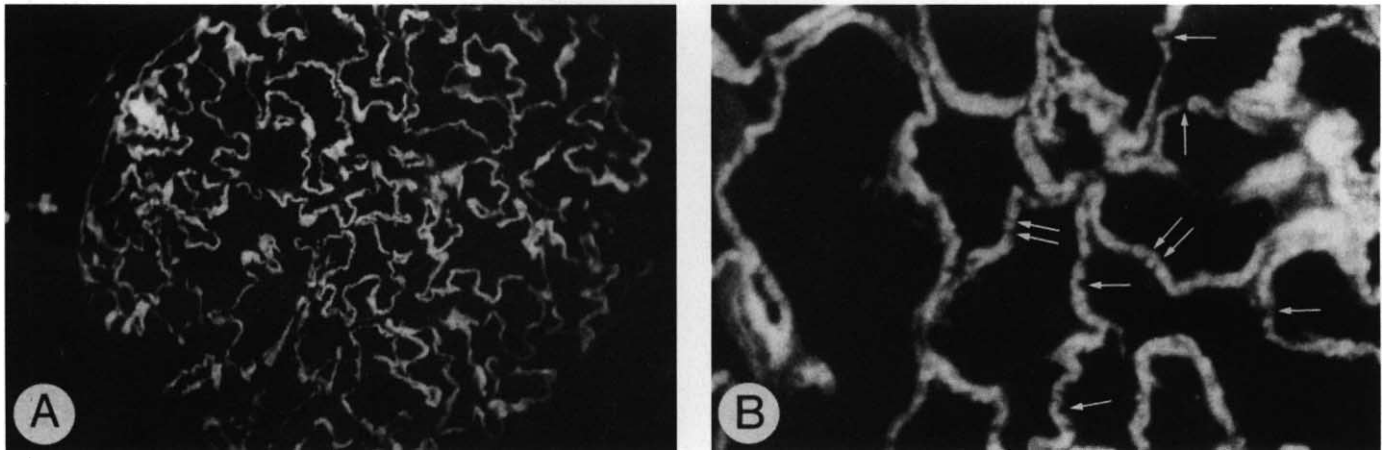

Fig. 6. *Distribution of HSPG-core protein in membranous GN.* Indirect IF with anti-HSPG-core mAb JM-72. Overview of a glomerulus (**A**).. Magnification of a part of a glomerulus (**B**), demonstrating the irregular, moth-eaten GBM staining as indicated by arrows (A × 330; B × 1180).

Table 4. Staining of the amyloid deposits with anti-HSPG-core mAb JM-72 and anti-HS mAb JM-403

Type	Anti-HSPG-core mAb JM-72	Anti-HS mAb JM-403
AL amyloidosis	7/7[a]	1/5
AA amyloidosis	16/16	9/14

[a] Number of positive specimens/specimens studied are given

renal AA amyloidosis by immunohistochemical methods using antibodies specific for the HS-side chain and the core protein of HSPG [77–81]. Our study confirms and extends these data, since we also demonstrated the presence of HSPG in AL amyloidosis. In amyloidosis, the presence of intrinsic basement membrane components is not restricted to HSPG, since several other basement membrane components like collagen type IV, fibronectin and laminin, have been found in amyloid deposits [76].

The irregular, moth-eaten GBM staining that we observed in stage II and III membranous GN with our anti-HSPG-core mAb JM-72 is in agreement with the findings of others, showing the presence of HSPG in the spikes, whereas the immune deposits were negative [46, 50], and suggests that HSPG is a constituent of the newly formed basement membrane material in this disease.

Except for membranous GN, we found in all glomerulopathies an unaltered linear GBM staining with the anti-HSPG-core mAb JM-72. The unaltered HSPG-core expression in the GBM in various glomerulopathies has been found by others [46, 47], although some reports described a reduced HSPG-core staining of the GBM in experimental nephrotic syndrome [45] and in diabetic nephropathy [51]. These differences may be due to differences in HSPG source and specificity of the antibodies.

The unique combination of mAbs against the HSPG-core protein and the HS-side chains can be useful to delineate further the involvement of HSPG and HS in renal pathology, and perhaps also in other basement membrane disorders and cell-matrix interactions *in vitro* and *in vivo*.

Acknowledgments

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