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Immunohistochemical localization of glomerular basement membrane antigens in various renal diseases

Masanori Hara [1], Daisuke Mase [1], Susumu Inaba [1], Akira Higuchi [1], Takakuni Tanizawa [1], Noriaki Yamanaka [2], Yuichi Sugisaki [2], Yoshikazu Sado [3], and Toshio Okada [1]

[1] Department of Pediatrics, School of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan
[2] Department of Pathology, Nippon Medical School, Tokyo 113, Japan
[3] Shigei Medical Research Institute, Division of Immunology, Okayama 701-02, Japan

Summary. The immunofluorescent localization of glomerular basement membrane (GBM) antigens was examined in 52 specimens from normal kidneys and in various renal diseases using antisera to human GBM (HGBM), IV type collagen (IV Col) and P3 antigen, a rat nephritogen. Anti-HGBM serum normally stained the GBM and the mesangium in a restrictive pattern, anti-IV Col serum stained the GBM and the mesangium in a wider pattern and anti-P3 serum stained only the GBM. In mesangial proliferative glomerulonephritis, including IgA nephropathy and Henoch-Schönlein nephritis, the widened mesangial areas were stained with anti-HGBM and anti-IV Col sera. In membranous nephropathy, the punched-out lesions of thickened GBM were demonstrated with the three antisera in moderate cases and a double linear distribution with fine granulation with anti-HGBM and anti-IV Col sera were revealed in one severe case. In membranoproliferative glomerulonephritis, the expanded mesangium and thickened capillary walls were stained with anti-HGBM and anti-IV Col sera, while the outer line of glomerular capillary walls was only positive with anti-P3 serum. In crescentic glomerulonephritis, the collapsed glomerular tufts were stained normally with anti-HGBM and anti-P3 sera and weakly with anti-IV Col serum. In diabetic nephropathy, anti-HGBM serum stained the GBM in a double linear distribution without reacting with the expanded mesangium; anti-IV Col serum stained the mesangium and the GBM in a less clear double linear fashion while anti-P3 serum stained the GBM as single line. Thin membrane disease and Alport's syndrome had normal reactivity with all antisera. However, in one case of Alport's syndrome anti-HGBM and anti-P3 sera stained the GBM in a focal and segmental pattern, while normal staining with anti-IV Col serum was found. In

Offprint requests to: M. Hara at the above address

lesions with adhesions and crescents the staining was positive for HGBM and IV Col and negative for P3; obsolescent glomeruli were stained with anti-HGBM and anti-P3 sera, and had diminished staining with anti-IV Col serum.

The identification of the various structural glomerular antigens is useful in the classification of certain types of glomerular diseases. Further insight into the mechanisms underlying these conditions may be obtained in this way.

Key words: GBM antigens – Renal diseases – Immunohistochemistry

Introduction

The glomerular basement membrane (GBM) is an unique extracellular matrix forming an essential part of the glomeruli and has an important role in glomerular filtration (Kefalides 1978; Martinez-Hernandez and Amenta 1983). Ultrastructural studies of the GBM have identified an electron-dense layer, the lamina densa, as well as two adjacent regions, the lamina rara interna and externa. During the last two decades numerous morphological studies have defined various GBM changes in human renal diseases. Recently, in addition to morphological studies, it has been possible to dissect various antigenic components of the glomerulus in normal and disease states using immunohistochemical methods (Michael et al. 1984; Neale and Wilson 1982). The immunochemical structure of the GBM is not entirely understood, but it is clearly a complex mixture of collagenous components and non-collagenous glycoproteins (Kefalides 1978; Spiro 1967). The former includes type IV and V collagens, and the latter classic GBM antigen, fibronectin, laminin, Goodpasture antigen, entactin and amyloid protein (Michael et al. 1984; Martinez-Hernandez and Amenta 1983). There are several papers concerning the distribution of these antigens in normal kidneys (Courtoy et al. 1982; Fish et al. 1979; Michael et al. 1984; Roll et al. 1980; Scheinman et al. 1978; Scheinman et al. 1974; Scheinman et al. 1980; Scheinman et al. 1980). However, relatively little is known about changes in distribution in various renal diseases (Olson et al. 1980; Scheinman et al. 1978; Scheinman et al. 1974; Schiffer et al. 1981; Striker et al. 1984). Therefore, the aim of this study is to examine immunochemical alterations of the GBM in various renal diseases, using antisera to GBM antigens that are available in our laboratory.

Materials and methods

1. Patients and tissue specimens

The materials studied included 52 renal specimens, obtained either by needle or open biopsy. The histological diagnoses of these cases defined by conventional light, immunofluorescent and electron microscopic examinations were as follows: IgA nephropathy (15 cases), nephritis of Henoch-Schönlein purpura (HSP nephritis, 7 cases), normal kidneys and minor glomerular abnormalities (5 cases), mesangial proliferative glomerulonephritis (4 cases), membranous

nephropathy (4 cases), membranoproliferative glomerulonephritis type I (4 cases), thin basement membrane disease (4 cases), crescentic glomerulonephritis (2 cases), Lupus nephritis (2 cases), focal segmental glomerulosclerosis (2 cases), Alport's syndrome (2 cases), and diabetic nephropathy (1 case).

2. Antisera

Anti-HGBM serum. The GBM was isolated from human cadaveric kidneys by mechanical sieving according to the methods of Krakower and Greenspon (1951) and successively ultrasonicated. Outbred rabbits were immunized intradermally four times, each time with 10 mg of the lyophylized GBM sonicates at an interval of 1 week. The animals were bled 10 days after the last immunization. In order to remove antibodies to plasma proteins which normally contaminate in the GBM preparations, the sera were absorbed with insolubilized human pooled plasma (Avrameas and Ternynck 1969).

Anti-IV Col serum. Type IV collagen was prepared from rabbit lens capsules by limited pepsin digestion, then purified by differential salt precipitation and further by DEAE-cellulose chromatography to remove contaminating non-collagenous proteins (Kresina and Miller 1979; Timple et al. 1979). Antisera to type IV collagen were raised in sheep and characterized as described previously (Sano et al. 1981; Sugisaki and Yamanaka 1984). The sera were also absorbed with insolubilized human pooled plasma.

Anti-P3 serum. We previously established a method for isolation of a potent nephritogenic antigen from bovine GBM, which was found to be highly nephritogenic in rats (Steblay type nephritis) following a single injection. Anti-P3 serum is rabbit antiserum against this nephritogenic antigen. Its characterization was previously reported (Sado et al. 1984).

3. Immunohistochemistry

Frozen sections. A fragment of the biopsy specimen was immediately frozen in liquid n-hexane and maintained at −70° C until sectioned in a cryostat. The air-dried sections were stained with antisera against various GBM antigens and fluorescein isothiocyanate (FITC) labeled antibodies.

Fixed embedded tissue. The renal biopsy samples were fixed in Dubosq-Brazil Alcoholic Bouin's fixative for 4 to 6 h and embedded in paraffin. Paraffin-embedded tissues remaining from the usual light microscopic samples were used for study. Deparaffinization was completed using graded xylene and alcohol solutions. The tissues then were rinsed in 0.01 M phosphate-buffered saline (PBS), pH 7.2. The sections were then placed in 0.1% trypsin (Sigma, type III, USA) solution, pH 7.8, containing 0.1% calcium chloride, at 37° C for 60 min. The slide were washed thoroughly in PBS and covered with antisera to HGBM (1/20 dilution in PBS), IV Col (1/20 dilution in PBS) and P3 (1/20 dilution in PBS) for 30 minutes in a moist chamber at 37° C. They were then washed in PBS and FITC labeled goat anti-rabbit IgG (1/10 dilution, Cappel Laboratories, USA) and FITC labeled rabbit anti-sheep IgG (1/10 dilution, Cappel Laboratories, USA) were applied for 30 min. After washing with PBS the slides were mounted in glycerol (pH 9.6). Controls included first antisera without second antibody, second antibody only and no antibody.

Results

1. Comparison between frozen and fixed tissues

No difference was found in the localization or relative intensity of antigens. The results and illustrations refer to fixed tissues, since consistently thin sections were obtained as compared with frozen sections and more precise examination was possible.

Table 1. Distribution of GBM antigens in normal kidney

Localization in normal kidney	Antisera		
	anti-HGBM	anti-IV Col	anti-P3
Glomerular basement membrane	+++[a]	+−++	++
Mesangium	+++ (restrictive)	+++ (extensive)	−
Bowman's basement membrane	+++	+++	Tr−+
Tubular basement membrane	+++	+++	Tr−+
Capillary basement membrane	++	++	−
Basement membrane of artery walls	++	++	−
Interstitium	−	−	−

[a] Intensity of staining ranges from trace (Tr) to +++

2. Findings in various renal diseases (Table 2A)

Normal kidney and minimal change glomerular abnormality. Normal immunochemical reactions of the three antisera on normal kidneys and specimens from minimal change glomerular alterations are shown in Table 1. Within the glomeruli, anti-HGBM serum stained the GBM and the mesangium equally intensely (Fig. 1A); anti-IV Col serum stained the mesangium in a wider pattern and the GBM with less intensity (Fig. 1B) and anti-P3 serum stained the GBM without reacting with the mesangium (Fig. 1C).

Mesangial proliferative glomerulonephritis, IgA nephropathy and HSP nephritis. In these diseases, studies with both anti-HGBM and anti-IV Col sera revealed a widened mesangium, the latter antiserum in a more extensive pattern. The GBM was normal with both antisera (Fig. 2A and B). Anti-P3 serum stained only the normal GBM.

Membranous nephropathy. Staining with the anti-HGBM serum demonstrated a thickened GBM with multiple punched-out projections. In more severe lesions an apparent splitting of the GBM was observed (Fig. 3A). The outer (epithelial) aspect of the GBM was outlined as a fine granular component which extended across the outer epithelial aspect of the mesangial waist. The staining of the inner layer was also a fine granular pattern and appeared to blend with the aggregates of the mesangial matrix. Results with anti-IV Col serum were similar, although splitting of the GBM was not so apparent (Fig. 3B). The staining of the GBM with anti-P3 serum demonstrated a multiple punched-out and spike-like appearance (Fig. 3C). Splitting of the GBM was not found.

Membranoproliferative glomerulonephritis. In MPGN, various patterns of reactivity were observed with staining with anti-HGBM serum. Thickened GBM and greatly expanded mesangial areas were seen in three cases. However, in one case staining of the GBM and the mesangium was diminished and occasionally only faintly positive staining of capillary loops was detect-

Table 2. Distribution of GBM antigens in various renal diseases

	Antisera					
	Anti-HGBM		Anti-IV Col		Anti-P3	
	GBM	Mesangium	GBM	Mesangium	GBM	Mesangium
A. Diseases (52 cases)						
1. Normal, Minimal Change Glomerular Abnormalities ($N=5$)	N	N	N	N	N	(−)
2. Mesangial Proliferative GN, IgA Nephropathy, SHP-Nephritis ($N=26$)	N	N (widened)	N	N (widened)	N	(−)
3. Membranous Nephropathy ($N=4$)	N (thickened, punched-out, bilaminar)	N	N (thickened punched-out)	N	N (thickened, punched-out)	(−)
4. Membrano-proliferative GN ($N=4$)	N-D (thickened)	N-D (expanded)	N (thickened)	N (expanded)	D-T (in only rim)	(−)
5. Crescentic GN ($N=2$)	N (collapsed)	N	D (collapsed)	D	N	(−)
6. Alport's Syndrome, TBMD ($N=6$)	N (1 case: FS)	N	N	N	N (1 case: FS)	(−)
7. Diabetic Nephropathy ($N=1$)	N (thickened, bilaminar)	T-(−)	D (thickened)	N	N	(−)
8. Others ($N=4$)	N	N	N	N	N	(−)
B. Specific Glomerular Changes						
1. Adhesion	Positive		Positive		Negative	
2. Crescent	Positive		Positive		Negative	
3. Obsolescence	Positive		Diminished		Positive	

Abbreviations. GN = Glomerulonephritis, TBMD = Thin Basement Membrane Disease, N = Normal in immunofluorescent intensity, (−) = Negative, T = Trace, D = Decreased, FS = Focal and segmental

able (Fig. 4A). Anti-IV Col serum also showed a thickened GBM and a widened mesangium (Fig. 4B). Its intensity was not changed in the case described above. The staining with anti-P3 serum was restricted in the GBM to a fine rim around the capillary loops (Fig. 4C). Occasionally there were areas not reacting with antigen. The expanded mesangial areas did not react with this antigen.

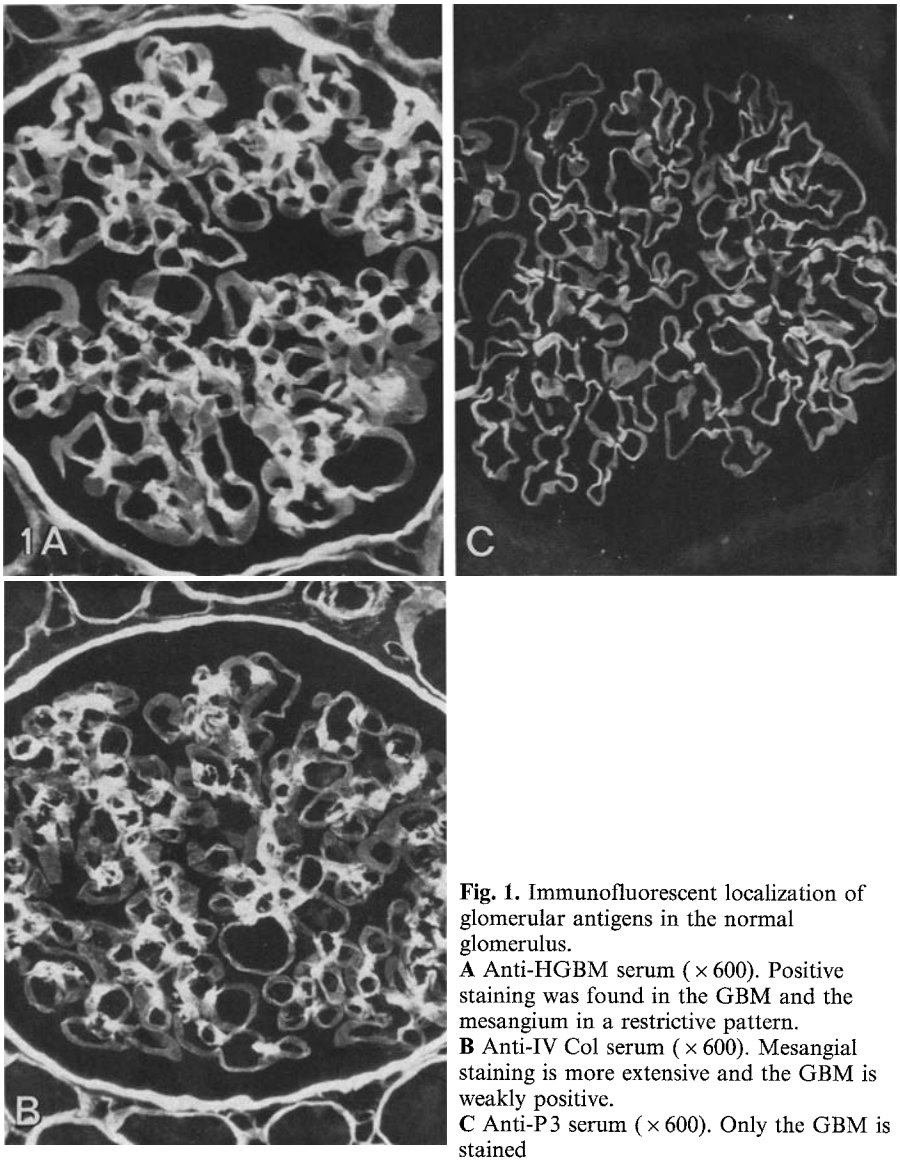

Fig. 1. Immunofluorescent localization of glomerular antigens in the normal glomerulus.
A Anti-HGBM serum (×600). Positive staining was found in the GBM and the mesangium in a restrictive pattern.
B Anti-IV Col serum (×600). Mesangial staining is more extensive and the GBM is weakly positive.
C Anti-P3 serum (×600). Only the GBM is stained

Crescentic glomerulonephritis. In crescentic glomerulonephritis, studies with anti-HGBM serum showed positive staining of the GBM, the mesangium and also of the crescent (Fig. 5A). Anti-IV Col serum stained the GBM and the mesangium with diminished intensity, although mesangial areas and capillary loops were difficult to identify in the collapsed glomeruli (Fig. 5B). Extracellular matrix in the crescent was also stained. Anti-P3 serum revealed only the GBM and no staining was observed in the crescentic lesions.

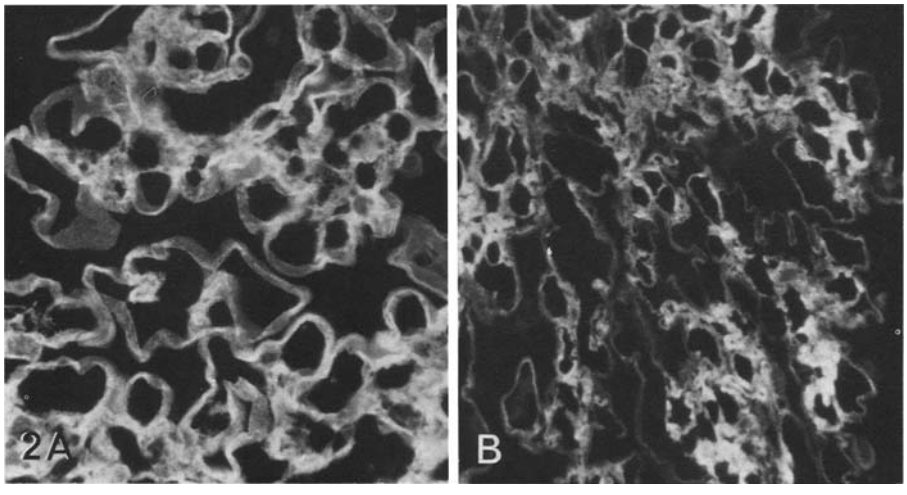

Fig. 2A, B. IgA nephropathy. The widened mesangial areas are stained. **A** Anti-HGBM serum (×1,000). **B** Anti-IV Col serum (×1,000)

Alport's syndrome and thin basement membrane disease. In these diseases the staining with all three antisera was normal. However, in one case with Alport's syndrome the staining with anti-P3 revealed a linear pattern with focal and segmental distribution (Fig. 6C). With anti-HGBM serum linear pattern with focal and segmental accentuation was found (Fig. 6A). The staining with anti-IV serum was normal in this case (Fig. 6B).

Diabetic nephropathy. The staining with anti-HGBM serum demonstrated thickened GBM in a double linear distribution. The staining intensity of the expanded mesangial areas were greatly diminished or negative (Fig. 7A and B). Anti-IV serum stained the mesangium predominantly while reactivity with the GBM was markedly diminished (Fig. 7C). A double linear distribution was occasionally detectable. Reactivity with the anti-P3 serum was restricted to the thickened GBM and a double linear distribution was not found (Fig. 7D).

Other diseases. In nephropathies other than the diseases described above, there were no significant alterations in the GBM and the mesangium detectable with the three antisera.

3. Findings in specific glomerular changes (Table 2B)

Adhesion. The portions with this change were stainable with anti-HGBM and anti-IV Col sera (Fig. 8A and B). There was no reaction with anti-P3 serum (Fig. 8C).

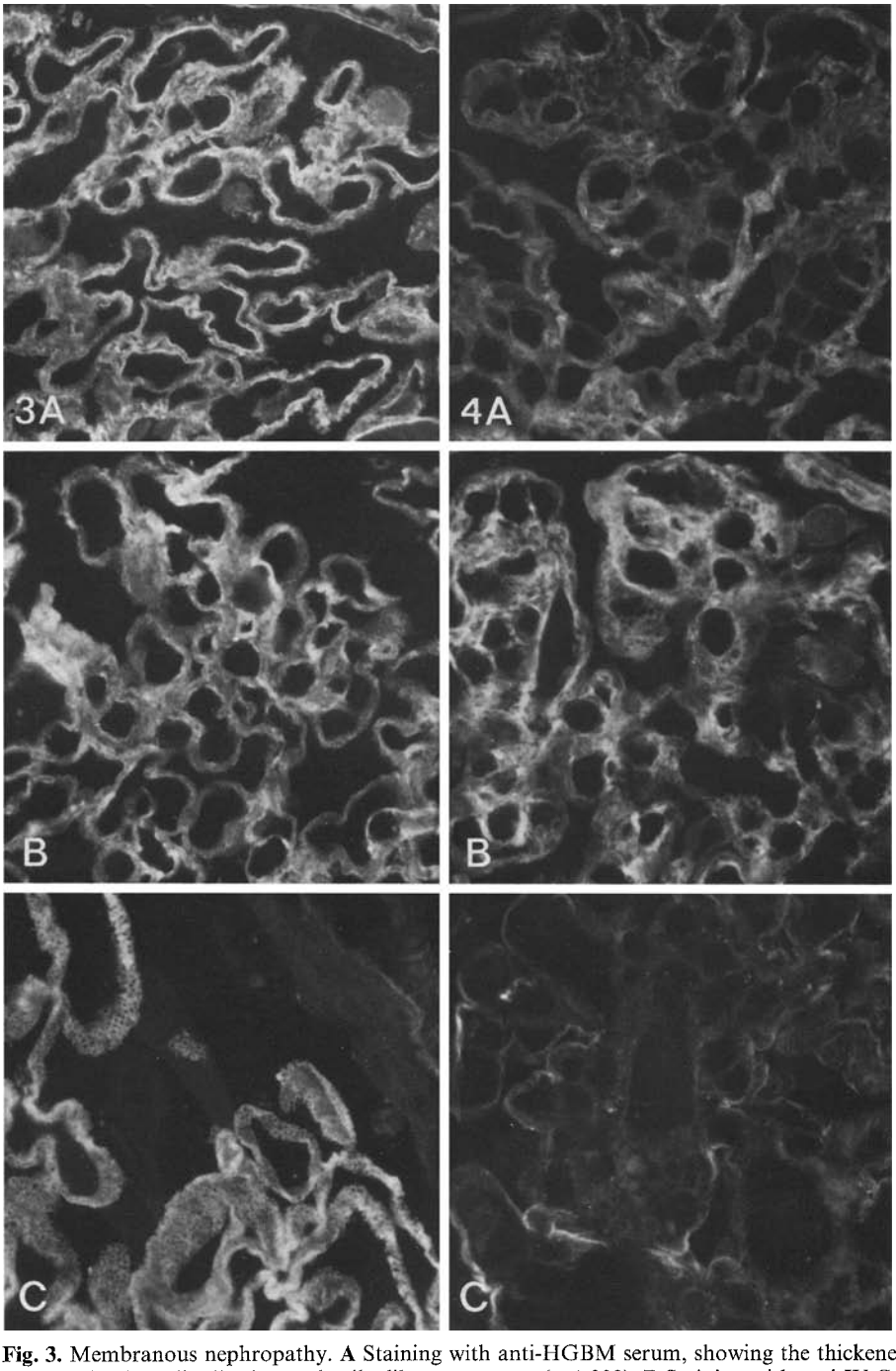

Fig. 3. Membranous nephropathy. **A** Staining with anti-HGBM serum, showing the thickened GBM, bilaminar distribution and spike like appearance (× 1,000). **B** Staining with anti-IV Col serum, showing the thickened GBM with irregular beading (× 1,000). **C** Staining with anti-P3 serum, showing multiple punched-out projections (× 1,500)

Fig. 4. Membranoproliferative glomerulonephritis. **A** Anti-HGBM serum. The staining of the thickened capillary walls and the expanded mesangium is diminished (× 1,000). **B** Anti-IV Col serum. The capillary loops and the mesangial areas are stained (× 1,000). **C** Anti-P3 serum. Only the rim of the capillary walls is stained (× 1,000)

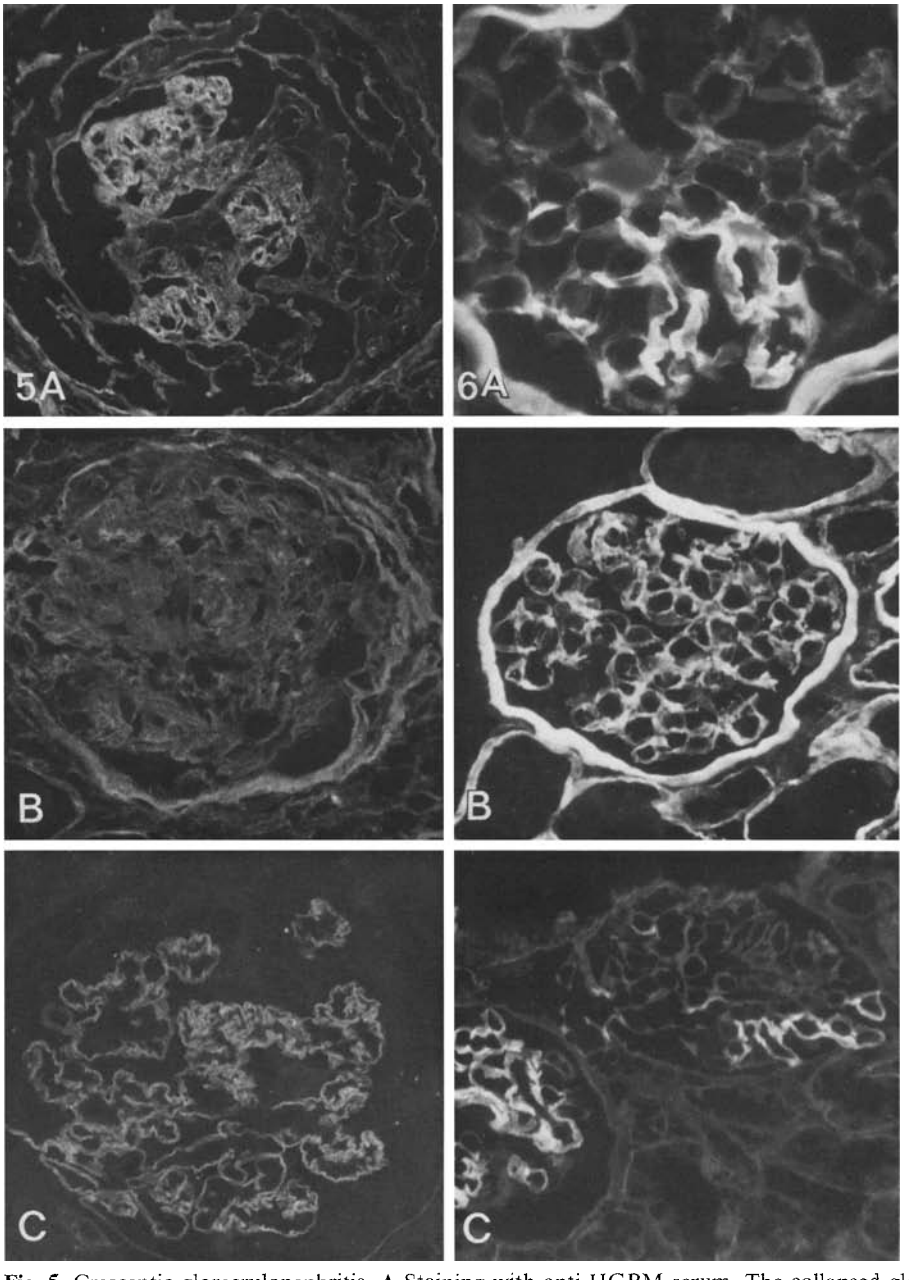

Fig. 5. Crescentic glomerulonephritis. **A** Staining with anti-HGBM serum. The collapsed glomerular tufts and crescent are positive (× 500). **B** Staining with anti-IV Col serum. Diminished reactivity with the glomerular tufts is observed (× 500). **C** Staining with anti-P3 serum. The GBM is stained but there is absence of reactivity with the crescent (× 500)

Fig. 6. Alport's syndrome. **A** Staining with anti-HGBM serum, demonstrating focal and segmental accentuation of the GBM (× 1,000). **B** Staining with anti-IV Col serum, showing normal distribution (× 300). **C** Staining with anti-P3 serum. The reactivity is observed focally and segmentally (× 300)

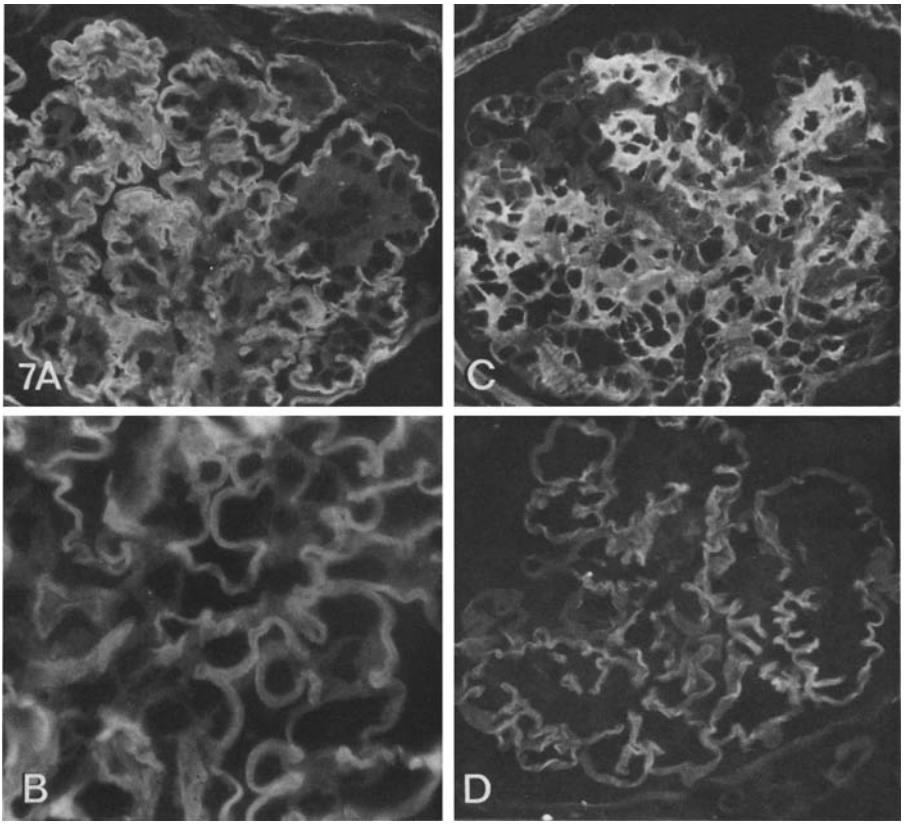

Fig. 7. Diabetic nephropathy. **A** Staining with anti-HGBM, demonstrating the GBM reactivity but not of the expanded mesangium (×500). **B** Double linear distribution of the GBM. Anti-HGBM serum (×1,000). **C** Staining with anti-IV Col serum, showing marked reactivity with the mesangial sclerotic areas (×500). **D** Thickened GBM without bilaminar fashion stained with anti-P3 serum. (×500)

Crescent. In the fibrous and fibro-cellular crescents in the cases with crescentic glomerulonephritis, IgA nephropathy and HSP nephritis, the extracellular matrix was stained with anti-HGBM and anti-IV Col sera, but not with anti-P3 serum.

Obsolescence. The hyalinized glomeruli showed positive staining with anti-HGBM and anti-P3 sera (Fig. 9A and C). Diminished reactivity with or without spotty positive areas was found with anti-IV Col serum (Fig. 9B).

Discussion

We have investigated the distribution of GBM antigens in various renal diseases using an immunohistochemical method with antisera to three kinds of GBM antigens. In this study we used formalin-fixed, paraffin-embedded

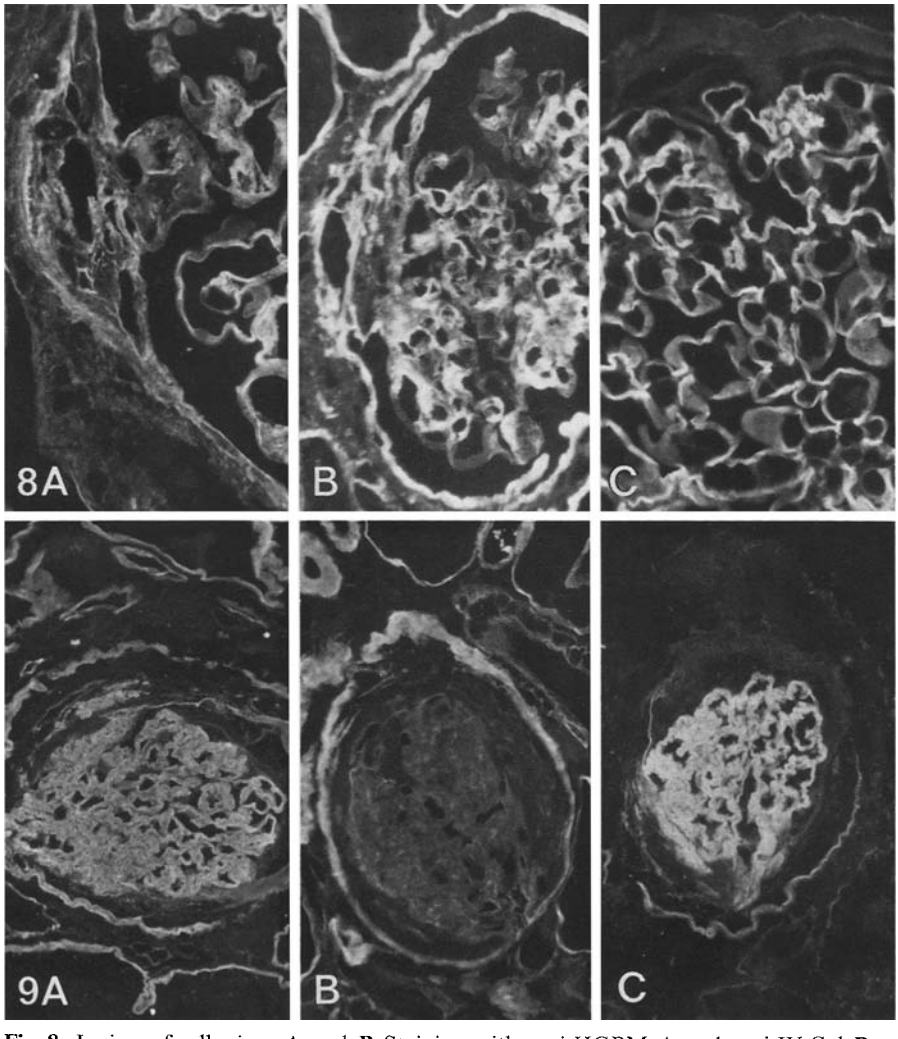

Fig. 8. Lesion of adhesion. **A** and **B** Staining with anti-HGBM **A** and anti-IV Col **B** sera. The positive staining is observed in this lesion (**A**: × 1,000, **B**: × 500). **C** No reactivity in the lesion of adhesion with anti-P3 serum (× 1,000)

Fig. 9. Obsolescent glomerulus. **A** and **C** Staining with anti-HGBM **A** and P3 **C** sera, showing preserved antigenicity (**A**: × 300, **C**: × 300). **B** Staining with anti-IV Col serum, showing diminished reactivity (× 300)

tissue sections pretreated with trypsin. The immunohistochemical demonstration of immunoproteins in formalin-fixed renal biopsy specimens is usually as reliable as in frozen specimens (Enerstom et al. 1980; MacIver et al. 1979; Qualman and Keren 1979). Various GBM antigens are also demonstrable using paraffin-embedded enzyme-treated sections (Striker et al. 1984; Suzuki et al. 1984). No discrepancies between the localization and intensity

of the antigens in this study were observed in the two specimens. Better morphology was obtained in paraffin-embedded enzyme-treated sections, enabling satisfactory investigation of the distribution of GBM antigens.

Anti-HGBM serum stained the GBM, the mesangium, the Bowman's basement membrane, the tubular basement membrane, the capillary basement membrane and the basement membrane of artery walls. There is some evidence that this antiserum reacts with non-collagen glycoprotein antigens, although the determinants have not been characterized (Marquardt et al. 1973). Within the glomeruli the staining pattern with anti-HGBM serum revealed some differences compared with that of anti-IV Col serum. The mesangial staining with anti-IV Col serum was more extensive, although both of the antibodies react with the mesangial matrix and the GBM of the mesangial area (Fish et al. 1979; Courtoy et al. 1982; Scheinman et al. 1982). This difference might be due to the heterogeneity of the mesangial matrix components. In the mesangial region there are three distinctive domains: 1) the endothelial-mesangial matrix, 2) intermesangial matrix, and 3) the portion of the GBM that covers the mesangial regions. Recent studies from the laboratory of Michael have revealed that the rabbit anti-human GBM antibody reacts with antigens along the inner and the outer aspects of the glomerular capillary wall, forming a double linear pattern along the GBM by immunofluorescence (Fish et al. 1979). The outer aspect takes a peripheral course and is continuous between glomerular capillaries, while inner staining is contiguous with antigenic components of the glomerular mesangium. We did not see this distribution in this study. This might be due to the poor resolution of the immunofluorescent microscope in our laboratory or to different properties of the antisera. However, the double linear pattern of the GBM was found in the diseases with thickened GBM (membranous nephropathy and diabetic nephropathy). Anti-HGBM serum is considered to react with some non-collagenous glycoproteins of the GBM, probably including laminin, a defined non-collagen basement membrane glycoprotein. This antigen is distributed in a double linear fashion (Scheinman et al. 1980), and is in accordance with our results. Altered distributions of HGBM antigens were found in some diseases with well-defined morphological alterations of the GBM (Schiffer et al. 1981). In membranous nephropathy, multiple punched-out projections of the GBM and bilaminar antigen distribution were detectable in our study with fine beading in severe lesions. These findings are similar to those of Schiffer et al. (1981). In MPGN, the expanded mesangium and thickened capillary walls had positive staining, with markedly diminished in both GBM and mesangium, in one case, a phenomenon noted by Schiffer et al. in some cases using anti-HGBM serum. We also observed a double linear distribution in diabetic nephropathy, which resembles their findings. As they discussed, the striking, well preserved separation of the antigenic and non-antigenic portions of the GBM support the concept that the antigens are partitioned in the inner and outer aspects of the glomerular capillary walls. In one case of Alport's syndrome anti-HGBM serum stained the GBM with a focal and segmental accentuation. Anti-HGBM serum reacts with several antigenic components of the GBM

and one or some of them are presumed to be distributed in this fashion. The responsible antigenic component(s) are now under analysis in our laboratory.

Anti-IV Col serum stained the GBM, the mesangium, the Bowman's basement membrane, the tubular basement membrane, the capillary basement membrane and the basement membrane of artery walls. The localization of IV Col is similar to the findings of several investigators (Courtoy et al. 1982; Roll et al. 1980; Scheinman et al. 1980; Striker et al. 1980; Sugisaki and Yamanaka 1984). In this study antiserum against rabbit IV Col was used. The distribution of this antigen in human kidney was similar (Sugisaki and Yamanaka 1984), so we consider that the use of this antiserum does not present a critical problem. In mesangial proliferative glomerulonephritis including IgA nephropathy and HSP nephritis with various degree of mesangial widening, associated increase of IV Col was found in the mesangium. IV Col is produced by mesangial cells as well as by glomerular epithelial cells. So increased IV Col in the mesangium is presumed to be due to the proliferation of mesangial cells. The glomerular tufts in crescentic glomerulonephritis showed diminished reactivity with anti-IV Col serum. Striker et al. (1984) reported similar results in anti-GBM type nephritis, although normal staining was observed in the crescentic nephritis associated with vasculitis. Thus, heterogeneity of the collapsed glomeruli stained for IV Col was demonstrated.

In diabetic nephropathy the double linear distribution of the capillary loops was detected with anti-IV Col serum, although it was not so evident. IV Col is found predominantly in the lamina densa (Courtoy et al. 1982) and moreover, bilaminar morphology was not seen in electron microscopic examination. This bilaminar distribution is thus very interesting but an explanation for this finding has to await further studies. Bright staining with anti-IV Col serum in the expanded mesangium was found in diabetic nephropathy. The most recent hypothesis proposes that basement membrane heparan sulfate proteoglycan synthesis is decreased in the diabetic state and there is compensatory hypersecretion of IV Col and laminin (Rohrbach et al. 1982). This may well explain the findings in diabetic nephropathy. Normal distribution of IV Col was observed in Alport's syndrome. These findings are compatible with the reports of Habib et al. (1982), in which Goodpasture antibody failed to react with the GBM and anti-IV Col serum showed normal reactivity.

Anti-P3 is rabbit antiserum against the nephritogenic antigen which was prepared from bovine GBM and induces of Steblay type nephritis in the rat when injected together with complete Freund's adjuvant (Sado et al. 1984). Amino acids and carbohydrate analyses revealed that this antigen is a glycoprotein which contains amino acids and sugars characteristic of collagen, namely hydroxyproline, glycine, glucose and galactose, although the relative amounts of these amino acids and sugars are less than those found in IV Col of the GBM. So we think that the P3 antigen is a complex of collagenous and non-collagenous components, or a part of IV Co. Its similarity to Goodpasture's antigen is supported by the following findings:

1) Immunoblotting analysis of the collagenase solubilized HGBM using anti-P3 serum showed 4 bands ranging from 20,000 to 50,000 of molecular weights (unpublished data). This finding is similar to the results of Fish et al. (1984) in which they used Goodpasture antibody. 2) Both anti-P3 serum and Goodpasture antibody (Schiffer et al. 1981) stained only the GBM (probably lamina densa) in a single linear distribution. 3) P3 is nephritogenic in rats and recent report showed the similarity of Goodpasture antibody with autoantibody to the GBM in Steblay type nephritis (Jeraj et al. 1982).

Recent studies have revealed that Goodpasture antibody fails to react with the GBM in Alport's syndrome, indicating an absence of nephritogenic Goodpasture antigen in the GBM (Jenis et al. 1981; Jeraj et al. 1983; McCoy et al. 1982; Olson et al. 1980). However, there are no reports about the antigen distribution in Alport's syndrome. Our patient did not suffer from bilateral hearing loss and not have a family history of renal disease and deafness. However, the GBM alterations on electron microscopic examination are quite compatible with Alport's syndrome (Zollinger and Mihatsch 1978) and failure of Goodpasture antibody to react with the GBM was confirmed, which are the grounds for its diagnosis in this case. This case may represent a new type of Alport's syndrome, possibly a new mutation. The focal and segmental distribution of the P3 antigen supports the possibility of mosaic localization of the cells that excrete this antigen. But further studies are necessary before a conclusion can be reached.

In glomerular lesions with adhesion and crescents, positive staining with anti-IV Col serum was obtained. It is assumed that laminin is bound to IV Col (Martinez-Hernandez and Amenta 1983), although the exact interaction remains to be defined. Laminin has been shown to play a role in cell adhesion and attachment. Thus, it is likely that both IV Col and laminin are present in these lesions. On the other hand, lack of staining with anti-P3 serum seems to indicate that this antigen plays either a minor or no role in these lesions. In obsolescent glomeruli, the staining was well preserved using anti-HGBM and anti-P3 sera and diminished with anti-IV Col serum. Scheinman et al. (1974) reported that the obsolescent glomeruli had a loss of antigens reactive with anti-HGBM serum. This discrepancy is probably due to the difference in the character of antibody. They speculated that the anti-HGBM serum in their study might react with the collagen related protein. However, IV Col was diminished in the obsolescent glomeruli. One explanation for this finding is a progressive replacement by other components, e.g. type III collagen (Striker et al. 1984).

Different GBM antigen distributions were observed in the following renal diseases, especially when serious morphological alterations were present, membranous nephropathy, membranoproliferative glomerulonephritis, crescentic glomerulonephritis, diabetic nephropathy, and Alport's syndrome. We think that these changes have significant meaning as follows:

1) A double linear pattern of the GBM with anti-HGBM and anti-IV Col sera was observed in the diseases with thickened GBM (membranous nephropathy and diabetic nephropathy). This supports the concept that the anti-

gens are partitioned to the inner and outer aspects of the glomerular capillary walls and furthermore might indicate the impairment of the partitioning process in such diseases.

2) In one case of membranoproliferative glomerulonephritis and diabetic nephropathy, anti-HGBM showed diminished reactivity in the mesangium indicating altered antigenicity of non-collagen glycoprotein antigens, while anti-IV Col showed normal reactivity at this site. These findings might mean that altered metabolism of the glycoprotein components is involved in the process of mesangial sclerosis.

3) Normal staining with anti-IV Col was observed in congenital renal basement membrane diseases, such as thin basement membrane disease and Alport's syndrome, although ultrastructural changes in the GBM were prominent. This suggests an important role for collagen IV as a basic structural protein.

4) Interesting findings using anti-HGBM and anti-P3 sera were obtained in 1 case of Alport's syndrome. Considering the complexity of the definition of Alport's syndrome and the related clinical entities, immunochemical approachs to the GBM may help in the definition and classification of these diseases.

5) In glomerular lesions with adhesions and crescents anti-P3 serum showed negative reactivity, while positive staining with anti-HGBM and anti-IV Col sera was observed. Extracellular matrices in these lesions presumably differ in some components when compared with the GBM of glomerular tufts.

6) Collagen IV was diminished in the obsolescent glomeruli, although well preserved antigenicity was found using anti-HGBM and anti-P3 sera. These findings may reveal a switch of IV collagen to type III collagen.

Identification of the various structural glomerular antigens is useful in the classification of certain types of glomerular diseases. Further insight into the mechanisms underlying these conditions may be obtained using this method.

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