ANTIBODIES TO BASEMENT MEMBRANE COLLAGEN AND TO LAMININ ARE PRESENT IN SERA FROM PATIENTS WITH POSTSTREPTOCOCCAL GLOMERULONEPHRITIS

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The presence of antibodies against basement membrane antigens of the glomerulus (GBM)[1] (1) and anterior lens capsule (ALC) (2) in the sera of patients with Goodpasture syndrome, and of the GBM in the sera of those with poststreptococcal glomerulonephritis (PSGN) (3) was recently reported. The first two studies show that antibodies in the patients' sera react with collagenase-resistant sequences of basement membrane (BM) collagen (type IV) corresponding to the globular NC-1 domain; the third study shows that the sera from patients with PSGN react with heparan sulfate proteoglycan (PG) and a galactosamine-containing PG. Anti-BM antibodies can produce a spectrum of diseases ranging from mild to severe and rapidly progressive glomerulonephritis, with or without pulmonary hemorrhage, a condition seen in Goodpasture syndrome (4). Unlike the situation with anti-BM–induced disease, where the antibody is deposited in a linear fashion on the GBM, in PSGN the immunoglobulin is found in granular deposits along the GBM (5). Ultrastructurally, the glomerular lesions in PSGN are characterized by large subepithelial deposits that contain antibody, complement, and possibly streptococcal antigens (6). BM collagen (type IV) has been isolated from several tissues, including the glomerulus (7, 8), the ALC (9–11), the placenta (12), and the mouse EHS tumor (13). When obtained by nondegradative methods, it consists of two identical polypeptide chains, $[\alpha 1(IV)]_2$, and a third chain, $\alpha 2(IV)$, having M_r of 185,000 and 170,000 respectively (13, 14). These chains form a triple helical molecule. Chains having lower M_r (125,000 and 115,000 as well as 95,000 and 75,000) have been obtained after pepsin digestion of basement membranes (12, 15). Rotary shadowing electron microscopy has revealed molecules having a filamentous, helical, collagenous domain terminating in a noncollagenous, globular domain at the carboxyl end (NC-1 domain); at the amino end, the molecule terminates in a highly crosslinked

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[1] *Abbreviations used in this paper:* ALC, anterior lens capsule; APSGN, acute PSGN; BM, basement membrane; GBM, glomerular BM; PBS-T, PBS-Tween buffer; PG, proteoglycan; PSGN, poststreptococcal glomerulonephritis.

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collagenous region termed 7-S domain (14, 16). Upon collagenase digestion at 37°C these two domains survive, one by virtue of its composition (NC-1), and the other by virtue of its high disulfide and lysyl-derived crosslinking (17).

Laminin is a large (M_r 900,000) glycoprotein component of BMs composed of at least three highly crosslinked subunits (18). The isolation of a large glycoprotein from GBM was first reported in 1966 (7). Immunochemical studies on a similar glycoprotein, isolated after collagenase digestion of GBM, were reported in 1972 (19). It is very likely that the latter studies were dealing with the glycoprotein we now refer to as laminin. The proportion of laminin with respect to type IV collagen varies among BM but no mature BM is totally devoid of it (20).

A number of studies (1–3, 21) suggest that the antibodies in the sera of patients with glomerulonephritis may be directed against a variety of glomerular and nonglomerular antigens. The demonstration by Fillit et al. (3) that sera from patients with acute PSGN (APSGN) react with heparan sulfate PG suggested that such patients may have antibodies against other BM components. We therefore decided to investigate this possibility. Since there is a high degree of immunologic crossreactivity among heterologous basement membranes (3, 19), and since sera from patients with Goodpasture syndrome react with bovine ALC collagen (2), we decided to test the reactivity of the latter with sera from patients with APSGN. Our data show that sera from patients with APSGN have antibodies that react not only with heparan sulfate PG but also with type IV collagen and laminin. No reaction was noted with fibronectin. We also present evidence that the collagenase-resistant fragment of type IV collagen corresponding to the 7-S domain contains the epitope or epitopes against which the anti–type IV collagen antibodies react. This is a significant difference in the reactivity of sera from patients with APSGN compared to those from patients with Goodpasture syndrome, and may explain the reported difference in the distribution of antibody on the GBM between the two diseases.

Materials and Methods

Reagents

Human sera from patients with APSGN and normal controls were obtained from Trinidad. Rabbit anti–mouse laminin (EHS tumor) was obtained from Bethesda Research Laboratories, Gaithersburg, MD (6265 SA). Normal rabbit serum was obtained from Cappel Laboratories, Malvern, PA (5012-1380), and rabbit anti–human fibronectin was purchased from Calbiochem-Behring Corp., San Diego, CA (341643).

Rabbit Antisera Against BM Collagen (type IV)

These sera are available at the laboratory of N. Kefalides, and were prepared against bovine ALC type IV collagen as described by Brinker et al. (14).

Preparation of Antigens

BM collagen (type IV). Native, undegraded type IV collagen was extracted from bovine ALC with 0.5 M acetic acid (22), and purified on a DEAE-cellulose column as described by Brinker et al. (14). Rotary shadowing electron microscopy revealed procollagen-like molecules having a helical domain (364 nm), a carboxyl terminal, a globular domain (11 nm), and tetramers of such molecules overlapping at the amino terminus to form the 7-S domain (35 nm) (14).

Bacterial collagenase-resistant fractions of BM collagen. The insoluble ALC that remained after extraction with 0.5 M acetic acid were subjected to bacterial collagenase digestion (19). ~500 mg of lyophilized ALC was suspended in 18 ml of 0.1 M Tris–0.4 M NaCl, pH 7.4 buffer containing protease inhibitors (*N*-ethylmaleimide, 3 mM; PMSF, 1 mM) and $CaCl_2$, 6 mM. Bacterial collagenase (CLSPA-Worthington Biochemicals, Freehold, NJ) was added at an enzyme/protein ratio of 1:100, and the digestion was allowed to proceed at 37°C for 20 h. At the end of this period, 1 ml of 100 mM EDTA was added, and the solubilized protein was separated by centrifugation at 30,000 *g* for 1 h at 4°C. The supernatant was dialyzed exhaustively against distilled water at 4°C and lyophilized. For gel filtration, 40 mg of the lyophilized digest was dissolved overnight at 4°C in a solution of 1 M $CaCl_2$ containing 50 mM Tris, pH 8.5, and 20 μg pepstatin (P-4265; Sigma Chemical Co., St. Louis, MO). The solution was warmed for 30 min at 45°C, and then centrifuged at 20,000 *g* for 30 min at 22°C. The pellet was discarded and the supernatant was placed on a 6% agarose A-5m column (1.6 cm × 145 cm). Proteins were eluted with a buffer containing 1 M $CaCl_2$, 50 mM Tris-HCl, pH 7.5, at room temperature at a flow rate of 14.8 ml/h (Fig. 1) (15). The fractions were dialyzed at 4°C against water and lyophilized.

Human fibronectin. Human fibronectin was isolated from human plasma on a gelatin-Sepharose column as described previously (23).

ELISA

Antibodies against type IV procollagen and its fractions, and against laminin in patients with APSGN were detected by using the ELISA. The assay was performed essentially according to previously described methods (24). The wells of Immunolon I microtiter plates (Dynatech, Inc., Alexandria, VA) were coated with the appropriate antigen. Native type IV procollagen was coated in Voller's buffer (0.15 M Na_2CO_3, 0.35 M $NaHCO_3$, 0.2% NaN_3) at a concentration of 600 ng/well. The noncollagenous NC-1 domain of type IV collagen (Fig. 1, peak V) as well as aggregates of NC-1, i.e., Peaks I and II of Fig. 1 were coated in Voller's buffer, 0.5 M acetic acid, or 6 M guanidine-HCl. ELISA was performed for human fibronectin and the 7-S fraction (Fig. 1, peak III) of type IV collagen, as described for the native type IV collagen molecule. Mouse laminin (6260LA; Bethesda Research Laboratories, Gaithersburg, MD) was coated in 0.5 M acetic acid, also at 600 ng/well. The plates were then incubated overnight at 4°C. The wells were washed three times over 30 min with 0.05% Tween 20 in PBS (PBS-T), followed by a 90-min incubation at room temperature with goat anti–human polyvalent Ig–alkaline phosphatase conjugate (A-5034; Sigma Chemical Co.) diluted 1:300 in PBS-T. The wells were washed again as above, the phosphatase substrate (*p*-nitrophenyl phosphate disodium, 104-0; Sigma Chemical Co.) was allowed to incubate for 30 min, the reaction was stopped by the addition of 2 M NaOH (50 μl/well), and the reaction product was quantitated by measuring absorbance at 405 nm with a Dynatech microELISA minireader.

Competitive ELISA

Inhibition experiments with type IV procollagen and laminin revealed inhibition of APSGN serum reactivity to both type IV procollagen and laminin. In this case, a competitive ELISA was performed. Wells were coated as above with either type IV procollagen or laminin at a concentration of 600 ng/well. Another plate was coated with 1% BSA albumin (Calbiochem-Behring Corp.) in Voller's buffer and left overnight at 4°C.

Various concentrations of type IV procollagen or laminin (0–100,000 ng/ml) were mixed separately with a constant amount of APSGN serum or control serum diluted 1:25 in PBS-T, were then placed in the BSA-treated wells, and were incubated overnight at 4°C. After the overnight incubation, these mixtures were placed in the antigen-coated wells (containing either type IV procollagen or laminin) and incubated for 30 min. The unbound antibody was removed by washing with PBS-T three times for a total of 30 min. The goat anti–human conjugate was then added and the assay continued as described above.

Immunoblot

To further confirm the presence of antibodies to type IV procollagen and laminin in the sera of patients with APSGN, immunoblots were performed. SDS-PAGE was performed according to Laemmli (25).

For type IV collagen, 30 μg protein was placed on each lane of a 5% polyacrylamide gel. For laminin, 2.2 μg protein was placed on each lane of a 4% polyacrylamide gel.

Following electrophoresis, the gels and nitrocellulose membrane (162-0115; Bio-Rad Laboratories were soaked in transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3) for ~30 min. The proteins were then allowed to transfer overnight using a Bio-Rad Trans-Blot apparatus (120 V, 170-3917; Bio-Rad Laboratories). Transfer was performed at 4°C, 30 V.

Immunodetection was performed using a protocol from Vector Laboratories (Burlingame, CA). After transfer, the membranes were incubated in PBS-T for 30 min. All incubations were carried out at room temperature with gentle agitation. Each membrane was then incubated in a solution of primary antibody in PBS-T for 60 min. In the case of type IV procollagen, a membrane was incubated with each of the following: rabbit anti–type IV procollagen antiserum, at a dilution of 1:1,000; normal whole rabbit serum, at a dilution of 1:1,000; APSGN serum, at a dilution of 1:100; and control human serum, at a dilution 1:100.

For laminin, a membrane was incubated with rabbit anti–mouse laminin at a dilution of 1:10. Control and patient sera were used as above.

The membranes were then washed with PBS-T with three changes over 30 min, followed by an incubation in biotinylated secondary antibody in PBS-T (Vectastain ABC Kit, Rabbit IgG, PK-4001 Vector Laboratories, Burlingame, CA) for 30 min. They were then incubated for 30 min in the Vectastain ABC reagent. Another wash was performed as above, but in PBS. Membranes were then incubated for ~15 min in the peroxidase substrate solution, which consisted of 2 ml of a 3 mg/ml solution of 4-chloro-1-naphthol in methanol, 10 ml of PBS, and 5 μl 30% H_2O_2, followed by a wash in distilled water with two changes over 10 min.

Results

Characterization of Antigenic Components of BM

BM Collagen. The material solubilized by extraction with 0.5 M acetic acid and purified by DEAE-cellulose chromatography was subjected to amino acid and carbohydrate analysis. Table I shows that the preparation has a high 4-hydroxyproline, 3-hydroxyproline, and hydroxylysine content, as well as a low alanine content, which is consistent with previous analyses of type IV collagen (14, 16). The equimolar amounts of glucose and galactose, as well as the low content of mannose and fucose further confirm the nature of the material as type IV collagen that still has its propeptide extensions intact (analogous to procollagen). Rotary shadowing electron microscopy revealed typical monomers and their tetramers overlapping at the amino terminus (7-S domain) (14, 16) (data not shown).

Bacterial collagenase–resistant fractions of BM. The bacterial collagenase–resistant fractions of ALC were resolved into seven fractions on an agarose A-5m column (Fig. 1). Amino acid analysis of peaks I and II revealed the noncollagenous nature of the fractions, containing only traces of hydroxyproline and hydroxylysine (data not shown). The analyses of peaks III and V are shown in Table I. Peak V has a noncollagenous amino acid composition. Note the collagenous nature of peak III. The 4-hydroxyproline, hydroxylysine, and glycine content is much higher than that seen in the intact type IV collagen, whereas

TABLE I

Amino Acid and Sugar Compositions of ALC Acetic Acid Extract and Collagenase-resistant Fractions

Amino acid*	Acetic acid extract DEAE flow-through volume	Collagenase digest	
		Peak III‡	Peak V‡
Cysteic acid			
3-Hydroxyproline	12.08	4.83	—
Methionine sulfoxide	—	2.26	1.70
4-Hydroxyproline	82.55	96.28	Trace
Aspartic	40.51	39.32	59.73
Threonine	31.78	35.04	64.80
Serine	51.85	32.50	87.30
Glutamic	105.42	89.63	93.33
Proline	67.34	66.96	76.45
Glycine	263.97	316.24	100.54
Alanine	44.38	25.76	94.57
Cystine (reduced)	9.48	32.08	34.36
Valine	30.72	50.04	53.25
Methionine	13.69	5.08	15.45
Isoleucine	28.93	27.86	45.04
Leucine	56.15	42.66	73.20
Tyrosine	17.30	17.35	36.63
Phenylalanine	33.93	17.76	43.28
Hydroxylysine	35.27	41.41	4.48
Lysine	21.16	11.13	30.24
Histidine	15.48	12.24	36.14
Arginine	38.02	41.09	57.10
Sugars§			
Mannose	6.84	34.07	1.85
Fucose	1.46	11.093	1.66
Galactose	30.77	44.40	2.57
Glucose	33.90	36.37	2.51

* Residues per thousand.
‡ Fractions isolated after bacterial collagenase digestion of ALC and gel filtration.
§ μg/mg protein.

the alanine content is now significantly lower. Note the high reduced cystine content, indicative of the high crosslinking within the 7-S domain. The carbohydrate content again is characterized by the equimolar amount of glucose and galactose, but one now sees a significantly higher content of mannose and fucose.

Rotary shadowing electron microscopy revealed small globular pieces in peak V and aggregates of these in peaks I and II of Fig. 1. Peak III was composed entirely of the 7-S domain. (data not shown).

Laminin. The amino acid and carbohydrate analyses of laminin from the mouse EHS tumor was similar to that published earlier (13) (data not shown).

Immunologic Studies of BM Components

Type IV collagen. The native, undegraded type IV collagen obtained after 0.5 M acetic acid extraction of ALC was tested by ELISA and by immunoblotting

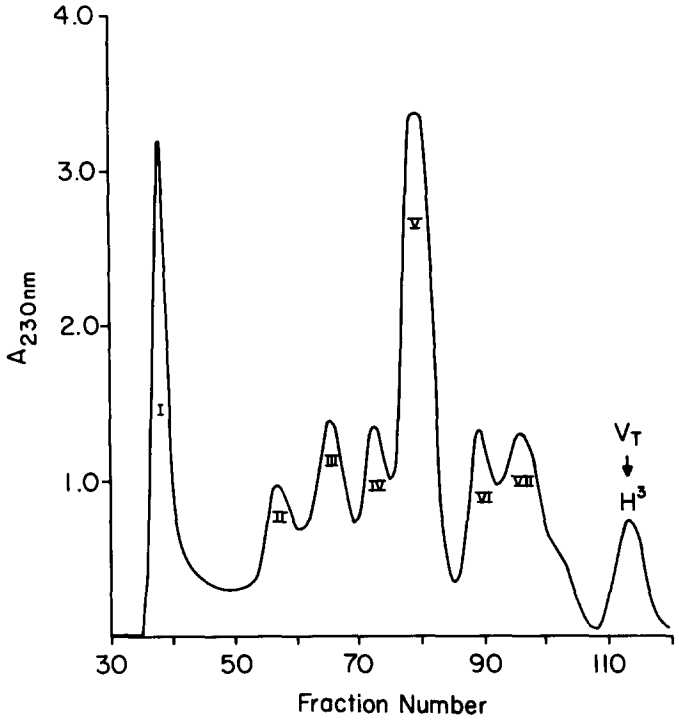

FIGURE 1. Gel filtration of bacterial collagenase digest of ALC on a 6% agarose A-5m column. Proteins were eluted with a buffer containing 1 M $CaCl_2$ in 50 mM Tris-HCl, pH 7.5. Peak I is the void volume.

using an antiserum prepared against it in rabbits, as well as human sera from controls and patients with APSGN.

Using an ELISA, type IV collagen, at a concentration of 600 ng/well, reacted with diluted sera from five patients with APSGN and five controls (Fig. 2). A clear titration curve of reactivity was observed as the serum was diluted and the type IV collagen antigen concentration was held constant. Except for the serum from patient 4, who had a much higher titer, the remainder of the patients' sera had comparable titers and presented similar titration curves. The sera from the five control individuals showed very little reactivity with the antigen, even at the lowest serum dilution. This experiment suggests that the sera contained antibodies against type IV collagen and that IgG in the serum being tested accounted for the observed antibody binding.

To further verify the nature of the antibody in the APSGN sera, a inhibition ELISA was performed using varying concentrations of ALC type IV collagen and a constant dilution of the patients' sera against the antiserum to ALC type IV collagen. In Fig. 3, sera from patients 3 and 4 were inhibited from reacting with type IV collagen up to an antigen concentration of 10,000 ng/ml, suggesting that antibodies in the patients' sera were indeed directed against determinants of the type IV collagen molecule.

To determine whether antibodies in the patients' sera were directed against any of the well-defined domains of type IV collagen, the ELISA was used with

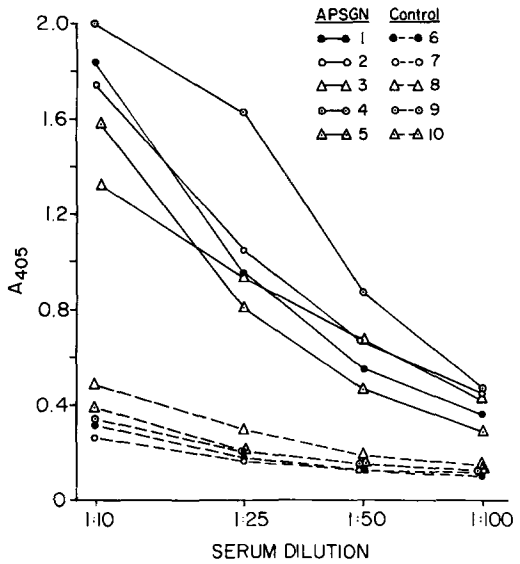

FIGURE 2. An ELISA study with type IV collagen isolated from ALC. Antigen at 600 ng/well. The reactivity of sera from five patients with APSGN and five controls are shown.

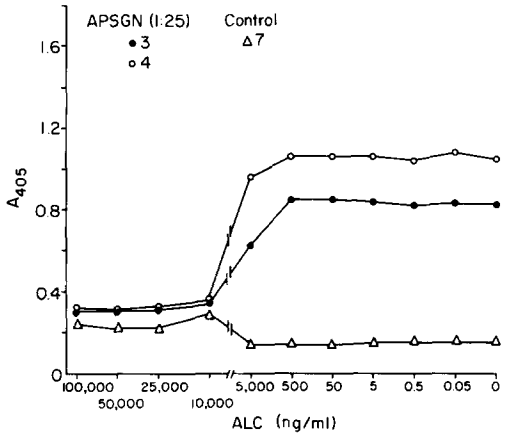

FIGURE 3. An ELISA study with type IV collagen from ALC. Two APSGN sera and a control serum (1:25 dilution) were inhibited with type IV collagen at varying concentrations. The APSGN sera were inhibited up to an antigen concentration of 10,000 ng/ml. The reaction with the control serum is unchanged throughout the range of antigen concentrations.

material from peaks III and V as well as peaks I and II of the collagenase digest (Fig. 1). Fig. 4*B* shows that the 7-S domain (peak III) has a much stronger reactivity compared to the noncollagenous domain NC-1 (peak V) and its aggregates in peaks I and II.

The reactivity of the APSGN sera with type IV collagen and its fractions was further confirmed by performing immunoblots. Fig. 5*A* clearly shows a positive reaction of type IV collagen with its rabbit antiserum, as well as with the serum from a patient with APSGN. Similarly, peak III (7-S domain) of the collagenase

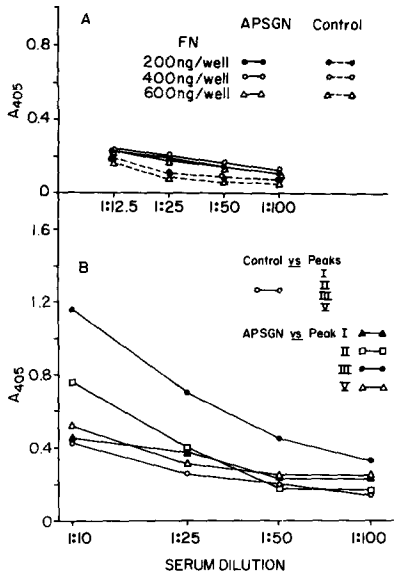

FIGURE 4. An ELISA study with fibronectin and collagenase-resistant fractions of type IV collagen. (*A*) Reaction of an APSGN serum wtih fibronectin at different concentrations. Patient and control sera show little or no reactivity with fibronectin. (*B*) Reactivity of an APSGN serum with collagenase-resistant domains of type IV collagen. Peak V, monomer of NC-1 domain; peaks I and II, aggregates of NC-1 domain; peak III, 7-S domain. The control serum was reacted with the same peak material as the APSGN serum (O—O), and the values represent the average from all four peaks.

digest of ALC shows a clear reaction with the rabbit antiserum to type IV collagen, as well as with serum from a patient with APSGN (Fig. 5*B*).

Laminin. The mouse laminin isolated from the EHS tumor was tested by ELISA and by immunoblotting using an antiserum prepared commercially against it, as well as with human sera from controls and patients with APSGN. Fig. 6 shows the reactivity of mouse laminin at a concentration of 600 ng/well with increasing dilutions of the control and patients' sera. As was noted with type IV collagen, a clear titration curve of reactivity was obtained with increasing serum dilution.

The presence of antibodies against laminin in the sera from patients with APSGN was further confirmed by carrying out an inhibition of ELISA (Fig. 7). The reaction of laminin with the APSGN sera was inhibited by the antigen up to a concentration of 10,000 ng/ml, suggesting that antibodies in the patients' sera were directed against determinants of the laminin molecule.

An experiment using ELISA with human laminin isolated from placental membranes (27) showed comparable reactivity of two APSGN sera with that of mouse laminin (data not shown).

Fig. 8 shows an immunoblot of mouse laminin with a serum from a patient with APSGN. The reactivity of the serum with laminin is clearly demonstrated.

Fibronectin. The reactivity by ELISA of human fibronectin with an APSGN serum was weak and no higher than that obtained with a control serum (Fig. 4*A*).

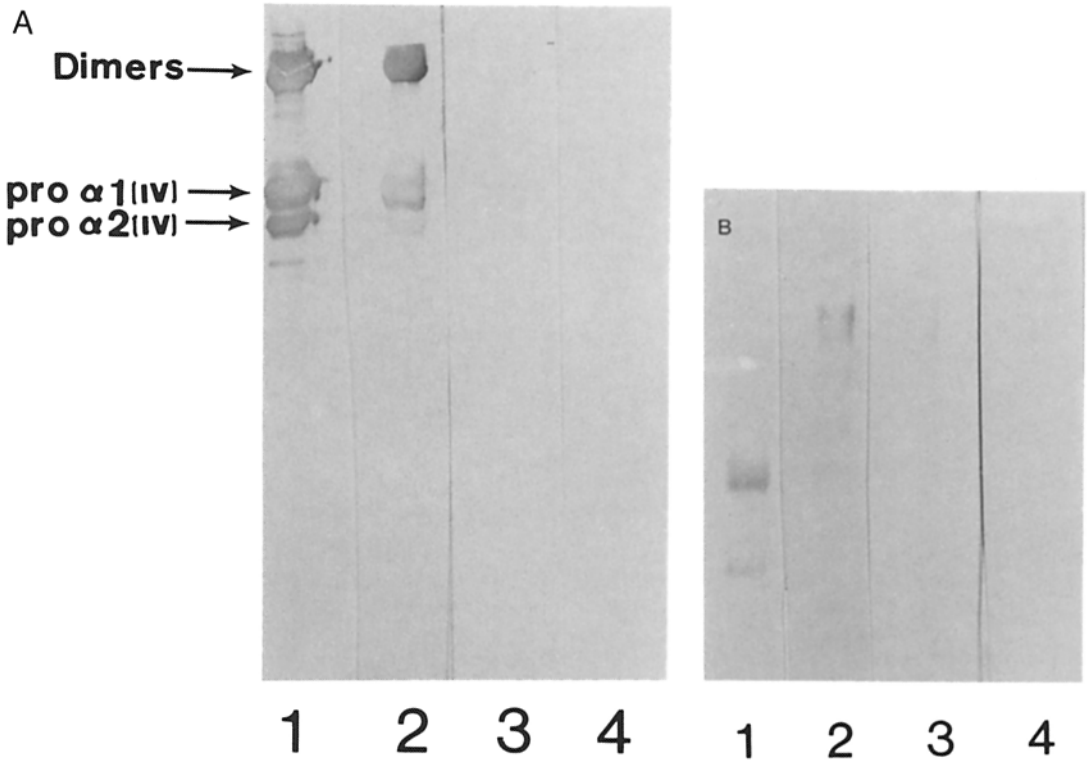

FIGURE 5. Immunoblots of type IV collagen and of the 7-S domain. (*A*) Reaction of reduced type IV collagen with (*1*) rabbit anti-Type IV serum, (*2*) APSGN serum, (*3*) control serum, and (*4*) normal rabbit serum. (*B*) Reaction of reduced collagenase resistant material from peak III (7-S domain) with (*1*) rabbit anti-type IV, (*2*) ASPGN serum, (*3*) control serum, and (*4*) normal rabbit serum.

Discussion

This study is an extension of the recently published work (3) which demonstrated that sera from patients with acute or chronic PSGN contain antibodies against proteoglycans derived from bovine and human glomeruli (3). This observation suggested that, in conditions in which tissue injury can occur, as in bacterial or viral infections, release into the circulation of antigenic fragments from critical tissue regions can take place, leading to the development of specific autologous antibodies. It was further suggested that, in the case of PSGN, autoantibodies to other antigenic components of the glomerulus, such as type IV collagen and laminin, can develop as well. The data obtained in the present study support this view.

Purified type IV collagen has been obtained by nondegradative methods from bovine lens capsule, the only type of normal BM that allows the isolation of intact type IV collagen molecules (14, 18). This has permitted the isolation of structural domains from such molecules and allowed their ultrastructural and immunochemical characterization (14, 28). The sera from patients with APSGN reacted with intact type IV collagen molecules as shown by ELISA (Fig. 2). The nature of the antibody in the APSGN sera was verified by performing a competitive

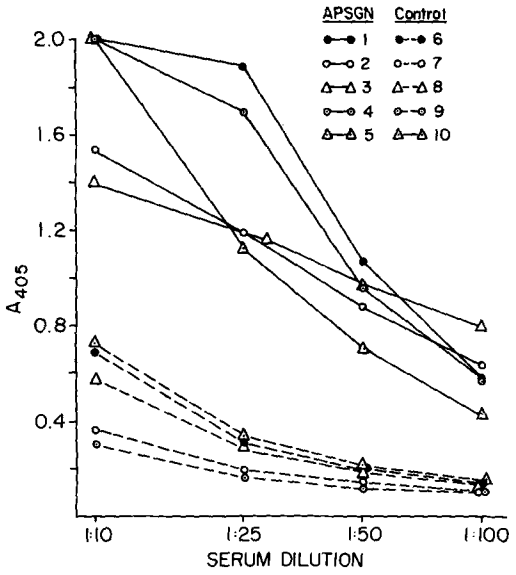

FIGURE 6. An ELISA study with mouse laminin. Antigen at 600 ng/well. The reactivity of sera from five patients with APSGN and five controls are shown.

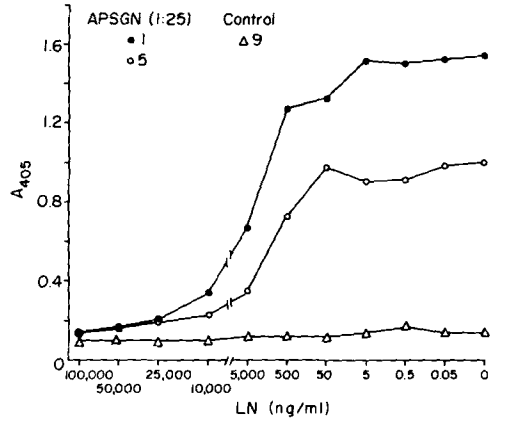

FIGURE 7. An ELISA study with mouse laminin. Two APSGN sera and a control serum (1:25 dilution) were inhibited with laminin at varying concentrations. The APSGN sera were inhibited up to an antigen concentration of 10,000 ng/ml. The reaction with the control serum is unchanged throughout the range of antigen concentrations.

ELISA. Sera from two patients were tested (Fig. 3) and exhibited inhibition of the immunologic reactivity of type IV collagen with its antiserum. The specificity of the antibodies against type IV collagen was confirmed by performing immunoblots (Fig. 5*A*).

The collagenase-resistant domains of type IV collagen were obtained from the insoluble ALC BM (Fig. 1). On the basis of chemical analyses and ultrastructural studies, the NC-1 domain is noncollagenous (Fig. 1, peaks I, II, IV and V and Table I) and globular (13, 14, 16), whereas the 7-S domain is collagenous (Fig. 1, peak III and Table I) and rod-like (13, 14, 16). The NC-1 domain is stabilized by interchain disulfide bonds (1, 2, 26, 29), whereas the 7-S collagenous domain

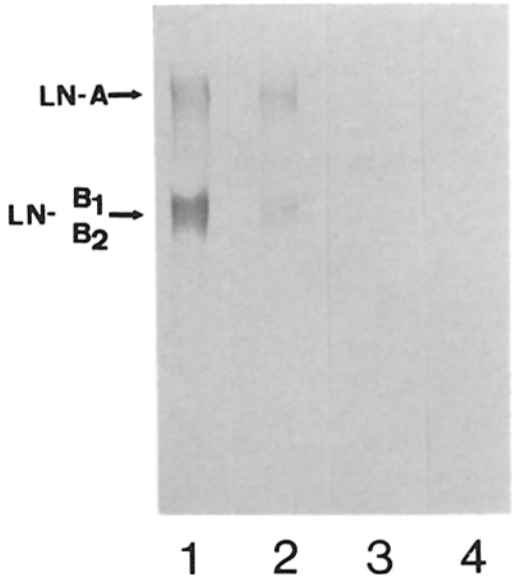

FIGURE 8. Immunoblot of mouse laminin (*LN*). The reduced antigen was reacted with (*1*) rabbit anti-mouse LN, (*2*) ASPGN serum, (*3*) control serum, and (*4*) normal rabbit serum. *A*, B_1 and B_2 denote position of the respective laminin subunits.

is stabilized by both disulfide and lysyl-derived covalent crosslinks (17, 30). Unlike the reports (1, 26) that used sera from Goodpasture syndrome patients and localized the antigenic determinant in the noncollagenous, globular domain (NC-1) of type IV collagen, in our present study, the sera from patients with APSGN react predominantly with the 7-S domain (Fig. 4). An earlier study by Kefalides et al. (2) indicated that sera from three patients with Goodpasture syndrome reacted predominantly with the 7-S domain (Fig. 1, peak III). In this study, compared to material in peaks I, II, and V, peak III reacted more strongly with the APSGN sera. However, neither the NC-1, nor the 7-S domain of the type IV collagen reacted as strongly as did the intact molecule (Figs. 2 and 4*A*). This suggests that a major portion of the antibodies in the APSGN sera must be directed against conformational determinants. The higher reactivity of the 7-S domain with the APSGN sera cannot yet be explained; however, carbohydrate analysis (Table I, peak III) shows a significantly higher sugar content compared to the NC-1 domain (Table I, peak V). It should also be noted that material in peak III contains 18 times more mannose and ~7 times more fucose than material in peak V, suggesting that, in addition to the glucosyl-galactose unit linked to hydroxylysine, the 7-S domain must contain mannose-rich oligosaccharide units. The latter property may render the 7-S domain more antigenic. Little or no reactivity was noted when pepsin-resistant α1(IV) and α2(IV) collagen chains (M_r 125,000 and 110,000, respectively) were tested against several APSGN sera (our unpublished observation).

The APSGN sera were reactive against mouse laminin both by ELISA and immunoblot (Figs. 6 and 8). This is not surprising, since this glycoprotein is an integral part of GBM and constitutes ~30–40% of its weight (20). The laminin

molecule, which has the shape of a cross, has several structural domains: three short arms with two globular domains in each, and a long arm with one globular domain. At present, it is not known against which of the domains the antibodies are directed. Unpublished studies in our laboratory (N. A. Kefalides) show that sera of patients with Goodpasture syndrome also react with mouse as well as human laminin. We have tested a number of sera from patients with APSGN by ELISA and immunoblot against a preparation of laminin from human placenta (27) and noted a significant degree of reactivity (our unpublished observation). It would appear, therefore, that autoantibodies associated with either APSGN or Goodpasture syndrome are directed against a variety of antigenic components present in the GBM. Sera of patients with the above disorders may contain autoantibodies against other renal or extrarenal antigens. Studies by Wilson and Dixon (31) show that sera of patients with Goodpasture syndrome react with capillaries of the choroid plexus. Clayman et al. (32) has recently reported the isolation of a tubular antigen producing antitubular BM disease. As other renal antigens become isolated and characterized, their relationship to immunologically-mediated renal disease could be ascertained. It has been reported (33) that the B-1 subunit of laminin consists of two polypeptide chains (50 nm long) having a coiled-coil α-helix. A similar structure has been reported for a surface protein (50 nm long) isolated from group A streptococci (34). It is intriguing to speculate that the immunologic crossreactivity observed between GBM and the β-hemolytic streptococcus may be related to the structural similarity between the B-1 subunit of laminin and the streptococcal surface M protein.

Cellular immune studies of glomerulonephritis by a number of investigators have previously demonstrated the presence of cellular reactivity to GBM antigens in these patients (reviewed in 35). However, the exact biochemical nature of the reactive BM antigens in these cellular assays has not been defined. The demonstration in the present study of antibodies to laminin and type IV collagen determinants, and in a previous report (3) to glomerular PG in poststreptococcal sera lends credence to the data derived from the cellular immune studies indicating that autoimmunity to a variety of glomerular antigens may occur in glomerulonephritides besides classic anti-GBM disease. These cellular studies have also consistently demonstrated cellular immune reactivity to streptococcal antigens in patients with glomerulonephritis (35). The possibility remains that streptococcal cellular reactivity in patients with glomerulonephritis is due to the presence of crossreactive determinants shared by the streptococcus and BM antigens (5). These crossreactive determinants may not only be protein in nature, but may also represent crossreactive carbohydrate epitopes (3).

In view of the observations by Fillit et al. (3) and our current findings, it can be stated that autoantibodies can be produced against a range of tissue-specific antigens after bacterial infection. It is becoming increasingly evident that in anti-BM disease, the production of antibodies is not against a single antigenic component or a single epitope on an antigen. Rather, antibodies are produced against all the known integral components of the BM, including type IV collagen, laminin, PG, and possibly entactin. Our ongoing studies with sera from patients with Goodpasture syndrome also support this view. It should be noted that fibronectin, which occasionally can be identified in the GBM as a result of

trapping, did not react with any of the APSGN sera, further indicating the apparent specificity of such autoimmune reactivity for true BM components. Additional work is required to elucidate the factors that contribute to the relative antigenicity of the various domains of type IV collagen and laminin, and the differences in the pathologic picture of the kidney between PSGN and Goodpasture syndrome. Finally, the role of anti-BM autoimmunity in APSGN and the subsequent development of chronic PSGN requires investigation.

Summary

Sera from patients with poststreptococcal glomerulonephritis (PSGN) known to have antibodies to proteoglycans were studied for the presence of antibodies against other basement membrane (BM) components. BM collagen (type IV) was isolated in the native state by extracting bovine anterior lens capsule (ALC) with 0.5 M acetic acid. The 7-S (collagenous) domain and the NC-1 (noncollagenous) domain of type IV collagen were obtained after bacterial collagenase digestion of ALC followed by gel filtration. Laminin was isolated from the mouse EHS tumor and fibronectin from human plasma.

Immunologic studies, using an ELISA and electroimmunoblot, revealed the presence of antibodies that reacted with intact, native type IV collagen and the 7-S collagenous domain of this molecule. Reaction with the NC-1 (noncollagenous) domain was minimal, and not higher than that obtained with control sera. Laminin reaction strongly with the patients' sera, but fibronectin did not.

Unlike sera from patients with Goodpasture syndrome, which contain antibodies primarily against the NC-1 (noncollagenous) domain of type IV collagen, sera from patients with acute PSGN contain antibodies against all the major macromolecular components of BM. This difference in immunologic reactivity may account for the observed differences in the pathologic picture at the glomerular level.

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