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Research Article

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Glomerulonephritis Mediated by Antibody to Glomerular Basement Membrane

IMMUNOLOGICAL, CLINICAL, AND HISTOPATHOLOGICAL CHARACTERISTICS

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ABSTRACT A prospective study was undertaken to establish the incidence of glomerular basement membrane (GBM) antibody-mediated glomerulonephritis and its histopathological characteristics in a clinical group of patients presenting with renal disease. Biopsies from 43 of 409 consecutive patients technically satisfactory for direct immunofluorescent (IF) examination had diffuse and generalized linear localization of host immunoglobulin (Ig); two other badly scarred kidneys tested negative to IF although GBM antibodies were eluted. Confirmatory evidence of GBM antibody-mediated disease in these patients came from whole kidney or biopsy elutions (15 patients), serologic assays for circulating GBM antibodies by indirect IF (9 of 38 patients), radioimmunoassay (26 of 34), and hemagglutination (31 of 32). Although sera were not tested from six patients, circulating antibodies were demonstrated by some test in 36 of 39 of the remainder. Histologically, half of the patients had minor and nonspecific glomerular abnormalities or mild focal proliferative glomerulonephritis. More severely involved kidneys had focal necrotizing (17%), rapidly progressive (7%), and chronic, usually sclerosing, glomerulonephritis (27%). Clinical courses of these patients comparably were quite variable, ranging from indolent microhematuria and/or gross hematuric bouts to progressive renal failure; nephrotic syndrome was observed in 11 patients. GBM antibody-mediated glomerulonephritis may be a relatively mild disease with apparently stable renal function, although 16 patients have experienced functional deterioration, and 11 have progressed to dialysis, renal transplantation, or death.

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INTRODUCTION

Models of experimental glomerulonephritis have validated two general types of immunologically mediated glomerulonephritis: the first due to circulating, macromolecular immune complexes and the other caused by antibodies with specificity for intrinsic structural antigens associated with the glomerular basement membrane ([GBM]¹ antibodies) (1). These latter antibodies that characteristically cause a smooth, linear fixation of Ig along GBM as detected by direct immunofluorescent IF examinations, are elutable, can be transferred, and cause glomerulonephritis in the secondary hosts. Comparable characteristics have been confirmed in cases of human glomerulonephritis, especially associated with Goodpasture's syndrome (GP) of rapidly progressive glomerulonephritis and intra-alveolar pulmonary hemorrhage (2).

Nevertheless, the frequency of GBM antibody-mediated glomerulonephritis and its clinical and serological characteristics in a general population have not been established. A prospective study was undertaken by nephrologists of cooperating military hospitals to attempt recognition of immunologically mediated human glomerulonephritis, including GBM antibody-mediated disease, to define its epidemiologic background, and to estimate its clinical course.

Our data suggest that GBM antibody-mediated glomerulonephritis, inferred from a linear pattern of host

¹ *Abbreviations used in this paper:* C, collagenase; EM, electron microscopy; GBM, glomerular basement membrane; GP, Goodpasture's syndrome; Hand E, Hematoxylin-Eosin; IF, immunofluorescent; Ig, immunoglobulin; NS nephrotic syndrome; PAS, periodic acid Schiff; PBS, phosphate buffered saline; PMN, polymorphonuclear; SRBC, sheep red blood cells; T, trypsin.

glomerular Ig fixation, comprises a minority of cases of human glomerulonephritis, but a substantially larger fraction than suggested heretofore. Moreover, onset of disease in these patients usually is unassociated with any discrete clinical event or obvious epidemiologic factor, and relatively few patients pursue a course of rapid progression to renal failure.

METHODS

Patients. Renal tissue adequate for immunohistochemical tests was obtained from 409 of 449 consecutive, diagnostic renal biopsies (Table I) between January 1970 and July 1973. 40 biopsies could not be cut for technical reasons or contained too few glomeruli. Excluded from all analyses were biopsies from other than the participating Centers, cases of suspected familial renal disease, and renal allografts.

Routine histologic staining. The renal tissue for light microscopy was fixed in 10% buffered formalin. 2-3 μ m thick sections were cut from the paraffin-embedded tissue and routinely stained with hematoxylin-eosin, (H and E), periodic acid-Schiff (PAS), and Gomori trichrome stains. A specimen was considered inadequate for evaluation if fewer than five viable glomeruli were present in sections. The majority of renal biopsies in the study contained an average of 10-15 glomeruli per section suitable for evaluation.

Electron microscopy (EM) was performed using an RCA EMU 3F model (RCA Electronic Components, Harrison, N. J.). Specimens for EM were fixed in purified paraformaldehyde, postfixed in osmium tetroxide, dehydrated at 4°C through graded alcohols, embedded in epoxy resin (Epon 812), and stained with uranyl acetate and lead citrate. There was adequate tissue for ultrastructural studies from 16 patients.

Terminology. Focal, lesions which affect some but not all the glomeruli present; segmental, lesions which affect only a portion of the capillary loops within an involved glomerulus; global, involvement of an entire glomerulus (synonymous with diffuse); sclerosis (segmental or global), the process of obliteration of capillary loops by proliferation of PAS-positive, mesangial, matrix-like material.

IF tests were done on snap-frozen kidney cores obtained by percutaneous or operative biopsy, cut at 4-6- μ m thickness and fixed as described (4). Sections were stained for

IgG, IgM, IgA, IgD, IgE, B₁C, and fibrinogen, as described by Coons and Kaplan (3). Controls were done as described (4).

Antisera for immunohistochemical tests were prepared in rabbits to antigens isolated from normal human or myeloma sera by salting out with ammonium sulfate, chromatography on DEAE-cellulose, and Pevikon electrophoresis (5). Antiserum to B₁C (C'3 component of complement) was the gift of H. J. Müller-Eberhard. (Scripps Clinic and Research Foundation, La Jolla, Calif.) Antiserum to IgE was purchased in a single lot from Hyland Div., Travenol Laboratories Inc., Costa Mesa, Calif. Its specificity has been reported in detail (6).

Fluoresceination of antibody globulin or IgG fractions was done by dialysis (7); nonspecific fluorescence was removed by subsequent rechromatography on DEAE-cellulose, and by mouse or rabbit liver powder absorption.

Radiolabeling of isolated proteins and glomerular antigens with ¹²⁵Iodine or ¹³¹Iodine was done by the technique of McConeahy and Dixon (8).

Serological tests. Antinuclear antibodies were tested on freshly snap-frozen mouse liver by indirect IF. Concentrations of C'3 and C'4 of serum complement were estimated by immunodiffusion, using commercially prepared kits, Meloy Laboratories, Inc., Springfield, Va. as were serum immunoglobulin concentrations. Sensitized sheep cell agglutination tests for rheumatoid factor were done by the method of Heller et al. (9).

Elution experiments. Whole human test kidneys were decorticated; the cortex was minced, washed extensively in phosphate-buffered saline (PBS), and then eluted in 0.02 M citrate buffer, pH 3.2 at 37°C (4). Biopsy tissue was eluted from the whole biopsy or from multiple sections in a bath of citrate buffer, and concentrated to a single drop before testing for GBM antibodies.

Transfer experiments. Transfers of radiolabeled, eluted globulins were performed in squirrel monkeys; simultaneous injection of acid-treated, human IgG radiolabeled with the alternate iodine isotope was done as control (4). Monkeys were sacrificed after 24-72 h and kidneys perfused with PBS, minced, and washed; particulate debris was counted in a well-type radiodetector.

Tests for circulating human GBM antibodies

Indirect IF. GBM antibodies were tested on normal, homologous kidney sections (5) using test sera neat and at 1:5 dilutions.

Hamagglutination assay. (a) Sheep red blood cells (SRBC) in Alsever's solution were purchased from Colorado Serum Co., Denver, Colo., washed in 0.9% NaCl, and packed before sensitization. (b) Sensitizing antigens: bovine albumin, human fraction II, collagenase-digested human GBM (C-GBM), trypsin-digested human GBM (T-GBM) (see below). (c) Control antisera: two separate sheep and one rabbit antisera made in animals immunized with particulate human GBM and absorbed extensively with lyophilized normal human serum and washed, packed normal human and SRBC. (d) Sensitizing conditions; 0.2% concentration of chromic chloride (10), 2-mg/ml concentration of T-GBM, or 1-mg/ml concentration of C-GBM. (e) Test conditions; microtiter plates, using 0.125% test cell concentration of sensitized SRBC, diluent of 0.5% absorbed normal rabbit serum in PBS, incubated 60 min at 37°C, spun 45 s at room temperature at 1,200 rpm, and read at a 45° angle after 10, 30, and 60 min. (f) Test sera; sera for testing were absorbed with equal volumes (50/50),

TABLE I
Biopsy Study Group by Age and Sex, and GBM Antibody Patients as Subgroup

Age	Study group as whole			GBM antibody patients		
	Total	Male	Female	Male	(%)	Female
<i> yrs</i>						
1-10	40	25	15	3	(12.1)	
11-20	98	64	34	5	(8.0)	4
21-30	113	74	39	13	(17.8)	1
31-40	91	51	40	9	(17.6)	1
41-50	47	28	19	7	(25.0)	
51-60	19	11	8	2	(18.2)	
61-70	1	1				
Total	409	254	155	39	(87%)	6 (13%)

washed, packed SRBC after heat inactivation for 60 min at 56°C. (g) Test sera selection; sera for testing included 40 normals, 40 patients whose direct IF indicated granular Ig deposition characteristic of immune complex pathogenesis, and sera from 32 patients whose biopsies showed glomerular Ig deposition of linear Ig pattern, and were suspected of GBM antibody pathogenesis. Samples were coded and analyzed concomitantly.

Radioimmunoassay. (a) Trypsin- and collagenase-digested human GBM were labeled separately with ¹²⁵Iodine. (b) 100- μ l samples of test sera, neat, and 1:10 dilutions, were incubated at 37°C for 1 h with deaggregated, radiolabeled antigen. (c) 1 ml of hyperimmune goat antihuman IgG was added to the incubating mix after 60 min, agitated, incubated another 60 min at 37°C, and placed in the cold at 4°C overnight. (d) Total counts were measured in a well detector, after which the supernate was discarded, the packed precipitate washed with PBS, and counted. (e) Percentages of labeled antigens precipitated by test sera were compared to those of a panel of 37 sera from normal donors analyzed concomitantly. (f) Concomitant controls included radiolabeled human IgG added to normal sera tested to assure complete precipitation of IgG by the goat serum.

Soluble glomerular antigens. GBM were isolated by Spiro's modification of the technique of Krakower and Greenspon (11), from kidneys of victims of accidental death. Washed, desalted, and lyophilized human GBM were then digested with C or T; solubilized GBM was used for sensitization, and labeling as described above.

Purer GBM antigens for radioassay were isolated as follows: serum from a patient with GP was prepared as an immunoadsorbent by the technique of Avrameas and Ternynck (12). Separate batches of immunoadsorbent were reacted with radiolabeled C- or T-GBM; the adsorbents were washed with PBS until supernates were free of radioactive material, and then eluted at 37°C for 1 h in 0.2 M glycine-HCl buffer, pH 2.2. Eluted, radiolabeled antigens then were centrifuged free of immunoadsorbent, neutralized, dialyzed against PBS overnight at 4°C, concentrated, and spun free of aggregates.

Comparison of GBM antigens eluted from immunoadsorbents was done as follows: (a) Separate immunoadsorbents were prepared from sera of four patients with circulating GBM antibodies; three of these patients had the GP syndrome. Radiolabeled C- and T-GBM were eluted from separate batches of the immunoadsorbents and four different C-GBM and two different T-GBM antigens were prepared. Comparative binding of sera from six patients with circulating GBM antibodies (three with GP syndrome) was tested against the panel of six radiolabeled antigens. (b) Additionally, eluates from kidneys of 12 different patients with GBM antibody nephritis (7 with GP syndrome) and from 6 patients without GBM antibody nephritis were tested against radiolabeled C- and T-GBM antigens isolated from the immunoadsorbents described in (a) above and pooled as C- or T-GBM.

RESULTS

Direct IF examinations. Biopsies from 409 of the consecutive patients were satisfactory for direct IF analyses; 43 patients had direct IF evidence of linear localization of host Ig in glomeruli, presumptive of anti-GBM antibody fixation. Another two patients with end-stage kidney disease had negative direct IF tests, but

elution of their kidneys yielded anti-GBM antibodies; they are included for survey purposes (patients 31 and 32, Table II). As indicated in Table I, the apparent incidence of linear Ig pattern did not differ significantly from decade to decade as a percentage of the biopsy population, although there was a distinct sex preference favoring males with linear Ig deposition.

In each case, IgG was detected as the predominant or sole Ig class binding in vivo to glomeruli; IgM was detected in biopsies of 22 of 45 patients, often in a patchy or discontinuous pattern, and IgA was detected in 11 patients. IgD was not detected in a single case, and IgE in a linear pattern was detected in 5 of 26 patients. B:C was seen in 30 instances; in many cases staining was not concordant with linear Ig staining and was more mesangial in distribution; fibrinogen localization in glomeruli occurred in 19 of the 45 patients. Fibrinogen staining often was focal and/or segmental; in numerous biopsies fibrinogen was mesangial in location rather than along GBM.

Clinical features (Table II). 39 of the 45 patients with apparent GBM antibody-mediated disease were males and 6 female. As Table I indicates this is a disproportionate representation of males, although males predominated in the series as a whole. Nephrotic syndrome (NS) was the presenting clinical problem in 11 patients. Hematuria, either gross or microscopic, was the initially detected abnormality or complaint of another 11 patients. Five patients pursued a relentless course of rapid progression to renal failure within 6-9 mo, and four others were seen for the first time in renal failure, although two had documented chronic renal disease for over 10 yr. In all, 11 patients have progressed to either dialysis or transplantation during the period of this study and another 5 have had functional deterioration. Persistent, relatively mild hypertension was a feature evident during clinical evaluation of 13 of the 45 patients. Three patients had documented hepatic cirrhosis associated with ethanolism; two of the latter were among the three patients with chemical and clinical diabetes mellitus in this group.

Elution results. Elutions were attempted on renal tissues obtained from 19 of the patients. In seven cases, elutions were attempted from organs obtained at nephrectomy or autopsy; nine elutions were attempted from biopsy specimens (six surgical biopsies and three needle biopsies) and three from whole organs as well as biopsy specimens. Eluates from tissue of 15 patients were positive for anti-GBM antibodies; the negative elutions were made from biopsy specimens, two from needle biopsies.

Assays for circulating anti-GBM antibodies

Indirect IF. 9 of 38 patients assayed for GBM antibodies were positive by this technique (24%).

TABLE II
GBM Antibody Patients

Patient no.	Age	Sex	Clinical syndrome	BUN	Creatinine	24 h urine protein	Hypertensive	Progressive loss of renal function
	<i>yr</i>				<i>mg/100 ml</i>	<i>gm</i>		
I 1	28	M		10	1.0	1.0	+	
2	45	M	NS†	12	1.3	7.0	0	
3	18	M	H	14	1.1	0.5	0	
4	28	M	H	12	1.0	0.120	+	
5	23	M	H	13	1.1	0.300	0	
6	18	M		13	1.3	+	0	
II 7	36	M		14	1.0	3.1	0	
8	38	M		16	1.4	1.7	0	
9	34	M	H	20	1.3	0.03	0	
10	19	M	H	13	1.0	0.700	0	
11	23	M	H	14	1.2	—	0	
12	36	M	H	17	1.4	0.040	0	
13	29	M	H	21	1.0	0.070	0	
14	20	M	H	11	1.0	0.064	0	
15	38	M		40	2.7	0.900	0	
III A 16	51	M	N	67	2.8	15.0	0	+
17	52	M	N	22	1.1	5.5	0	
B 18	37	M		12	1.2	1.6	0	+
19	43	M	N	35	2.4	3.5	0	+
20§	21	M		29	2.3	2.3	0	+
IV 21§	44	M		45	2.9	1.5	+	+
22	8	M	NS	17	1.0	4.0	+	+
V A 23	8	M	NS	88	6.8	+	0	+
24	18	F		Renal failure			+	+
B 25	39	M	NS	22	2.6	3.1	+	+
26	35	M	NS	29	3.0	5.0	0	+
27	38	M		42	4.0	2.6	+	
28	33	M	NS	19	2.5	6.0	0	+
29	19	F		End stage			+	+
30	13	F		23	2.6	0.400	+	+
End stage 31	28	F		End stage			+	+
32	38	F		End stage			+	+

* On scale 1-4 plus.

† H, hematuria; NS, nephrotic syndrome.

§ GP syndrome.

|| End stage kidneys histologically; not described in text. ND not done; T, trace; F, focal; M, mesangial.

Hemagglutinating antibodies. Normal sera never gave an agglutinating reaction beyond the first test well (titer 1:4). Sera from patients with granular, discontinuous Ig glomerular deposits also did not agglutinate the test cells. Sera from patients with linear glomerular Ig staining caused agglutination of GBM antigen-sensitized SRBC in 31 of 32 cases. Heterologous antihuman GBM sera gave strong agglutinating reactions.

Radioimmunoassay. Sera from 26 of 34 patients tested (76%) were positive for circulating antibodies

against one or both glomerular antigens: 5 bound the C-solubilized antigens (C-GBM) only; another 5 bound the T-solubilized antigens (T-GBM) only. Binding percentage ranged from 0.5 to 7.8% with these sera; the maximum binding observed with any serum tested to date has been 22%. Strong binding is characteristic of sera from patients with GP and rapidly progressive glomerulonephritis.

As indicated in Table III, the several GBM antibody containing sera differed in their degrees of binding to

Direct immunofluorescent tests of renal biopsies						Circulating anti-GBM antibodies								
						Positive eluate	Immuno-fluorescent, serum	Hemagglutination titers		Radioimmunoassay binding of antigen				
								C-GBM	T-GBM	C-GBM		T-GBM		
IgG	IgM	IgA	IgE	B ₁ C	Fibrin					Neat	1:10	Neat	1:10	
1*	—	—	—	—	M									
1-2	T	—	—	T, M	—		—	16	16		+		+	
1-2	—	T, M	T	1, M	—									
1-2	T	—	—	1	—			—	16		+	+	+	+
1	T	—	—	—	—			16	8					
1	—	—	—	—	—			16	16	2.0		1.10		
1	T	—	—	T	—									
1	1	—	—	1	2-3			64	32	1.1		ND		
1	—	1	—	1, M	1			16	8	—		—		
1	—	—	—	—	—			32	128					
2	—	—	—	1	1-2		+			—		—		
1	—	—	—	T	T			16	8	2.0		—		
1-2	1	—	—	1	—			—	16	—		—		
1-2	—	—	—	—	1-2									
1	—	—	2, M	1, M	1-2					—		1.0		
1-2	1	—	—	1-2	2, F		+	16	32	1.61		1.07		
2	T	—	—	1-2	1									
3	—	1	—	—	—						0.30		0.70	
3-4	1	1	—	T	T-3+		+	16	16	2.0		0.5		
4	1-2	—	—	—	—		+	64	—	7.47	7.1	5.86	1.10	
4	1-2	—	—	1, M	2					7.80	3.3	4.18	1.3	
2	1	1	—	1	3		+	64	64	2.9		—		
2	2, F	—	1, F	2	T-2+, M		+	16	8		+	+	+	+
1	1, F	T, F	—	1	—		+	32	32		+	+	—	—
1	1, M	—	—	—	—		+	8	16		1.1		0.5	
2-3	1	T	—	1, F	1		+	—	16		1.30	1.23	0.5	
1	T, M	T	T	1	—			16	—	—	—	+	+	
2	1, M	—	—	1-2M	1, F, M			8	16	—	—	—	—	
1	—	—	—	—	—		+	16	—	—	—	1.3	3.0	
1	—	—	—	1	—		+	32	8		0.6		—	
—	—	—	—	—	—		+	—	—		+	+	+	—
—	—	—	—	—	—		+	—	16		+	—	+	—

the panel of antigens eluted from different immune adsorbents. At the dilution tested, non-GP sera demonstrated a more restricted antigenic specificity and 3- to 10-fold less binding capacity. In contrast, GP sera bound a broader spectrum of antigens.

All eluates from GBM antibody-mediated glomerulonephritic kidneys tested bound pooled C-GBM antigens; in addition, five also bound T-GBM antigens (three were eluates from GP kidneys).

Transfer experiments. Nephrotoxic antibodies were

demonstrated in eluates from seven patients, none with GP; contents of kidney fixing antibodies ranged from 0.5 to 2.0%.

Other serological tests

Antinuclear antibodies. 8 of 41 patients tested had weakly positive antinuclear antibodies: 2 with a titer of 1:10, and none greater.

Complement protein analyses. Measurements of C'3 were normal in 38 of the 40 patients tested; one of the

TABLE III
Comparison of Specificities of Sera Containing GBM Antibodies
Tested Against a Variety of GBM Antigens

Patient	Binding of radiolabeled, immunoadsorbed human GBM antigens					
	C-GBM				T-GBM	
	A*	B	C*	D*	E*	F*
	%					
GP†						
1	9.4	6.8	11.7	4.4	4.8	—
2	10.5	5.4	16.1	3.7	6.5	1.4
3	9.3	4.9	14.2	3.7	3.2	—
Non-GP						
1	1.1	—	—	0.7	—	—
2	1.3	—	—	0.7	—	—
3	—	—	—	—	1.2	3.3

All sera were tested at 1:10 dilution.

* Denotes antigen eluted from immunoadsorbent made from serum of different patients with GP syndrome.

† GP, Goodpasture patient; non-GP, non-Goodpasture.

two subnormal values was detected in a patient already substantially azotemic. C'4 concentrations were normal in 34 of 35 patients tested.

Sensitized sheep red cell agglutinations to detect rheumatoid factors were positive in 7 of 40 patients. None of these patients had histories or signs of rheumatic disease; six of the seven had detectable glomerular IgM deposition, including both patients with GP.

Data confirmatory of GBM antibody pathogenesis. Evidence was adduced in support of GBM antibody activity by assay of circulating antibodies using indirect IF test, hemagglutination, and radioimmunoassay with a soluble GBM antigen-binding test. Circulating antibodies were demonstrated in 9 of 38 patients whose sera were tested by indirect IF, 26 of 34 tested by radioimmunoassay, and 31 of 32 tested by hemagglutination. Eluates from whole kidneys or biopsies contained demonstrable GBM antibodies in 15 cases; in 1 patient the eluate was positive in the absence of demonstrable circulating antibodies. No sera were available for testing in 6 of the 45 patients.

Results of pathological examinations. Analyses compiled on 32 of 45 patients with apparent GBM antibody-mediated glomerulonephritis are shown in Table II. Three cases were excluded because of the possibility that morphologic manifestations of diabetes mellitus might distract from definition of uncomplicated GBM disease. Another 10 patients were excluded by reason of insufficient tissue for adequate histopathological review: paraffin blocks were lost from three cases; although tissue, including EM material from several samples, was available from the rest, it was judged that light microscopy did not afford a representative sample of kidney parenchyma.

Pathologic description. Histologically, the lesions in this study could be divided into five broad pathologic groups. Two cases (31 and 32) were examined at the time of nephrectomy before transplantation; morphologic changes were advanced, and only a diagnosis of end-stage kidney could be rendered. They are not considered below.

Normal glomeruli or minor abnormalities (cases 1-6)

LIGHT MICROSCOPY

Glomeruli in all cases appeared normal or showed focal, minimal hypertrophy of mesangial matrix without definite hypercellularity or polymorphonuclear (PMN) exudation. The tubules were well preserved; hyaline degeneration of proximal tubules was prominent in the case with NS (case 2).

EM

Four cases, including the patient with NS, demonstrated minimal, nonspecific changes consisting of: local

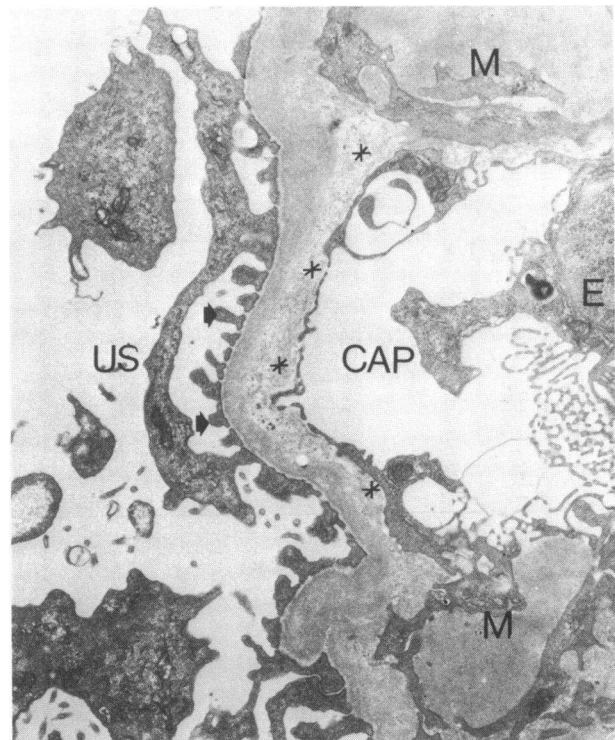


FIGURE 1 EM demonstrating nonspecific alterations in peripheral capillary wall. The most prominent change is widening of the lamina rara interna (marked by asterisks) which has a moth-eaten appearance. The overlying lamina densa appears relatively normal with intact epithelial cell foot processes (arrows). Cap, capillary lumen; E, endothelial cell; M, mesangial region and US, urinary space. × 12,000.

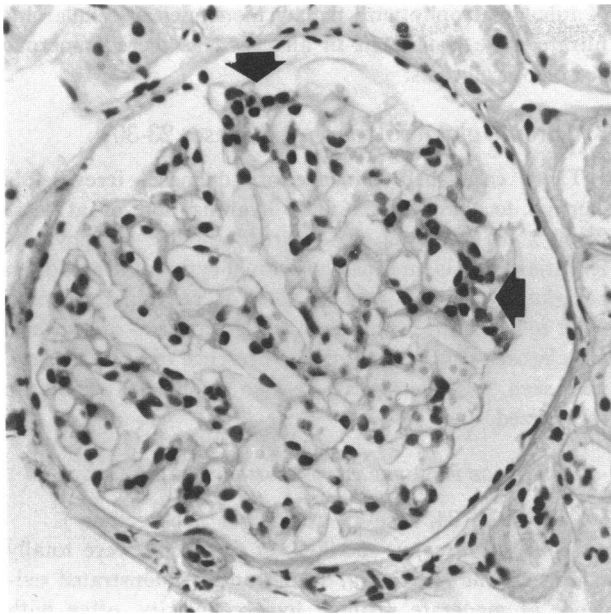


FIGURE 2 Mild segmental proliferative glomerulonephritis. The glomerulus appears normal except for two segmental areas of mild hypercellularity (arrows). H and E. $\times 342$.

fusion of foot processes (extensive only in case 2), hypertrophy of epithelial cells with foci of villous transformation, and focal areas of widening of the lamina rara interna (Fig. 1).

Focal proliferative glomerulonephritis (cases 7-15)

LIGHT MICROSCOPY

Cases demonstrated a mild focal, segmental, proliferative glomerulonephritis (Fig. 2) involving from 20 to 60% of the glomeruli; proliferating cells appeared to be mainly mesangial. The areas of hypercellularity were associated with a mild segmental increase in mesangial matrix without evidence of necrosis, PMN exudation, or crescent formation. In a single case (case eight), 10-20% of glomeruli had segmental mesangial sclerosis, associated with areas of hypercellularity and adhesions to Bowman's capsule without crescents, foam cells, or hyaline deposits (13-16); mild focal periglomerular fibrosis occurred in three cases.

EM

Seven cases (including case 11) were studied; findings were minimal and nonspecific, differing from group 1 only by the presence of focal mesangial hypercellularity and matrix hypertrophy in four of the seven. Virus-like particles were observed in two patients in this group: an extracellular collection of "spherical microparticles"

(17) was seen in the subepithelial region in one biopsy (case 9), while intracellular myxovirus-like particles (18) were seen in several endothelial cells of another (case 10).

Focal necrotizing glomerulonephritis (five cases)

LIGHT MICROSCOPY

Cases of this group had focal proliferative glomerulonephritis with areas of segmental necrosis involving 5-40% of the glomeruli. In all areas of active necrosis there was epithelial cell proliferation with production of cellular crescents of variable size (Fig. 3).

Segmental areas of sclerosis with adhesions to Bowman's capsule were commonly present in glomeruli free of necrotic lesions. Glomeruli showing both segmental necrosis and/or sclerosis often demonstrated periglomerular fibrosis, often associated with prominent periglomerular mononuclear cell infiltrate. There were significant interstitial changes in all cases: focal areas of edema and early fibrosis, variable degrees of tubular atrophy, and patchy mononuclear cell infiltrate. Red blood cell casts, often numerous, were demonstrated in dilated, atrophic tubules of all cases. Variable degrees of hyaline degeneration of proximal tubules occurred in three cases associated with clinical nephrotic syndrome.

The severity of the necrotizing process differed significantly among members of this group.

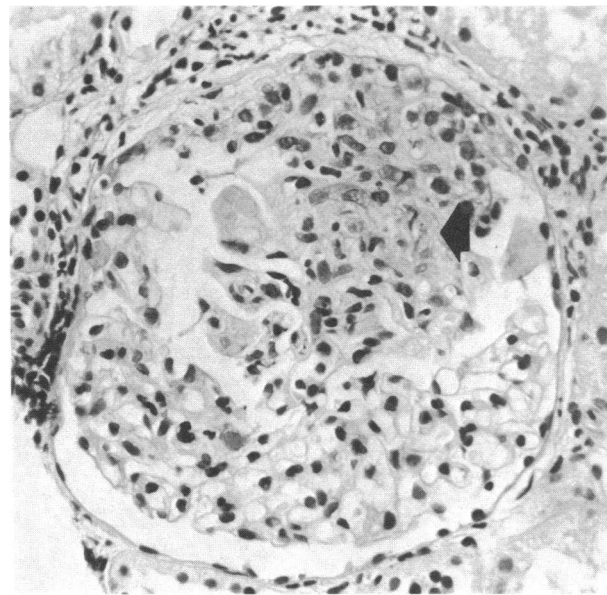


FIGURE 3 Focal necrotizing glomerulonephritis. Note the segmental area of tuft necrosis (arrow) and cellular proliferation associated with a small, cellular, epithelial crescent. H and E. $\times 342$.

Minimal involvement. (Cases 16, 17): 5 to 10% of glomeruli showed segmental necrosis, with small crescents and minimal PMN exudation.

Moderate involvement. (Cases 18-20): 20 to 40% of glomeruli had necrotizing lesions, larger than those in the preceding group, with more cellular epithelial crescents and more PMN exudation. Serial biopsies were available in two cases of this group, including one with GP (case 20).

EM

Adequate EM studies were available only in case 18; the changes in two well-preserved glomeruli were mild and nonspecific, similar to those in groups 1 and 2.

Rapidly progressive glomerulonephritis (Fig. 4) (cases 21, 22)

More than 80% of glomeruli contained large epithelial crescents typical of rapidly progressive glomerulonephritis (19, 20); they varied from cellular to fibrocellular, with fibrin and PMNs often admixed between the proliferating epithelial cells. Atrophic-appearing glomeruli showed focal areas of residual tuft necrosis associated with PMN exudation and nuclear dust, and varying degrees of mesangial matrix hypertrophy with widespread segmental and global sclerosis. Periglomerular fibrosis associated with mononuclear cellular infiltrate, widespread interstitial edema and nearly fibrosis, and areas

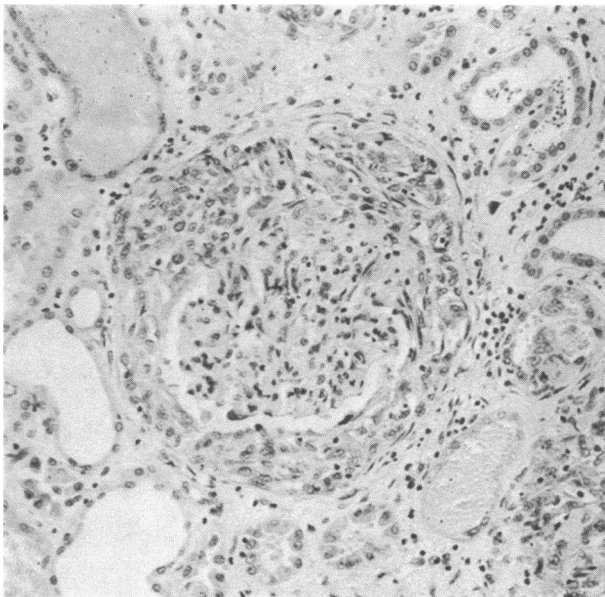


FIGURE 4 Rapidly progressive glomerulonephritis. The central atrophic glomerular tuft is completely surrounded and compressed by the large cellular epithelial crescent. H and E. $\times 180$.

of tubular atrophy with patchy mononuclear cellular infiltrate were prominent in all cases, as were numerous red blood cell casts.

Chronic glomerulonephritis (cases 23-30)

These cases had evidence of advanced, irreversible damage to parenchyma with hyalinization of at least 20-30% of glomeruli. Widespread tubular atrophy, interstitial fibrosis, and patchy lymphocytic infiltrate, often periglomerular, involved 30-80% of renal parenchyma. Mild to moderate changes of benign arterio- and arteriosclerosis, consistent with the presence of hypertension, was seen in several cases. Two subgroups could be recognized.

END-STAGE PROLIFERATIVE GLOMERULONEPHRITIS

(Cases 23, 24, case 19 at time of nephrectomy).

Light microscopy. 50-80% of glomeruli were totally sclerosed, but remaining, viable ones demonstrated evidence of moderate residual hypercellularity, often with small fibrocellular crescents. Cases 23 and 24 still had foci of segmental-necrotizing glomerulonephritis with PMN exudation; PAS staining showed focal thickening of GBM without splitting or significant lobulation.

EM. Studies, available only in case 19, were nonspecific, with focal thickening of the GBM and widening of lamina rara interna. Also noted: hypertrophy with focal villous transformation of epithelial cells and extracellular collections of spherical microparticles in the subepithelial space of several capillary loops.

CHRONIC SCLEROSING (21) GLOMERULONEPHRITIS (CASES 25-30)

Light microscopy. 30 to 80% of glomeruli had global sclerosis without evidence of hypercellularity or crescents, often with prominent periglomerular fibrosis and mononuclear cellular infiltrate. Many of the remaining glomeruli appeared relatively normal while areas of segmental sclerosis were present in all except cases 28 and 29.

EM. Studies were done in three cases, including two of three associated with NS. Viable glomeruli from both latter cases had mild mesangial matrix hypertrophy: small electron-dense deposits limited to the mesangial regions were seen in one case (Fig. 5, case 28). Peripheral GBM in both cases was relatively normal, with extensive fusion of foot processes (50-70% of loop area) and associated hypertrophy of epithelial cells with focal villous change; no peripheral loop deposits were detected. The case without NS had areas of segmental mesangial sclerosis with collagen deposition, focal thickening of the GBM with widening of the lamina rara interna, and no loop deposits.

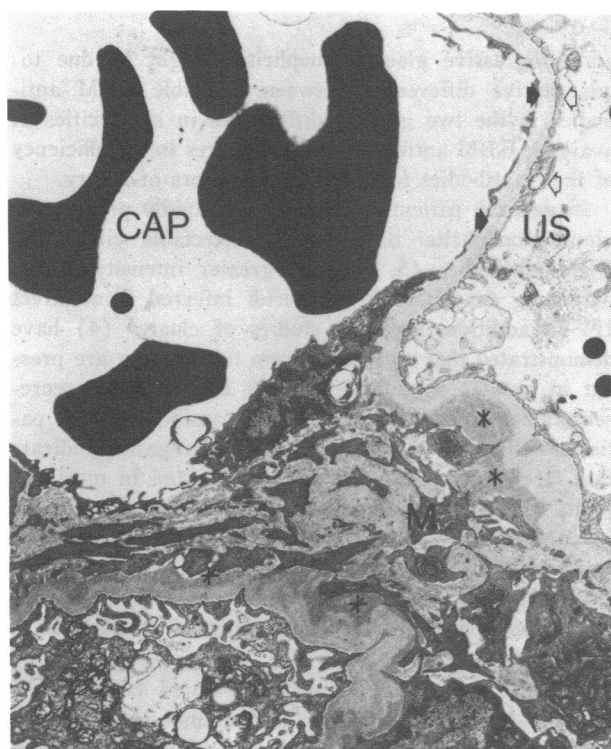


FIGURE 5 EM of electron-dense mesangial deposits. These electron-dense deposits (marked by asterisks) are limited to mesangium (*M*). The peripheral GBM (solid arrows) appears of normal thickness with focal fusion of overlying epithelial foot process (open arrows). *Cap*, capillary lumen. $\times 6,400$.

DISCUSSION

Direct IF tests indicated significant glomerular localization of host immunoglobulins in 71% of all native kidney biopsies examined in this prospective survey. Presumptive evidence suggesting GBM antibody etiology in 16% of Ig-positive biopsies (11% of the entire group) was inferred from diffuse and generalized, linear IgG deposition clearly perceived in biopsies from 43 patients.

Confirmation of GBM antibody mediation was derived from successful elutions of native kidney tissue in 15 (33%) of the patients; all seven of the eluates tested in primates contained kidney-fixing antibodies. An additional 22 patients had circulating GBM antibodies detected by indirect IF assay, hemagglutination, or radioimmunoassay of serum. In all, data were supportive of GBM antibody activity in 37 of the 45 patients (82%); such tests were not attempted in 6 patients.

The greater frequency of antibody detection by radioimmunoassay and hemagglutination techniques is due to their 10- to 40-fold greater sensitivity compared to the indirect IF assay. When heterologous basement membrane antisera were tested in a comparison of hemag-

glutination and radioimmunoassay, they were approximately equivalent in sensitivity. Despite this relative equivalence, circulating antibodies were detected in sera of 4 patients by hemagglutination but not by immunoassay, and in 1 by radioimmunoassay and not by hemagglutination; in 21 patients, antibodies were detected by both.

Biochemical analyses of solubilized human GBM (11, 22) have indicated that it is composed principally of two different proteins, a special form of collagen and glycoprotein. Digestion of GBM with two different enzymes was done to harvest antigens with different determinants for testing. Radioimmunoassay with such soluble GBM antigens eluted from immunoadsorbents indicated that approximately one-third of the patients with circulating GBM antibodies bound only one of the two antigens. Comparison of several sera against a panel of adsorbed antigens confirmed that varying specificities of GBM antibodies occurs, and wider specificity is more typical of sera with greater antigen binding capacity. Eluate binding experiments showed that all eluates tested reacted with pooled C-GBM antigens, whereas fewer than half reacted with T-GBM. These data do not resolve conflicting conclusions of other investigators (23, 24) as to the precise nature of nephritogenic antigen(s), but support the position that binding of these different antigens by sera of patients presumed to have GBM antibody-mediated disease is pathogenetically meaningful.

Histopathological characteristics of the 32 patients reviewed present a wide histological spectrum from virtually normal morphology (group 1) to that of rapidly progressive glomerulonephritis. The minimal changes present in almost 50% of patients in this study are in marked contrast to findings in the great majority of documented human anti-GBM glomerulonephritis cases reported in the literature (25-28) and are compatible with lesser amounts of host Ig fixed to glomeruli and in the sera of these patients. However, occasional cases of documented human anti-GBM glomerulonephritis with mild morphologic changes have been reported (28, 29).

The focal necrotizing glomerulonephritis demonstrated in cases 16-20 occurs in several systemic diseases (30) and is also the typical histological lesion seen early in the course of GP. Cases 19, 23, and 24 demonstrated slowly progressive renal deterioration similar to the course in 4 of 21 cases of non-GP anti-GBM glomerulonephritis reported by Wilson and Dixon (29). Histological examination in these three cases demonstrated evidence of a chronic proliferative glomerulonephritis often with necrosis, especially well documented in serial biopsies in case 19.

The histology in cases 25-30 was that of a chronic sclerosing glomerulonephritis (21) similar to 10 adult

cases with linear Ig deposition and glomerular sclerosis recently reported (31). The finding of prominent global sclerosis without significant hypercellularity at times presents a difficult diagnostic problem with respect to focal sclerosing glomerulopathy of the global variety (13). However, intracapillary foam cells and prominent subendothelial hyaline loop deposits were uniformly absent in our study group (13-16). Three of our 6 cases with chronic sclerosing glomerulonephritis had associated NS and significant fusion of epithelial foot processes, but not to the degree (70% or greater) commonly seen in focal sclerosing glomerulopathy (16).

One biopsy from the group of 16 patients studied by EM showed electron-dense deposits (case 28: mesangial location); these are not explicable by the hypothesized GBM antibody mechanism. Direct IF in this patient showed mesangial deposits of IgM and B₁C in contrast to the smooth localization of IgG to the peripheral GBM. Protein aggregates swept from the GBM or antiglobulin aggregates due to a superimposed or associated autoimmune process (this patient had circulating rheumatoid factor) may be the basis of the observed ultrastructural abnormality.

Although the histopathological material analyzed in this study (32 of the 45 cases) is not complete, nevertheless the case material excluded is comparable to that analyzed in terms of patient ages, clinical problems, and functional severity.

Clinically, GBM antibody-mediated glomerulonephritis documented in this study has several noteworthy facets. Its apparent frequency in the general nephritic population has already been discussed, but its appearance as a relatively constant fraction of patients first diagnosed in any age range (Table I) has not been appreciated generally. The preponderant occurrence in males, a feature documented in GP, is evident clearly in our data, although the biopsy population as a whole is male dominated. No epidemiologic factor was evident in the clinical histories or medical backgrounds of these patients to suggest an etiology or precipitating cause of the immunopathogenetic process.

The course of the glomerulonephritis in these patients has varied greatly. The rapid progression to renal failure observed in 5 patients, including 2 with GP, and slower progression documented in 11 other patients are compatible with published experience in patients proven to have GBM antibody-mediated disease. However, mild disease without progression has not been apparent in other reports; 11 patients, 3 of whom had occasional gross hematuria, had persistent microhematuria documented from 2 to 5 yr before their biopsies.

The indolent character of clinical disease in these latter patients as contrasted with patients having more serious

and progressive glomerulonephritis, might be due to: quantitative differences between available GBM antibodies in the two groups, differences in specificities of available GBM antibodies, or differences in the efficiency of their antibodies for eliciting mediators of injury.

In general, patients with GP and rapidly progressive glomerulonephritis have readily detectable circulating GBM antibodies (5, 32) and greater intensity of apparent Ig deposition in glomeruli inferred from direct IF. In addition, previous studies of eluates (4) have demonstrated that antibodies from GP kidneys are present in greater abundance and fix to a broader representation of renal antigens than do antibodies from patients without GP. Data from this investigation indicate that circulating antibodies can be detected in most patients with GBM antibody-mediated nephritis, but there is more antibody in the circulation of patients with more aggressive syndromes. Moreover, differences exist among these circulating antibodies as regards their antigenic specificities; the broadest range of specificities is expressed in sera from GP.

No substantial differences were noted between the groups as regards presence of immunohistochemically demonstrable B₁C and fibrinogen, and hence, no data suggest that complement activation was faulty or that local coagulation did not occur in hematuric patients at sites in injured glomeruli.

In summary, a prospective study undertaken to define the apparent incidence of GBM antibody-mediated glomerulonephritis suggests an overall incidence of 11%, adjusted to 16% when calculated as a fraction of patients with significant glomerular Ig fixation. Direct IF results were substantiated by elution studies and assays for circulating GBM antibodies. Patients with anti-GBM glomerulonephritis had variable clinical courses as regards clinical signs and functional deterioration. Although our data do not define all factors for the differing severity between patients with GBM antibody-mediated glomerulonephritis, they are compatible with the hypothesis that two major variables are operational: (a) the amount of nephrotoxic antibody and (b) broader or more restricted antigen specificities of the available GBM antibodies.

The overall frequency of GBM antibody nephritis in this study is higher than generally recognized (29); the reason(s) for this is not apparent. It is possible that this prospective investigation, including all patients undergoing renal biopsy irrespective of severity, reflects more accurately the real incidence of GBM antibody disease. Alternatively, this biopsy population of military members and dependents, a highly immunized group, may have a greater incidence of GBM antibody glomerulonephritis.

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