Application of the Solid Phase C1q and Raji Cell Radioimmune Assays for the Detection of Circulating Immune Complexes in Glomerulonephritis

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ABSTRACT The C1q solid phase and Raji cell radioimmune assays were used to determine the frequency of detectable circulating immune complexes in patients with glomerulonephritis. In this study, 46% of 56 patients with glomerulonephritis had evidence of circulating immune complexes. More important, circulating immune complexes were associated with some, but not other, types of glomerulonephritis. Thus, immune complexes were detected in lupus glomerulonephritis (9/9 patients), rapidly progressive glomerulonephritis (5/6 patients), and acute nephritis (5/6 patients), but not in IgA-IgG glomerulonephritis (0/7 patients), or membranous glomerulonephritis (0/8 patients). The Raji cell radioimmune assay and the C1q solid phase radioimmune assay showed concordance of 79% in the detection of circulating immune complexes. Serial determinations, in general, showed either persistence of a negative or positive result or conversion of positive to negative.

INTRODUCTION

Experimental (1–5) and immunofluorescence studies (6–12) strongly support the concept that most glomerulonephritis in man is immune complex mediated. Assays to detect circulating immune complexes would permit objective evaluation of the concept of immune complex-mediated glomerulonephritis, and several such tests have now been reported and recently reviewed (13). Initial success with assays for circulating immune complexes in systemic immune complex diseases, such as systemic lupus erythematosus (14–16), has spurred interest in applying these tests to the study of serum from patients with primary immune complex glomerulonephritis. Immune complexes have been detected in sera from some but not all patients with presumed immune complex glomerulonephritis by the microcomplement consumption test (17, 18), the platelet aggregation assay (19), the radiolabeled C1q binding assay (18, 20–23), the macrophage uptake assay (24), the C1q deviation test (25), the bovine conglutinin assay (26), and the Raji cell radioimmune assay (*IRCA) (18).[1]

The solid phase C1q assay (C1qSPA) (27) and the *IRCA (28) were employed in the present study to provide quantitative information regarding the frequency of detection of circulating immune complexes in very well-characterized individuals with glomerulonephritis. Both assays are sensitive and IgG specific, but their reactivities are different with the *IRCA detecting immune complex-bound complement components via cell surface complement receptors and the C1qSPA detecting immune complexes which can fix exogenous C1q. These different reactivities were exploited by using the assays in combination.

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[1] *Abbreviations used in this paper*: AHG, aggregated human gamma globulin; C1qSPA, solid phase C1q assay; GBM, glomerular basement membrane; *IRCA, Raji cell radioimmunoassay; PBS, phosphate-buffered saline; RPGN, rapidly progressive glomerulonephritis; SLE, systemic lupus erythematosus.

METHODS

Patients

56 patients whose renal biopsies were studied at the University of New Mexico between 1973 and 1977 were selected for this study. 30 patients were male and 26 were female. Their ages ranged from 5 to 75 yr. 46 patients were Caucasian (and of these 14 were Spanish American), 8 were American Indians, 1 was Negro, and 1 was Filipino. The duration of disease at the time of renal biopsy ranged from 2 wk to 13 yr. The patients' medical records provided presenting signs and symptoms of the patients' illness, and laboratory data including urinalysis, serum proteins, serum creatinine, antinuclear antibodies, antistreptolysin 0 titers, and C3 levels.

Renal biopsy

Histologic study of needle or wedge biopsy specimens was done on formalin-fixed, paraffin embedded tissue sections (4 μm thick) stained with hematoxylin and eosin, periodic acid-Schiff, Jones silver methenamine, and Masson's stains. Immunofluorescence study was done on frozen sections of snap frozen tissue after fixation in an ether:95% ethanol mixture and rinsing in phosphate-buffered saline (PBS) (0.01 M sodium phosphate-buffered 0.15 M NaCl, pH 7.2). Fluorescein isothiocyanate-conjugated antisera to human γ-, μ-, α-, C3-, and fibrin-fibrinogen were used (29). The sections were viewed with a Leitz Ortholux II UV microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with the Phloem epi-illuminator. For electron microscopy, small blocks of renal cortex were fixed in glutaraldehyde and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with the Hitachi 11A electron microscope (Hitachi Ltd., Tokyo, Japan).

Serum samples

Blood for immune complex determinations was allowed to clot at room temperature for 2–3 h and was then centrifuged at 400 *g* for 20 min at 20°C. Aliquots of sera were stored and shipped at −70°C. The use of aliquots allowed the assays to be performed on previously unthawed serum samples.

Sera from 36 of the 56 patients were obtained within 2 wk of renal biopsy, and 42 were obtained within 2 mo. In 27 patients, follow-up sera (obtained after an interval of 13 to 583 days) were studied for circulating immune complexes, and a third serum sample was studied in 5 of these patients. Sera from 29 healthy laboratory technicians were obtained as normal controls. The sera were studied under double-blind conditions.

Quantitation of circulating immune complexes

**IRCA.* The Raji cell radioimmune assay was modified from that described by Theofilopoulos et al., (28) by the addition of radiolabeled bovine serum albumin to correct for nonspecific entrapment. The statistical analysis of the test was also changed by daily comparison to a panel of normal sera, and has recently been described in detail (18). Results were expressed as micrograms aggregated human gamma globulin (AHG) equivalent per milliliter serum above the mean±2 SD value of control sera.

The AHG was prepared from human Cohn Fraction II by the method of Mauer et al. (30), which uses an ultracentrifugation step to remove unaggregated gamma globulin. Sucrose density gradient fractionation of ^{125}I-labeled AHG showed the aggregates to be 40–100 S. The AHG was stored in aliquots at −70°C and centrifuged at 10,000 g for 5 min before use (18).

ClqSPA. The method was modified from that described by Hay et al. (27). Clq was isolated from fresh normal human serum by the method of Yonemasu and Stroud (31), except that relative salt concentrations of 0.04 M and 0.078 M were used for the first and second precipitation steps, respectively. The purity of the isolate was confirmed by immunoelectrophoresis with rabbit antisera to whole human serum and to human Clq.

12 × 75-mm polystyrene tubes (Falcon Plastics, Oxnard, Calif.) were coated with freshly isolated Clq by incubation with approximately 4 μg Clq in 1.0 ml PBS for 20 h at 4°C. The tubes were then washed three times with 5.0 ml PBS, incubated with 2.0 ml 1% bovine serum albumin in PBS for 2 h at 25°C, washed a further three times with PBS, and then stored at −70°C until use. (With the above method, 500–1,000 tubes can be coated with the Clq isolated from 100 ml serum.)

The assay was performed as follows: 50 μl test serum was incubated with 100 μl 0.2 M EDTA, pH 7.5, for 30 min at 37°C. 60 μl of the EDTA-treated serum was added to Clq-coated tubes in 1.0 ml PBS. Each test was performed in duplicate. The tubes were incubated for 60 min at 37°C and for 20 h at 4°C, and were then washed three times with PBS. Approximately 2.5 μg ^{125}I-labeled (32) goat anti-human IgG antibody was added in 1.0 ml 1% bovine serum albumin-PBS and incubated for 60 min at 37°C and 30 min at 4°C. The tubes were then washed three times with PBS and radioactivity bound to the tubes was counted in a gamma counter. The result was referred to a standard curve of the binding obtained with AHG in normal human serum and expressed as micrograms AHG equivalent per milliliter serum above the mean±2 SD value of control sera.

Preliminary experiments were performed to evaluate the specificity, sensitivity, and reproducibility of the assay. Thus, the binding obtained with 1–1,000 μg AHG per milliliter normal human serum was determined with fresh or heat-inactivated (56°C for 30 min) Clq-coated tubes and compared with the binding with monomeric IgG (1–1,000 μg 7S human IgG added per milliliter normal human serum). The effect of EDTA treatment of the test serum was assessed by comparing the detection of AHG in fresh normal human serum, heat-inactivated (56°C for 30 min) normal human serum, and 0.13 M EDTA treated normal human serum. The influence of certain nonimmune complex reactants was evaluated by testing normal human serum preincubated with heparin (Riker Laboratories, Northridge, Calif.) 500 U/ml normal human serum, DNA (Worthington Biochemical Corp., Freehold, N. J.) 100 μg/ml normal human serum, or *Escherichia coli* endotoxin (Difco Laboratories, Detroit, Mich.) 20 μg/ml normal human serum. Finally, the stability of Clq-coated tubes with −70°C storage was determined by testing AHG and a panel of six sera on four occasions during a 2-mo period.

Detection of cytotoxic anti-lymphocyte antibody

The patients' sera were tested against a panel of lymphocytes isolated from the peripheral blood of 20 normal individuals. The histocompatibility antigens of the donor lymphocytes were determined to assure that a wide spectrum of antigens was included in the panel. The microcytotoxicity method of Terasaki and McClelland (33) was used with 15°C incubation for 3 h after the addition of rabbit serum as a source of complement. An individual test was considered positive if 20% or more of the lymphocytes

from one donor were killed by a given serum. A serum was considered positive only if it killed 20% or more of the cells from at least 50% of the donors in the panel. Normal human serum and human serum albumin consistently showed no more than 0–10% death of the target lymphocytes.

Diagnostic categories (Table I)

Group I: Systemic lupus erythematosus (SLE) (patients 1–9). Eight of the nine patients were female. The other patient (no. 8), a 61-yr-old man, had received procaine amide for cardiac arrhythmia for 4 mo. All patients presented with two or more systemic findings of SLE (malar rash, pleuritis, arthralgia, hemolytic anemia, or Raynaud's syndrome) and were subsequently found to have hematuria and (or) proteinuria. All had strongly positive serum antinuclear antibody and the serum C3 levels (eight patients) were reduced, ranging from 50 to 100 mg/100 ml (normal range = 123–167 mg/100 ml). Four patients (nos. 1–4) had diffuse proliferative glomerulonephritis with subendothelial electron-dense deposits. Three of these patients also had subepithelial dense deposits and two had mesangial deposits. Immunofluorescence studies (two patients) showed granular deposits of immunoglobulins (Ig) and C3 along glomerular capillary loops. Of the other five patients, four (nos. 5–8) had mesangial proliferative glomerulonephritis with mesangial dense deposits and mesangial deposition of Ig and C3 by immunofluorescence, and one (no. 9) had only minimal glomerular changes with no deposits by electron microscopy or immunofluorescence.

Group II: Antiglomerular basement membrane (GBM) antibody glomerulonephritis (patients 10–12). All patients were teenage females with circulating antiGBM antibody detectable by radioimmunoassay (13, 34). The assay employed a radiolabeled extract of collagenase solubilized human GBM. A double antibody radioimmunoassay was utilized with specific binding >1% considered positive. Two of these patients had linear IgG and C3 deposition along the GBM; one (no. 10) developed severe hemoptysis which was controlled by lung bypass, and the other (no. 11) developed pulmonary infiltrates. The immunofluorescence findings in the third patient were indeterminant because of severe glomerular damage. These patients did not have systemic infections and were not on hemodialysis at the time of the initial study for circulating immune complexes.

Group III: Rapidly progressive glomerulonephritis (RPGN) (patients 13–18). RPGN was defined as glomerulonephritis in patients with rapid deterioration of renal function, negative serum antinuclear antibody, negative antibody to GBM, and the finding of epithelial crescents in over 50% of the renal glomeruli. Of the patients (four males and two females in this group) two (nos. 13 and 14) had no extrarenal manifestations, two (nos. 15 and 16) had skin rash and arthralgia, one (no. 17) had evidence of recent β-hemolytic streptococcal pharyngitis, and one (no. 18) had sinusitis and vasculitis. Diagnoses of Henoch-Schönlein purpura, poststreptococcal glomerulonephritis, and Wegener's granulomatosis, respectively, were considered in the last four patients but could not be firmly established. Four of the six patients in this group had glomerular deposits by electron microscopy and (or) immunofluorescence consistent with immune complexes.

Group IV: Acute nephritis (patients 19–24). Of the patients in this group (four males and two females), four (nos. 19–22) had typical acute poststreptococcal glomerulonephritis with proven β-hemolytic streptococcal pharyngitis or skin infection, hypertension, hematuria and erythrocyte casts, decreased serum C3 levels, increasing or high antistreptolysin 0 titer, proliferative glomerular changes and granular deposits of Ig and (or) C3, and they recovered with conservative management. The other two patients (nos. 23 and 24) had acute nephritis with diffuse proliferative glomerular changes and granular deposits of C3.

Group V: IgA-IgG disease (patients 25–31). All patients (four males and three females) presented with hematuria. Serum creatinine levels of six patients were below 1.3 mg/100 ml, serum antinuclear antibodies were negative, and serum C3 levels (five patients) were normal in three and slightly reduced in two. Renal biopsy in all patients showed mesangial proliferative changes, intense mesangial deposition of IgA, IgG, and C3, and mesangial electron-dense deposits.

Group VI: Membranous glomerulonephritis (patients 32–39). All patients (six males and two females) presented with heavy proteinuria (range 5–11 g/24 h). Typical subepithelial electron-dense deposits and closely opposed granular IgG along the capillary loops were present in all renal biopsies studied. One patient (no. 37) was in the third trimester of her pregnancy, and two patients (nos. 38 and 39) had renal vein thrombosis.

Group VII: Other glomerulonephritis (patients 40–48). In this group were three patients (nos. 40–42) with the nephrotic syndrome and diffuse proliferative glomerulonephritis. All three patients had granular deposits of Ig and C3 and electron-dense deposits consistent with glomerular immune complexes. One patient (no. 43) had chronic glomerulonephritis and hypertension with mesangial deposits of IgA alone. One (no. 44) had proteinuria, minimal mesangial proliferative changes, and scanty deposits of IgG. The other four patients in this group lacked immunofluorescent or electron microscopic evidence of glomerular deposits. One of these (no. 45) was a patient with recurrent hematuria and previous poststreptococcal glomerulonephritis, and three (nos. 46–48) had proteinuria with nil or minimal glomerular lesions.

Group VIII: Miscellaneous (patients 49–56). This group contains three patients with electron microscopic and (or) immunofluorescent evidence consistent with glomerular immune complexes; patient 49 with Henoch-Schönlein purpura, patient 50 with acute renal failure of uncertain etiology, and patient 51 with diabetes mellitus, Kimmelstiel-Wilson disease, and membranoproliferative glomerulonephritis. Five patients without evidence of glomerular immune complexes were also in this group; patients 52 and 53 with monoclonal gammopathy, patient 54 with amyloidosis, patient 55 with medullary cystic disease, and patient 56 with flank pain, hematuria, and possible renal infarction. The glomerular immunofluorescent deposits seen in patients 52–54 were considered atypical for immune complexes. Crystalloid material was detected by electron microscopy in patient 52 with monoclonal gammopathy and a cryoglobulin (IgG, κ) was demonstrated in the glomerular deposits with an anti-idiotype antiserum (35, Case 1). Electron microscopy in patient 54 showed characteristic amyloid fibrils in the deposits.

RESULTS

In vitro evaluation of the immune complex assays

**IRCA.* These studies have already been reported (18, 28) and are summarized here for comparison with results from similar studies with the C1qSPA. The assay detects as little as 5 μg AHG/ml serum, is not influenced by heparin, DNA, or endotoxin, and appears to be little affected by excess monomeric IgG or by

TABLE I

Morphologic, Immunopathologic, and Clinical Features in Relationship to the Levels of Circulating Immune Complexes (IC)

Patient	Age	Sex	Histologic diagnosis	Additional clinical features	Duration of renal disease at biopsy	IC in glomeruli by IF: Ig	IC in glomeruli by IF: C3	Glomerular dense deposits by EM	Serum C3	Circulating IC: IRCA*	Circulating IC: ClqSPA	Days after biopsy
					mo				*mg/100 ml*	*μg AHG eq/ml*		
Group I: SLE												
1	36	F	Diffuse proliferative GN		2	3*	2*	En M	55	335 a	56	(0)
										42	11	(438)
2	20	F	Diffuse proliferative GN		4	ND		En Ep	ND	379 a	871	(0)
3	20	F	Diffuse proliferative GN		132	2	1	En Ep	85	71	221	(14)
4	58	F	Diffuse proliferative GN		3	ND		En Ep M	50	93 a	51	(0)
5	12	F	Mesangial proliferative GN		36	2	2	M	70	164	191	(1)
6	33	F	Mesangial proliferative GN		120	1	1	M	73	3 b	Neg	(0)
										11	0	(304)
										0	0	(347)
7	31	F	Mesangial proliferative GN		3	2	2	M	92	10 b	61	(56)
										40	21	(615)
8	61	M	Mesangial proliferative GN	Procaine amide	.5	1	2	M	73	40 a	0	(11)
										0	21	(159)
9	35	F	Minimal change	Diabetes mellitus	2	0	0	None	100	5	0	(1)
Group II: AntiGBM antibody GN												
10	17	F	Crescentic GN	Severe hemoptysis	3	3(lin)	2	None	165	41 b	0	(43)
11	16	F	Crescentic GN	Pulmonary infiltrates	8	2(lin)	2	None	165	36	0	(0)
										1	0	(583)
12	20	F	Crescentic GN		3	1(ind)	0	ND	56	0 a	0	(0)
										0	0	(395)
Group III: RPGN												
13	60	F	Crescentic GN		3	1	0	M	130	38 b	4	(1)
										0	0	(424)
14	15	M	Crescentic GN		1	3	3	M	55	0 b	19	(0)
										0	0	(19)
										0	0	(144)
15	55	M	Crescentic GN	Rash arthralgia	3	1	1	None	ND	0 b	0	(136)
										0	0	(149)
										0	0	(245)
16	54	F	Crescentic GN	Rash arthralgia	6	3	1	M	80	13 b	0	(166)
										47	0	(702)
17	52	M	Crescentic GN	PSGN	6	0	0	None	83	110 b	19	(0)

TABLE I (*Continued*)

Patient	Age	Sex	Histologic diagnosis	Additional clinical features	Duration of renal disease at biopsy	IC in glomeruli by IF: Ig	C3	Glomerular dense deposits by EM	Serum C3	Circulating IC: IRCA*		ClqSPA	Days after biopsy
					mo				*mg/ 100 ml*	*µg AHG eq/ml*			
18	27	M	Crescentic GN	Sinusitis vasculitis	1	ND		None	160	45	a	81	(1094)
Group IV: Acute nephritis													
19	49	M	Mesangial proliferative GN	PSGN	1.5	3	1	M	115	72	b	4	(702)
20	29	F	Diffuse proliferative GN	PSGN	1	1	1	En Ep	90	17	b	1	(869)
										0		0	(1090)
21	24	M	Diffuse proliferative GN	PSGN	6	3	1	M	ND	3	b	4	(32)
22	44	M	Diffuse proliferative GN	PSGN	1	0	2	Ep M	18	18	a	56	(5)
23	5	M	Diffuse proliferative GN		.5	0	2	None	64	0	b	0	(0)
24	9	F	Diffuse proliferative GN	Interstitial nephritis	2.5	0	1	None	ND	0	b	1	(0)
										0		16	(188)
										0		0	(263)
Group V: IgA-IgG disease													
25	5	M	Mesangial proliferative GN		1	3	1	M En Ep	150	0		0	(44)
26	33	M	Mesangial proliferative GN		18	3	1	M	ND	0	b	0	(0)
27	31	F	Mesangial proliferative GN		1	2	1	M	162	0	b	0	(395)
										0		0	(910)
28	18	F	Mesangial proliferative GN		6	3	2	M	110	0	b	0	(0)
29	26	F	Mesangial proliferative GN		9	3	2	M	ND	0		0	(0)
30	28	M	Mesangial proliferative GN		—	3	1	M	167	0	b	0	(498)
31	53	M	Mesangial proliferative GN	Cirrhosis	.5	3	2	M	110	0	b	0	(0)
Group VI: Membranous GN													
32	43	M	Membranous GN		84	2	0	ND	160	0	b	0	(555)
										0		0	(1060)
33	24	M	Membranous GN		24	3	1	Ep	120	0	b	0	(20)
										0		16	(550)
34	23	M	Membranous GN		3	2	2	Ep	114	0	b	0	(0)
										0		0	(540)

TABLE I (*Continued*)

Patient	Age	Sex	Histologic diagnosis	Additional clinical features	Duration of renal disease at biopsy	IC in glomeruli by IF		Glomerular dense deposits by EM	Serum C3	Circulating IC		Days after biopsy
						Ig	C3			IRCA*	ClqSPA	
					mo				*mg/ 100 ml*	*μg AHG eq/ml*		
35	70	M	Membranous GN		16	2	0	Ep	80	0 b 0	0 0	(0) (46)
36	30	F	Membranous GN		3	ND		Ep	ND	0	0	(366)
37	17	F	Membranous GN	Pregnant	4	3	1	Ep	ND	0	0	(0)
38	63	M	Membranous GN	Recent renal vein thrombosis	15	3	1	Ep	110	0 b 0	0 0	(0) (160)
39	55	M	Membranous GN	Renal vein thrombosis	6	3	1	Ep	ND	0	0	(5)
Group VII: Other GN												
40	31	F	Diffuse proliferative GN	Nephrotic	33	2	3	En	50	0 b 0	0 0	(516) (1019)
41	30	M	Diffuse proliferative GN	Nephrotic	3	1	2	En Ep M	130	0 b 0	0 0	(98) (584)
42	28	F	Diffuse proliferative GN	Nephrotic	.5	.5	.5	En Ep	ND	0 b 0	16 0	(13) (534)
43	27	F	Chronic GN Arteriosclerosis	Hypertension	1	3	0	ND	95	10 b	0	(0)
44	32	M	Minimal mesangial proliferation	Proteinuria	156	.5	0	None	108	0 b	0	(0)
45	17	M	Minimal change	Hematuria Previous PSGN	7	0	0	None	ND	0 a	0	(1)
46	56	M	Nil disease	Proteinuria	1	0	0	None (Foot process fusion)	ND	0 b	0	(0)
47	62	M	Nil disease	+ANA Proteinuria	3	0	0	ND	119	0 b 0 0	16 0 0	(0) (459) (491)
48	53	M	Minimal membranous thickening	Discoid lupus	—	0	0	None	127	0 b 0	0 0	(0) (421)
Group VIII: Miscellaneous												
49	51	M	Mesangial proliferative GN	HSP	.5	1	0	En	120	0	0	(433)
50	68	M	Minimal histologic change	Acute renal failure	2	.5	0	None	ND	86 b	0	(0)

TABLE I (*Continued*)

Patient	Age	Sex	Histologic diagnosis	Additional clinical features	Duration of renal disease at biopsy	IC in glomeruli by IF: Ig	IC in glomeruli by IF: C3	Glomerular dense deposits by EM	Serum C3	Circulating IC: IRCA*	Circulating IC: ClqSPA	Days after biopsy
					mo				*mg/ 100 ml*	*μg AHG eq/ml*		
51	75	M	K-W disease MPGN	Diabetes mellitus	.5	3	2	En Ep M	ND	0 b	101	(0)
										31	11	(95)
52	47	M	Lobular MPGN	Monoclonal IgG	3	atypical 1	3	Ep Crystalloid material	125	0 b	0	(680)
53	50	F	Lobular MPGN	Monoclonal IgG	—	atypical 1	0	None	ND	0 a	0	(3)
54	61	M	Amyloidosis		10	atypical 1	1	Amyloid fibrils	ND	0 b	0	(54)
										0	0	(104)
55	26	F	Medullary cystic disease	Familial	—	0	0	None	ND	0 b	0	(755)
										0	0	(1305)
56	32	F	Interstitial fibrosis Arteriosclerosis	Flank pain Hematuria	24	0	0	None	ND	0 a	0	(0)

Abbreviations used in this table: lin, linear; ind, indeterminate; En, subendothelial; Ep, subepithelial; M, mesangial; a, +ALA; b, −ALA; ND, not done.

* Refers to greatest quantity of Ig (IgG, IgA, or IgM) and C3 graded on a scale from 1 to 4. Granular deposits unless otherwise noted.

the storage and handling conditions of the test sera. It is possible that serum antilymphocyte antibody (36) might influence the assay but this does not appear to be a major limitation under the test conditions (18).

ClqSPA. The binding of ^{125}I-goat anti-human IgG antibody to Clq-coated tubes incubated with AHG or monomeric IgG in EDTA-treated normal human serum is shown in Fig. 1. Increased binding of the ^{125}I-anti-human IgG antibody was observed with as little as 5 to 10 μg AHG/ml normal human serum and was maximal (12.5% binding) with 4,000 μg/ml. The mean binding (±1 SD) for the control sera was 2.9±0.6%. Binding was not affected by monomeric IgG. The increased binding with AHG was prevented when heat-activated Clq tubes were used. Binding of AHG incubated in fresh normal human serum was up to 35% less than with AHG in heat-activated or EDTA-treated normal human serum. The reduced sensitivity with fresh serum may be due to saturation of AHG Clq binding sites by serum Clq, or to masking of solid phase Clq sites by the formation of Clq complex. EDTA treatment of test sera was subsequently adopted; this approach (37) obviates the problems associated with heat inactivation (specifically heat-induced aggregation of serum gamma globulin and destruction of thermolabile immune complex determinants), and the sensitivity of the modified assay (AHG + normal human serum in 0.13 M EDTA) is 5–10 μg AHG/ml. This assay was performed in conjunction with the *IRCA and, since the same AHG standards were used, quantitation of the two assays could be compared directly.

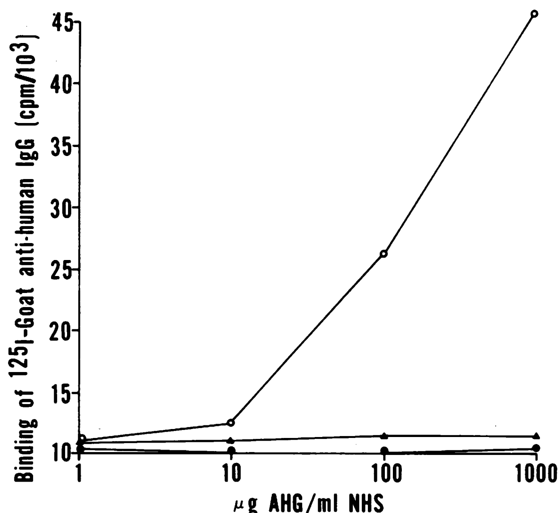

FIGURE 1 ClqSPA. Binding of AHG (or 7S IgG) in NHS. ○——○, binding of AHG; ●——●, binding of 7S IgG; ▲——▲, binding of AHG to heat-inactivated Clq-coated tubes.

The assay was not affected by the in vitro addition of heparin, DNA (native or denatured), or *E. coli* endotoxin to normal human serum. These substances bind C1q and can produce false positive results in the C1q deviation test (38) and the radiolabeled C1q binding assay (18). The IgG specificity of the C1qSPA is a major advantage in this regard. Unlike the microcomplement consumption test (18, 39), the C1qSPA appears to be little affected by suboptimal storage and handling conditions of test sera; no alteration in binding was detected after repeated (×10) freeze-thawing of four normal human sera. C1q-coated tubes were stable with storage at −70°C for at least 2 mo. With 1 mg of the standard AHG the percent binding was 11.3% initially and 10.5% after 2-mo storage. During this period, the same six sera were tested on four different occasions. The results of the four determinations were very reproducible, with the mean (±1 SD) binding (%) of the four runs on the six individual sera being 3.5±0.1, 2.6±0.3, 4.7±0.2, 2.0±0.1, 2.2±0.3, and 3.0±0.2.

*Comparison between the *IRCA and the C1qSPA.* Of the 119 sera obtained from patients with glomerulonephritis and from normal adults (Fig. 2), 94 (79%) were found to be either positive in 15 or negative in 79 with both assays. The correlation coefficient (r) of the two assays was 0.68 (based on the chi square and the 2 × 2 contingency table analysis).

Studies on patients with glomerulonephritis

Incidence of circulating immune complexes (Table I). The overall frequency of detecting circulating immune complexes in initial serum samples from the 56 patients was 46% as determined by both assays. The *IRCA was more frequently positive (38%) than the C1qSPA (32%). In comparison, the *IRCA was positive in 3 and the C1qSPA in none of the 29 control sera.

All nine patients with SLE were positive in one or both assays. The patients with diffuse proliferative lesions and widespread glomerular immune complex deposits (nos. 1–4) had higher levels of circulating immune complexes than patients with immune complex deposits confined to the mesangium (nos. 5–8). Patient 9 without glomerular immune complex deposits was negative in the C1qSPA and only weakly positive in the *IRCA. Follow-up serum immune complex determinations in four patients remained positive in three. The fourth patient (no. 6) had two sera which were weakly positive in the *IRCA, and a third sample which was negative.

Two of the three patients with antiGBM antibody glomerulonephritis were positive in the *IRCA. This is an unusually high frequency of positivity since in previous studies with antiGBM antibody glomerulonephritis, none of 10 (18), and 2 of 26 sera[2] were

[2] Wilson, C. B. Unpublished observations.

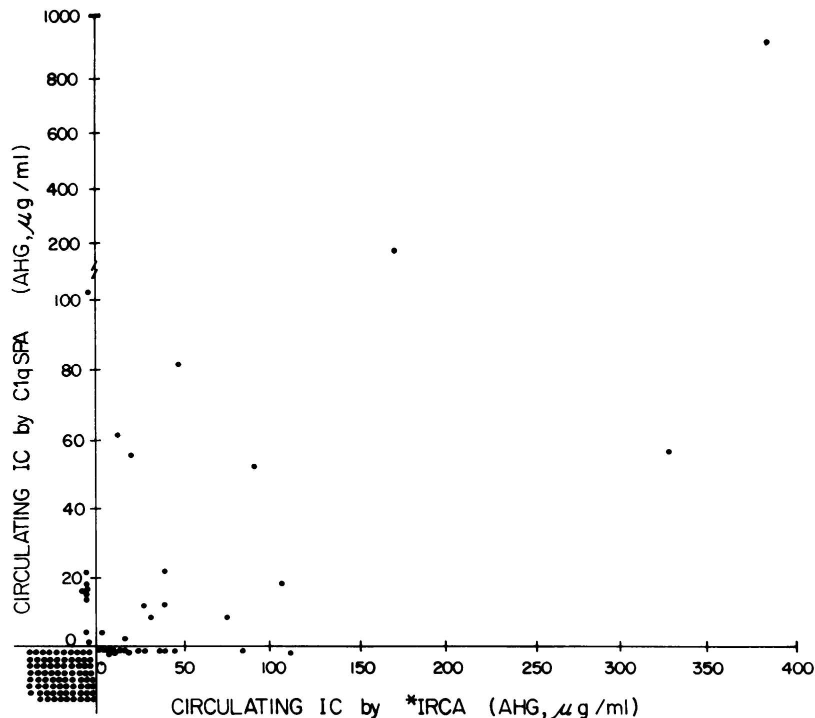

FIGURE 2 Correlation between C1qSPA and *IRCA based on 119 serum samples. Results of both studies are expressed as micrograms per milliliter of AHG equivalent above the normal range. The two techniques show a correlation (r) of 0.68.

positive in the *IRCA. The detection of circulating immune complexes in an occasional patient with anti-GBM antibody disease is perhaps not unexpected. GBM antigens have been demonstrated in serum (40), and it is conceivable that circulating GBM-antiGBM immune complexes might be present in the sera of patients with circulating antiGBM antibody.

Five of the six patients withRPGN had detectable circulating immune complexes. These included the two patients (nos. 17 and 18) lacking documented glomerular immune complexes. In general, the amounts of glomerular and circulating immune complexes were less than in the SLE group. Increased reactivity was still detected late in the course of RPGN and one patient (no. 18) was positive 3 yr after biopsy. Follow-up serum immune complex determinations (four patients) converted from positive to negative in two (nos. 13 and 14), remained negative in one (no. 15), and positive in one (no. 16).

Similarly, five of six patients with acute nephritis were positive. These include all four patients with acute poststreptococcal glomerulonephritis, in spite of delayed sampling (over 2 yr) in two. Repeat immune complex determinations in two patients (nos. 20 and 21) showed conversion from positive to negative.

Patients with IgA-IgG disease or membranous glomerulonephritis were free of circulating immune complexes. Follow-up sera were tested in six such patients and remained negative in five. The absence of circulating immune complexes in membranous glomerulonephritis has been noted previously (18) but is still unexplained, whereas the failure to detect circulating immune complexes in IgA-IgG disease must be qualified since both the *IRCA and the C1qSPA have IgG specific end points. Since all these patients had IgG deposits in addition to IgA in their glomeruli, this explanation seems unlikely.

Of the remaining patients (Groups VII and VIII), no. 42 with the nephrotic syndrome and diffuse proliferative glomerulonephritis was positive in the C1qSPA, no. 43 with chronic glomerulonephritis, hypertension, and IgA deposits was positive in the *IRCA, no. 47 with nil disease or minimal change glomerulonephritis was positive in the C1qSPA, no. 50 with acute renal failure and minimal glomerular lesions was positive in the *IRCA, and no. 51 with diabetes mellitus and glomerular immune complexes was positive in the C1qSPA. Circulating immune complexes were not detected in patients 45, 46, 48, and 52–56, who lacked glomerular immune complexes.

Attempts were made to correlate the results of the immune complex studies with the ultrastructural localization of electron-dense deposits within the glomerulus. As a rule, circulating immune complexes were absent when immune complexes were exclusively subepithelial. However, this observation is based on findings in idiopathic membranous glomerulonephritis, and it would be important to study the prevalence of circulating immune complexes in patients with the membranous form of SLE nephritis before any conclusion is drawn. Mesangial immune complexes may or may not be associated with detectable circulating immune complexes. Thus, seven patients with IgA-IgG disease were negative for circulating immune complexes, whereas three patients with acute poststreptococcal glomerulonephritis, four patients with SLE, and three patients with RPGN, whose glomerular immune complexes were exclusively mesangial in location, were positive. These results indicate that the detection of circulating immune complexes correlates better with the type of glomerulonephritis than with the sites of glomerular immune complexes.

Correlation between disease activity and immune complex detection. Serial immune complex determinations (Table I) in general showed either a persistence of a negative or positive result or conversion of positive to negative. However, the number of patients was too small to permit any conclusion regarding the correlation between clinical activity and circulating immune complexes. A relationship between the level of serum C3 and the positivity of the assays for immune complexes appeared to exist (Table I). Of the 27 patients with low C3 levels, 52 and 41% were positive in the *IRCA and the C1qSPA, respectively, compared to 36 and 18% for their normocomplementemic counterparts. In the 17 most severely hypocomplementemic patients (C3 < 100 mg/100 ml), 70 and 53% were positive in the *IRCA and C1qSPA, respectively. When the frequency of positive reactions in this group were compared to that in patients with C3 levels > 100 mg/100 ml, the difference was significant ($P < 0.05$).

Prevalence of antilymphocyte antibody in glomerulonephritis patients. This study was included to determine whether antilymphocyte antibody might account for positivity in the *IRCA. Antilymphocyte antibodies were detected in 10 of the 46 patients tested (22%). The results indicate that antilymphocyte antibodies probably did not contribute significantly to *IRCA positivity since, with the exception of SLE, circulating immune complexes occurred as frequently in the absence as in the presence of antilymphocyte antibodies. In addition, three of the four SLE patients with antilymphocyte antibodies and high levels of circulating immune complexes by *IRCA were also strongly positive in the C1qSPA.

DISCUSSION

Studies dealing with the detection of circulating immune complexes in glomerulonephritis are influenced by the type of immune complex assay and by patient selection. In the present study, the *IRCA

and the C1qSPA with a sensitivity of 5–10 μg AHG/ml serum were used to test sera from patients with a spectrum of carefully documented forms of glomerulonephritis. It should be noted that the immune complex assays used in this study only detect complexes capable of reacting with complement. Disparity might develop between detection of complexes in the circulation and in renal tissue, because complement fixation may not be prerequisite for deposition of the latter. The assays also preferentially react with large complexes while experimental evidence suggests that complexes of near 19 S sedimentation characteristically may be most nephritogenic (41).

Circulating immune complexes were readily detectable in SLE but were present less frequently and in smaller amounts in primary glomerulonephritis. When this latter group was analyzed further, a striking relationship between the type of glomerulonephritis and the presence of detectable circulating immune complexes was apparent. Immune complexes were detected frequently in acute nephritis, but not in membranous glomerulonephritis or IgA-IgG glomerulonephritis. Immune complexes were also found in most of the patients with RPGN; however, the frequency of circulating immune complexes in this group should not be generalized from this small sample. We have, for example, found other groups of patients with RPGN to be generally free of detectable circulating immune complexes (18).

Quantitative differences in the load of circulating immune complexes might also contribute to differences in the frequency of detection of circulating immune complexes in different forms of presumed immune complex-induced glomerulonephritis. Thus, large amounts of circulating immune complexes are present in systemic (SLE) and acute or fulminant glomerulonephritis. It is possible that the more indolent forms of glomerulonephritis (membranous glomerulonephritis, IgA-IgG glomerulonephritis) are mediated by much smaller quantities of circulating immune complexes and that the limited sensitivity of the assays precludes their detection. In addition, such patients may have inherent abnormalities causing them to handle low or even physiologic amounts of circulating immune complexes in a nephritogenic manner. Factors such as variable or impaired phagocytic function or relative deficiencies in the immune response might be involved.

Alternatively, circulating immune complexes may be present only intermittently or with changing composition in some forms of glomerulonephritis. The detection of circulating immune complexes would then be influenced by the timing of such determinations. For example, circulating immune complexes are found in the acute phase of poststreptococcal glomerulonephritis and might only be found during exacerbations in IgA-IgG glomerulonephritis. Glomerular immune complex deposits are probably more persistent and could therefore be present in the absence of randomly detectable circulating immune complexes.

As noted earlier, the size and composition of circulating immune complexes may influence both their detection and their size of localization within the glomerulus. Most immune complex assays detect large immune complexes more readily than small ones (42), and it has been suggested that small immune complexes are responsible for membranous glomerulonephritis (4). This concept may explain the absence of detectable immune complexes in membranous glomerulonephritis. With the exception of IgA-IgG glomerulonephritis, circulating immune complexes were detected in patients with mesangial deposits which are thought by some to be a result of large immune complexes.

In the chronic serum sickness experimental model of immune complex glomerulonephritis it is clear that the glomerular immune complex deposits are in dynamic equilibrium with immune complexes in the circulation, and that once initiated the disease can be perpetuated by "layering" of free (nonimmune complex) antigen or antibody on to existing binding sites in the glomerular immune complex deposits (13). More extensive studies with serial immune complex determinations may identify a similar process in human glomerulonephritis. Finally, it is possible that the glomerular immune deposits in some forms of glomerulonephritis in fact represent in situ formed immune complexes. Mauer et al. (43) have described in situ formation of mesangial immune complexes in rats given AHG followed by antibody. Similarly, Izui et al. (44) postulated in situ formation of glomerular DNA-antiDNA immune complexes in mice; in this model, endogenous DNA released into the circulation after administration of bacterial lipopolysaccharide binds to the GBM, where it is readily accessible to antibody. This Arthus-like phenomenon has also been described in experimental thyroiditis (45), tubulointerstitial nephritis (46), and postvasectomy orchitis in the rabbit (47, 48). The participation of nonGBM glomerular antigens in glomerulonephritis has been proposed by Van Damme et al. (49), who have reported the presence of fixed glomerular antigens in a rat model of heterologous immune complex glomerulonephritis. This disease is induced by a single injection of rabbit antiserum to a rat renal tubule preparation and fixation of this antibody to a component of the subepithelial aspect of the GBM could be demonstrated in vitro after treatment of normal rat kidney sections with neuraminidase and acid buffers. Similar mechanisms could conceivably be involved in certain forms of human glomerulonephritis. In these situations, glomerular immune complexes would be present in the absence of circulating immune complexes.

It is concluded that circulating immune complexes

are present in at least a proportion of patients with glomerulonephritis. The finding of immune complexes in patients with some but not other forms of glomerulonephritis indicates that serial immune complex determinations may prove to be of diagnostic and prognostic value clinically. However, it is clear that as yet these serologic studies cannot replace renal immunofluorescence in studies to establish the immunopathogenesis of immune complex glomerulonephritis. The techniques do provide the opportunity to study the composition of immune complexes and can be used in conjunction with renal biopsy studies in the identification of antigens in immune complexes.

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