Acute poststreptococcal glomerulonephritis in Maracaibo. II: Studies on the incidence, nature, and significance of circulating anti-immunoglobulins

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SUMMARY Antiglobulins were measured in the sera of 82 patients with acute poststreptococcal glomerulonephritis and correlated with C1q binding activity, serum levels of immunoglobulins and C3, site of infection, antibody titres to streptococcal enzymes, and the duration of the nephritis and interval from infection. Raised serum antiglobulins were found in 89.2% of the patients. In addition, raised titres were detected at any time during the course of the disease and very early after streptococcal infection. The finding of antiglobulins soon after the infection suggests the possibility that they appear as a primary event in the disease.

Several mechanisms have been proposed for the immune renal injury following infection with certain strains of group A β haemolytic streptococcus (Zabriskie *et al.*, 1973). Even though many morphological, clinical, and serological features strongly suggest that acute poststreptococcal glomerulonephritis (APSGN) is an immune complex disease, the precise nature of the antigen-antibody system(s) operating in the disease remains undefined (Michael *et al.*, 1966; Lange and Treser, 1973; Zabriskie *et al.*, 1973). The evidence suggest that antibody reacting with streptococcal antigen(s) in the circulation or locally in the glomerulus incites renal inflammatory disease (Michael *et al.*, 1966; Lange and Treser, 1973; McCluskey and Klassen, 1973; Zabriskie *et al.*, 1973).

From a series of clinical and laboratory observations in man and experimental animals we proposed that autologous immune complex systems may be of primary or secondary immunopathogenic significance in renal disease related to streptococcal infection (McIntosh *et al.*, 1970; McIntosh *et al.*, 1971a, 1971b; McIntosh *et al.*, 1972; McIntosh *et al.*,

1975; Griswold *et al.*, 1975). In this postulated mechanism, immunoglobulins are rendered auto-immunogenic by modification or interaction with streptococcal products or by incorporation into an immune complex. Antibodies formed to this neoautoimmunogen combine with the neoantigen forming pathogenic immune complexes. This antigen-antibody interaction may occur in the circulation or in situ in the renal glomerulus.

Patients and methods

Studies were performed on 82 patients admitted to the Hospital Universitario of Maracaibo, Venezuela, for evaluation of acute glomerulonephritis. The age of the patients ranged between 2 and 37 years; 38% were less than 7 years of age, 50% between 7 and 15 years, and 12% older than 15 years. All patients presented with an acute nephritic syndrome (acute onset of haematuria, urinary red blood cell casts, hypertension, oedema) without concurrent systemic disease. There was evidence of antecedent streptococcal infection in all patients. The renal disease was preceded by skin infection in 45 patients, pharyngeal in 20, and both skin and pharyngeal in 5. The site of infection was unknown in 12 patients. The approximate time of onset of clinical mani-

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festations of infection was known in 64 patients. Serum antibody titres to 1 or more streptococcal enzymes were raised in all but 1 patient.

Careful histories, serial complete physical examinations, assessment of clinical status and renal function, and routine serology were obtained in all patients. In addition, serum was obtained for special studies.

SERUM STUDIES

A single serum sample was obtained from each patient during the course of admission to hospital for the special immunological studies. The sample was obtained as early as the day of onset of the first sign or symptom of renal disease and up to 30 days after the onset of clinical evidence of nephritis. The interval between the reported date of the first signs or symptoms of infection and the obtaining of serum samples for special study varied between 2 and 8 weeks.

The serum sample from each patient was immediately frozen and stored at -70°C prior to testing. Levels of immunoglobulin G, M, and A, as well as C3 were quantitated by radial immunodiffusion. Titres of antistreptolysin O (ASO), anti-hyaluronidase (AH), and streptozyme were determined. Immune complexes were measured by the C1q binding assay of Zubler *et al.* (1976) using ^{125}I C1q and polyethylene glycol. The method of C1q determination and the C1q binding activity in these patients are detailed in another communication (Rodriguez-Iturbe *et al.*, 1978). C1q was isolated from normal serum radiolabelled with ^{125}I and reacted with the test sera. Separation of free from complex bound ^{125}I C1q was achieved by selective precipitation with polyethylene glycol. C1q binding activity (C1qBA) was calculated as percent of ^{125}I C1q precipitated. All samples were tested in duplicate. Serum samples from apparently normal healthy individuals were included on each test run.

The procedure was also performed reacting ^{125}I C1q with varying amounts of aggregated IgG (aggregated by heat and precipitation with Na_2SO_4). C1qBA was measured in over 100 apparently healthy subjects. The mean binding obtained was 9.5 ± 2.6 . Therefore the mean ± 2 SD was 14.7 , and in our laboratories C1q greater than 15% is considered abnormal. Antiglobulin titres were determined by agglutination using IgG coated latex particles. In selected samples anti-IgG was isolated by elution from immunoabsorbents: glutaraldehyde cross-linked human IgG, and IgG coupled to cyanogen bromide activated sepharose (Miller *et al.*, 1977).

The isolated antiglobulin was characterised by double immunodiffusion in agar gel using rabbit

antisera to human IgG, IgM, IgA, and albumin. The eluate was further characterised by Sephadex G 200 gel filtration and polyacrylamide gel electrophoresis. The IgG used in the immunoabsorbent studies was prepared from normal human serum by ammonium sulphate precipitation, ion exchange chromatography on diethyl-aminoethyl cellulose, and Sephadex G 200 gel filtration. The serum used was obtained from apparently healthy individuals with no recent illness and normal physical examination, urine analysis, and glomerular filtration rate. The donor sera had no detectable anti-IgG activity, negative antistreptolysin O (ASO), and antihyaluronidase (AH) titres, no C1qBA, and normal levels of serum IgG, IgM, IgA, and C3.

Results

The serological findings of the patients are summarised in Table 1. The distribution of antiglobulin titres is illustrated in Table 2. Rheumatoid factor titres were greater than 1:32 in 89.2% of patients. Levels exceeding 1:256 were found in 64.6% and titres as high as 1:4096 were found in 8.5%. Immunoabsorbent studies revealed anti-IgG activity in both IgM and IgG classes. No IgA was detected in the materials tested. There was no relationship between age or sex of the patients, the site of infection, or the levels of antibody titres to streptococcal antigens. Although correlation could not be

Table 1 Summary of serological findings in acute poststreptococcal glomerulonephritis

	Number of patients	% Positive	Total number of patients studied
↑ anti-streptococcal Ab*	81	98.8	82
↑ IgG (1600 mg/100 ml)	68	93.2	73
↑ IgM (150 mg/100 ml)	77	98.7	78
↑ IgA (300 mg/100 ml)	21	26.9	78
↑ C3 (100 mg/100 ml)	65	85.5	76
↑ C1qBA (15%)	45	55.5	80

*Streptozyme, anti-streptolysin O, anti-hyaluronidase, or combination. C1qBA = C1q binding activity.

Table 2 Incidence of rheumatoid factor in acute poststreptococcal glomerulonephritis

Rheumatoid factor titres	Number of patients	%
1:8	2	2.4
1:16	3	3.6
1:32	9	10.9
1:64	10	12.2
1:128	5	6.1
1:256	10	12.2
1:512	11	13.4
1:1024	20	24.4
1:2048	5	6.1
1:4096	7	8.5

made between the titres of antiglobulins and the interval between the onset of infection or nephritis (Tables 3 and 4), very high titres of rheumatoid factor were observed quite early. Of 10 cases studied less than 2 weeks after the streptococcal infection, seven had titres above 1:256. Similarly, 33 of 38 patients had titres greater than 1:256 within the first week of onset of symptoms.

There was no apparent association between rheumatoid factor titres and serum levels of immunoglobulins (Table 5). Similarly there was no apparent

relationship between C1qBA or C3 levels and rheumatoid factor titres (Table 6).

Discussion

Most observers agree that poststreptococcal glomerulonephritis is the result of immune complex injury to the renal glomerulus. Several streptococcal products have been implicated as the putative antigen in the pathogenic immune complexes (Seegal *et al.*, 1965; Yoshizawa *et al.*, 1973; Zabriskie *et al.*, 1973; Treser *et al.*, 1974; Lange *et al.*, 1976). The inconsistency of demonstration of these antigens in the kidney and the failure to localise them along IgG in the subepithelial 'humps', as well as the inability to detect streptococcal antigen and antibody simultaneously in the cryoprecipitable complexes (McIntosh *et al.*, 1971a), has led to the suggestion that alternative or additional immune complex systems may be involved.

We have reported cryoprecipitates containing IgM antibody and IgG antigen in poststreptococcal glomerulonephritis (McIntosh *et al.*, 1970, 1971a, 1975). In one instance the IgG component lacked sialic acid, raising the possibility that the IgG fraction was an antigen rendered autoimmunogenic by absence of sialic acid (McIntosh *et al.*, 1971a). Zinneman *et al.* (1968) studied the cryoprotein of a patient with IgM-IgG cryoglobulinaemia in which the IgG component of the cryoprotein lacked sialic acid. The IgM fraction possessed anti-IgG activity and gave a stronger reaction with neuraminidase-treated (sialic acid-depleted) IgG than with normal unmodified IgG. The IgM portion of the cryoglobulin fixed to the glomeruli of patients with chronic glomerulonephritis. Davis *et al.* (1977) found that 14 out of 120 strains of group A streptococcus produced neuraminidase. We have demonstrated decrease in the sialic acid content of human

Table 3 Rheumatoid factor in relation to time of onset of symptoms of nephritis

Rheumatoid factor titres	Time (days)			
	0-7	8-14	15-21	>21
1:8	2	0	0	0
1:16	2	0	1	0
1:32	4	5	0	0
1:64	5	5	0	0
1:128	2	2	1	0
1:256	5	2	1	1
1:512	5	4	2	0
1:1024	10	8	1	0
1:2048	1	2	2	0
1:4096	2	3	1	1

Table 4 Rheumatoid factor in relation to time elapsed from streptococcal infection

Rheumatoid factor titres	Time (weeks)			
	<2	2-4	4-6	>6
1:8	0	1	0	1
1:16	0	1	2	0
1:32	2	4	1	0
1:64	1	1	6	1
1:128	0	3	1	0
1:256	0	1	3	0
1:512	1	6	3	0
1:1024	4	7	5	0
1:2048	2	1	1	0
1:4096	0	4	1	0

Table 5 Rheumatoid factor titres in relation to serum immunoglobulin levels

Immunoglobulin class	Serum concentration (mg/100 ml)	Total patients	Rheumatoid factor titres									
			1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
IgG	> 2400	51	0	2	5	7	4	6	8	9	4	6
	2000-2400	17	1	1	2	2	0	3	2	5	1	0
	1600-2000	2	1	0	0	0	1	0	0	0	0	0
	1000-1600	3	0	0	0	1	0	0	0	1	0	1
	< 1000	2	0	0	0	0	0	0	0	2	0	0
IgM	> 450	6	1	0	0	1	1	1	0	1	0	1
	301-450	53	0	3	5	8	3	7	6	14	3	4
	151-300	18	1	0	3	1	1	1	5	3	2	1
	< 150	1	0	0	0	0	0	0	0	0	0	1
IgA	> 400	3	0	0	0	0	0	0	0	1	1	1
	301-400	18	0	0	3	3	1	3	2	5	0	1
	200-300	40	0	1	3	5	4	4	6	9	3	5
	< 200	17	2	2	2	2	0	2	3	3	1	0

Table 6 Rheumatoid factor titres in relation to serum C1q binding activity and C3 levels

		Total patients	Rheumatoid factor titres									
			1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
C1qBA (%)	< 15	32	1	0	2	4	1	8	6	9	2	2
	>15-20	35	0	3	5	3	3	1	5	10	2	3
	>20-30	7	1	0	0	1	1	1	0	0	1	2
	> 30	3	0	0	1	2	0	0	0	0	0	0
C3 (mg/100 ml)	< 40	44	1	2	4	6	3	4	6	10	2	6
	>40-70	12	1	0	0	2	2	1	4	1	1	0
	>70-100	9	0	1	1	2	0	2	1	1	0	1
	> 100	11	0	0	3	0	0	2	0	4	2	0

IgG incubated with a culture of nephritogenic streptococcus (McIntosh *et al.*, 1971b). At least partial immunological identity between IgG modified in this manner and normal IgG was demonstrated.

Anecdotal reports from Eastern Europe claim early observation of rises of serum and urinary levels of neuraminic acid in patients with acute post-streptococcal glomerulonephritis. Autologous IgG modified by incubation with streptococci is capable of inducing cryoglobulins and glomerular IgG and C3 deposition in the rabbit, in the absence of streptococcal antigens, infection, or antistreptococcal antibodies (McIntosh *et al.*, 1972). These findings suggest that streptococci induce an antiglobulin response like that seen in cryoglobulinaemia, which, interestingly, is a feature of APSGN. Streptococcal vaccine has also been reported to induce monoclonal antibody responses with anti-IgG activity and production of cryoglobulins (Sheagren *et al.*, 1970).

Immune deposit disease characterised by granular glomerular accumulation of immunoglobulin G, C3, and fibrinogen, as well as circulating anti-IgG antibodies, was produced in inbred rats from injection of homologous IgG treated with coxtrial and influenzal neuraminidase and, to a less extent, neuraminidase alone.

In the present study antiglobulins were detected in the sera of the majority of patients with APSGN. The origin of these antiglobulins and their role in development and/or progression of glomerulonephritis is speculative, but in the context of the rest of available evidence it may be suggested that the antibodies may develop against IgG altered by streptococcal enzymes or IgG incorporated in immune complexes of streptococcal antigen and antibody. These autoantibodies may react with the neoantigen in the circulation or in the glomerulus. Since there is cross-reactivity with normal IgG, the antibody could also react with unmodified IgG in the circulation. However, in this instance the situation would be one of extreme antigen excess.

It is noteworthy that titres of rheumatoid factor were consistently raised independently of the

duration of symptoms before the study, appearing in some cases within 8 days after the infection and on the first day of symptoms of nephritis. This observation differs from those of immune complex nephritis associated with subacute bacterial endocarditis (Bokish *et al.*, 1972) and suggests that the production of antiglobulins may have occurred as a primary event rather than as an antibody to IgG incorporated in an exogenous immune complex.

Immunoglobulin complexes (IgG-IgM, IgG-IgG, etc.) have been implicated in the pathogenesis of the renal lesion in systemic lupus erythematosus (Agnello *et al.*, 1973), progressive systemic sclerosis (McCoy *et al.*, 1976), and essential cryoglobulinaemia (Golde and Epstein, 1968). It is of interest that ultrastructural studies of glomeruli of some patients with IgM-anti-IgG cryoglobulinaemia have shown subepithelial humps indistinguishable from those observed in APSGN (Skrifvars *et al.*, 1973).

Glomerular-fixed antiglobulins have been found in some patients with nephritis of various aetiologies (Rossen *et al.*, 1977). These antiglobulins may have different properties from those found in rheumatoid arthritis. It has been suggested that antiglobulins may bind to immune complexes in the circulation, diverting the immune complex from the renal glomerulus (Lightfoot *et al.*, 1969), a suggestion borne out by the infrequency of development of glomerulonephritis in patients with rheumatoid arthritis. In fact soluble immune complexes are frequently found in the sera of these patients (Zubler *et al.*, 1976). It is possible that poststreptococcal glomerulonephritis involves a variety of immune complex systems with exogenous, as well as endogenous, immune complexes, antigen-antibody interactions occurring both in the circulation and locally (in situ) in the kidney, a situation analogous to that of the nephritis of systemic lupus erythematosus (Agnello *et al.*, 1973).

Serial determinations of antiglobulins and correlations with clinical serological and immunological parameters may provide insight into the factors determining the development and progression

of renal disease associated with streptococcal infection.

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