ORIGINAL ARTICLE

Circulating Immune Complex in Glomerular Diseases

by

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Abstract

Circulating Immune Complex (CIC) in the serum have been studied in 75 patients with glomerular diseases. The highest positive rate in patients with post streptococcal glomerulonephritis and lupus nephritis were respectively 83.3% and 75%.

In nephrotic syndrome : 33.3% (5/15) was positive in diffuse mesangial proliferative cases, and in all 3 patients with membranoproliferative glomerulonephritis, membranous nephropathy and focal glomerulosclerosis. All of them had detectable immune deposits in the glomeruli.

In minimal lesion nephrotic syndrome (MLNS) without glomerular immune deposits, CIC was positive in 2 out of 17 cases (12%). This support the hypothesis that in MLNS a humoral immune mechanism might take part in the pathogenesis of the disease.

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Introduction

A subject of considerable scientific interest and importance to nephrologist, as well as immunologist was the recent development of tecniques for the detection of Circulating Immune Complex (CIC) in the serum.

Studies performed in animals and humans have established the important role of antigen antibody complexs in the pathogenesis of immune mediated glomerulonephritis. The glomerular deposition of CIC has been studied by light, immunofluorescence and electron microscopy in various animal models and in human renal diseases (Wilson and Dixon 1976).

Mc Cluskey et al. (1960) could induce glomerulonephritis by injection of preformed immune complexes in mice.

Due to these observations, there seems to be some hope that CIC detection might serve as a new clinical tool for the diagnosis, prognosis as well as management in glomerular diseases.

However, up till now there is still inconsistency on the relationship between the occurrence of glomerular deposition and the demonstration of CIC in human subjects. For instance despite morphologic evidence of glomerular immune complex deposition in patients with membranous glomerulonephritis and IgA nephropathy, CIC have been difficult to demonstrate in these patients, even in studies where serial serum samples were obtained (Pussell et al, 1978).

On the otherhand in a number of patients with minimal change nephrotic syndrome and some patients with idiopathic crescentic glomerulonephritis despite severe renal damage and no detectable glomerular immune deposits, CIC examination in the serum showed positive results (Stachura, et al, 1981).

Due to these discripencies, a study of CIC in the serum was conducted in 75 children suffering from glomerular diseases seen during an interval of two years, to correlate the finding of CIC and the deposits in renal biopsy tissue and the severity of the clinical picture of the disease.

Materials and methods

Circulating immune complex examination of the serum was conducted in 75 children, 40 cases suffering from acute glomerulonephritis and 35 from nephrotic syndrome, during a period of two years, namely from June 1980 until June 1982. The age of the patients were between 1½ and 12 years old, comprising of 29 girls and 46 boys.

Serum samples for CIC examination were obtained on the day of admission and renal biopsy were performed on the first or second week of hospitalization and was sent for pathological examination by means of light, immunofluorescence and in some patients for electron microscopy examination.

Circulating immune complexes were examined by detecting the inhibition effect on the capacity of RF (rheumatoid factor) to agglutinate latex particles coated with human immunoglobulins (IgG).

The procedure was a modification to the method describe elswhere (Luhurma, et al, 1976).

Briefly, 5 μl of two — fold dilutions of the patient serum was mixed (V/V) with RF containing serum of optimal dilution and incubated at 37° C for 15 minutes.

The mixture (5 μl) was then tested for inhibition effect by mixing V/V with indicator particles, namely latex particles coated with human immunoglobulins on a glass slide. The agglutination pattern was evaluated after 2 - 5 minutes at room temperature.

The titer of CIC was determined as the reciprocal of the dilution of the patient serum providing significant inhibition, represented by subagglutination patpern.

Patients with a clinical entity of acute glomerulonephritis, were consisted of :

36 cases with post streptococcal glomerulonephritis.

3 cases of SLE nephritis and

1 case with Henoch Schönlein nephritis.

All patients with post streptococcal GN showed elevation of ASO titer and lowering of the C3 complement. Serum CIC examination showed positive results in 83% of these cases.

In the SLE patients, beside the low C3 examination, antinuclear factor was positive and clinically a butterfly rash was detected.

CIC was positive in 2 out of 3 cases of the SLE patients and on the single patient with Henoch Schönlein nephritis it was negative.

TABLE 1 : *CIC finding in Acute Glomerulonephritis.*

Etiology	No cases	CIC		%	
		+	—		
Post strept	36	30	6	30/36	(83)
S L E	3	2	1	2/3	(75)
Henoch Schönlein	1	—	1	0/1	(0)
Total	40	32	8	32/40	(80)

Renal biopsy of the nephrotic syndrome patients showed :
minimal lesion in 17 cases, diffuse mesangial proliferative in 15 cases, membranoproliferative G.N. in 1 case, membranous nephropathy in 1 case and focal and segmental glomerulosclerosis in 1 case.

In patients with minimal lesion 2 cases (12%) showed CIC positive, while

in mesangial proliferative cases it was positive in 5 (33%).

The result was positive in all of the 3 cases with MPGN, membranous nephropathy, and focal glomerulosclerosis. So that the overall positive rate in non minimal lesion of the nephrotic syndrome was 50%. (Table 2).

TABLE 2 : *CIC finding in nephrotic syndrome*

Histo Pathology	No cases	C I C		%	
		+	—		
Menimal lesion	17	2	15	12	
Masangial Prolif. G.N	15	5	10	33	
Membranoprolif. G.N	1	1	—		
Membranous Neph.	1	1	—		50
Focal glomerulo. Scl.	1	1	—		
T o t a l	15	10	25	28.5	

In 1 case who was hospitalized due to AGN, acute renal failure and disseminated intravascular cougulation, the general condition was further deteriorated and blood urea rose up to 250 mg%, necessitating a peritoneal dialysis intervention. Post dialysis the condition improved only temporarily so the second and third dialysis were performed.

Due to financial reason the dialysis could not be continued and the patient died one week afterwards. CIC was detected before dialysis, but during the dialysis it was negative and became positive again 2 days thereafter. From this finding it seemed that CIC was a dialyzable substance.

Discussion

Many different CIC assays have been used and reported in the literature to clarify the pathogenesis of immune mechanism in glomerular diseases.

Due to these various methods, results of the examination sometimes differs markedly. (Border, 1979).

For instance in cases of membranous nephropathy, using the Cl q deviation method Stachura, et al. (1981) reported 69% positive rate (18 of 26 cases), while Woodroffe, et al. (1979) using Cl q binding assay found CIC only positive in 1 out of 7 cases (14%).

In this study we have used the inhibitory effect on the agglutination of IgG coated particles by the rheumatoid factor to detect the CIC.

Rheumatoid factor, Cl q and conglutinin are the three molecules that is capable to recognize an immune complex (IC) and distinguish it from unbound antigen and antibody. (Border, 1979).

The positive rate in this study was highest in acute poststreptococcal glomerulonephritis patients namely 83%. Border (1979) who reviewed the CIC assays found 58% positive in acute glomerulonephritis.

In the SLE patients 2 out of 3 cases (75%) showed CIC positive. Since the number of the SLE patients is small it was difficult to interprete in this study. In patients with minimal lesion nephrotic syndrome (MLNS) without detectable immune deposit in the glomerulus, 2 out of 17 cases (12%) showed CIC positive.

This finding is in accordance with the literature. Border (1979) found a positive rate of 45% ranging from 19% to 50% in MLNS patients using several CIC methods of assays. Levinsky et al. (1978) using rabbit IgM antihuman IgG reagent reported a positive rate of 100%.

These findings and the prompt response to steroid treatment suggested an immunologic pathogenesis for minimal lesion N.S.

It was possible that CIC may play a role by interacting with cellular receptors causing a release of mediators or interfering with helper or suppressor functions without accumulation of glomerular deposit (Border, 1979).

On the other hand only 33% of the mesangial proliferative N.S. cases with detectable immune deposits in the glomeruli showed positive results.

This could be due to the single method used in this study. Higher positive rates could be achieved by using more than one method of CIC assay since each has its own special reactivity and sensitivity (Woodroffe, et al, 1977).

Other explanation for the discrepancy between CIC in the serum and in the glomeruli, are the report of Causer and Salant (1980), that CIC deposit are not always trapped preformed IC but could also be formed in situ in the glomeruli. Of particular interest was the disappearance of CIC in the serum in one of the patient during peritoneal dialysis. This was detected repeatedly on the same patient, who had undergone three times peritoneal dialysis.

This finding supported the report of Ahlin, et al. (1978) who found a significant decrease in detectable immune complexes which, in turn, was associated with improvement and stabilization of the renal function in patient undergoing hemodialysis.

They suggest that dialysis may remove immune complexes from the circulation and it could be of therapeutic benefit in selected patients with presumed immune complex — mediated glomerulonephritis.

Despite this, we think that it is too early to take any definite conclusion at this moment. Further study should be made to confirm this assumption.

REFERENCES

1. AHLIN, T.D.; TUNG, K.S.K.; WALHER, L..C.; LEONARD, C.D.; WILLIAMS, R.C. : Decrease of Circulating immune complexes during Hemodialisis. Amer. J. Med. 64 : 672 (1978).
2. BORDER, W.A. : Immune complex detection in Glomerular Diseases. Nephron. 24 : 105 - 113 (1979).
3. COUSER, W.G.; SALANT, D.J. : In situ Immune complex formation and blomerular Injury. Kidney Int. 17 : 1-13 (1980).
4. LEVINSKY, R.J.; MALLESON, P.N.; BARRATT, T.M.; SOOTHILL, J.F. : Circulating immune complexes in steroid responsive nephrotic syndrome. N. Engl. J. Med. 298 : 128 - 129 (1978).
5. LUHURMA, A.Z.; COMBLASO, C.I.; MASSON, P.L.; HEREMANS, J.F. : Detection of Circulating antigen-antibody complexes by their inhibitory effect on the agglutination of IgG-coated particles by rheumatoid factor or Clq. Clin. Exp. Immunol. 25 : 212 - 2616 (1976).
6. Mc CLUSKEY, R.T.; BENACERRAF, B.; PATTER, J.L.; MILLER, F. : The pathologic effects of intravenously administered soluble antigen-antibody complexes. I. Positive serum sickness in mice. J. Exp. Med. 111 : 181 - 194 (1960).
7. PUSSELL, B.A.; LOCKWOOD, C.M.; SCOTT, D.M.; PINCHING, A.J.; PETERS, D.K. : Value of immune-complex assays in diagnosis and management. Lancet. ii : 359 - 364 (1978).
8. STACHURA, I.; WHITESIDE, T.; KELLY, R.H.; : Circulating and Deposited Immune Complexes in Patients with Glomcrular Diseases. Immunophatologic Correlations. Amer. J. Pathol. 103:21 (1981).
9. WILSON, C.B.; DIXON, F.J. : Renal response to immunologic injury, in Brenner and Rector. The Kidney (Saunders, Philadelphia, 1976).
10. WOODROFFE, A.J.; BORDER, W.A.; THEOFILOPONIOS, A.N.; GOTZE, O.; GLUSSOCK, R.J.; DIXON, F.J.; WILSON, C.B. : Detection of circulating immune complexes in patients witth glomerulonephritis. Kidney Int. 12 : 268 (1977).