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Correlation between the Renal C1q Deposition and Serum Anti-C1q Antibody: A Potential Role of Anti-C1q Antibody in Lupus Nephritis

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SUMMARY The anti-C1q antibody has been shown to be associated with lupus patients with renal involvement. We conducted a study to determine the relationship between the serum anti-C1q titer and the renal deposition of C1q. The serum anti-C1q was measured in 26 healthy controls and 47 systemic lupus erythematosus (SLE) patients who were divided into 2 groups as non-nephritis and nephritis SLE. We analyzed the relationship between the anti-C1q titers and SLE, renal C1q staining and the WHO classification for lupus nephritis. The result revealed that the serum anti-C1q was present in 50.8% of the SLE patients, that its levels in those with renal involvement were significantly higher than in the normal control group (61.540 ± 87.720 U/ml *vs* 15.750 ± 2.530 U/ml, $p = 0.005$). Besides, the serum anti-C1q levels were higher in the patients with lupus nephritis with C1q deposition in the kidney tissue (66.038 ± 91.141 U/ml *vs* 16.652 ± 3.097 U/ml, $p < 0.01$). There seems to be evidence supporting that the autoantibody anti-C1q might play a pathogenic role in lupus nephritis.

Systemic lupus erythematosus (SLE) is a disease with protean clinical manifestations characterized by the production of a wide range of autoantibodies produced to ubiquitous self-antigens. Immune complexes, yielded and deposited systemically, contribute to the tissue inflammation and multi-organ damage following complement fixation and activation of phagocytic cells. The autoantibodies contributing to the immunopathogenesis of SLE have been investigated extensively.[1,2] The presence of antibody against C1q (anti-C1q) in patients with SLE has been demonstrated to be associated with lupus nephritis.[1-4] Increasing levels of anti-C1q precede the flare of glomerulonephritis in lupus patients.[4-7] Besides, there is also a significant inverse correlation between the levels of C1q and anti-C1q.[7] Nevertheless, to the best of our knowledge, the relation between serum anti-C1q and renal C1q deposition has not been analyzed yet. In the present study, we not only investigated the serum titers of anti-C1q in SLE with and without nephritis but also correlated anti-C1q titers with renal C1q deposition determined from renal biopsy specimens.

MATERIALS AND METHODS

Patients

From August 1999 to July 2001, forty-seven SLE patients meeting the American College of Rheumatology 1982 revised criteria for SLE, together with 26 sex-and-age matched healthy controls (Group 1), were recruited from the Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan. The SLE patients were divided into two subsets: one group of 22 non-nephritis patients without evidence of renal involvement for at least one year (Group 2) and another group of 25 patients with biopsy-proved lupus nephritis (Group 3). This study was approved by the investigational review board and informed consent from these patients

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were obtained before we conducted the serum anti-C1q measurement.

Anti-dsDNA measurement

Indirect immunofluorescence with the *Crithedia luciliae* test was used for detection of anti-dsDNA. The levels were expressed in titers of two-fold dilution, starting at a dilution of 1/10.

Serum anti-C1q measurement

For anti-C1q determination, a commercial enzyme-linked immunosorbent assay (ELISA) kit (IMTEC, Germany) was used according to the manufacturer's instructions. Briefly, the antibodies were measured with microtiter plates coated with C1q. The binding of plasma immunoglobulin was performed at a high ionic strength (phosphate buffered saline containing 1 M NaCl). The cut-off limit for a positive reaction was defined as 20 U/ml. The serum anti-C1q levels were tested at two different occasions over a period of one year in 16 patients of Group 3 and totally 41 samples were obtained in this group.

Renal biopsies

Renal biopsies were graded according to the WHO classification for lupus nephritis.

Renal C1q deposition

Renal C1q staining was assessed by direct immunofluorescence of the renal biopsy specimen by FITC-conjugated anti-C1q. The assay results were expressed as 0, 1+, 2+, and 3+. A representative fig-

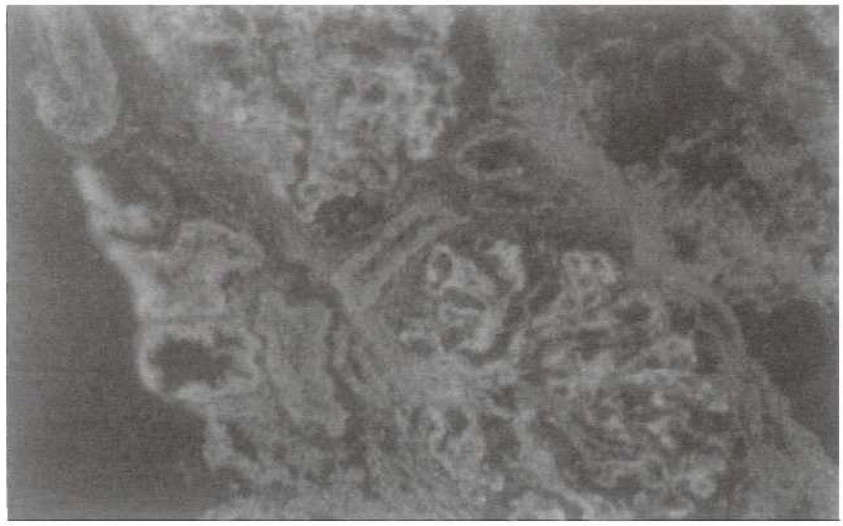

Fig. 1 A representative figure of the grade 3+ renal C1q staining.

Table 1 Characteristics of SLE patients

	Group 2	Group 3	*p*-value
Patient numbers	22	25	
Age	31.0 ± 12.2	27.3 ± 8.0	0.448
Male/Female	5/17	4/21	0.715
Mean duration SLE in years	2.79 ± 2.2	3.93 ± 3.70	0.241
Extra-renal manifestations (%)			
CNS	2 (9.1)	2 (8.0)	1.000
Mucocutaneous	3 (13.6)	7 (28.0)	0.706
Musculoskeletal	1 (4.5)	1 (4.0)	1.000
Serositis	1 (4.5)	5 (20.0)	0.194
Hematologic	1 (4.5)	2 (8.0)	1.000

Group 2: without lupus nephritis, Group 3: with lupus nephritis

ure of the grade 3+ renal C1q staining is shown in Fig. 1. The relationship between the serum anti-C1q titers and SLE, renal C1q staining, and WHO classification of lupus nephritis were analyzed.

Statistical methods

For statistical analysis, Fisher's Exact test, two samples t-test, F test for one way ANOVA (comparison of specific pairs of groups with t test), Wilcoxon Rank-Sum test, and Spearman rank-correlation coefficient with t test were used. A p-value below 0.05 was considered to be significant.

RESULTS

The clinical and demographic data of the patients studied are shown in Table 1. The extra-renal manifestations, such as in the central nervous system, skin, musculoskeletal system, serous membranes, and hematologic system were also shown. Compared to Group 2, the levels of serum albumin and C3 of Group 3 were lower and the titers of anti-dsDNA and SLE-disease activity index (SLE-DAI) score were higher, as shown in Table 2.

Thirty-two (50.8%) out of 63 SLE sera exhibited anti-C1q. The serum anti-C1q levels of the lupus patients were significantly higher than those of the normal controls (53.187 ± 73.616 U/ml *vs* 15.750 ± 2.530 U/ml, $p < 0.0005$; Two-sample t-test). Though the anti-C1q levels of Group 2 and Group 3 were both higher than those of Group 1 (Fig. 2), only the patients with nephritis (Group 3) were elevated by a statistical significance (61.540 ± 87.720 U/ml *vs* 15.750 ± 2.530 U/ml, $p = 0.00492$; F test for one way ANOVA).

Among the patients who underwent the renal biopsy, the se-

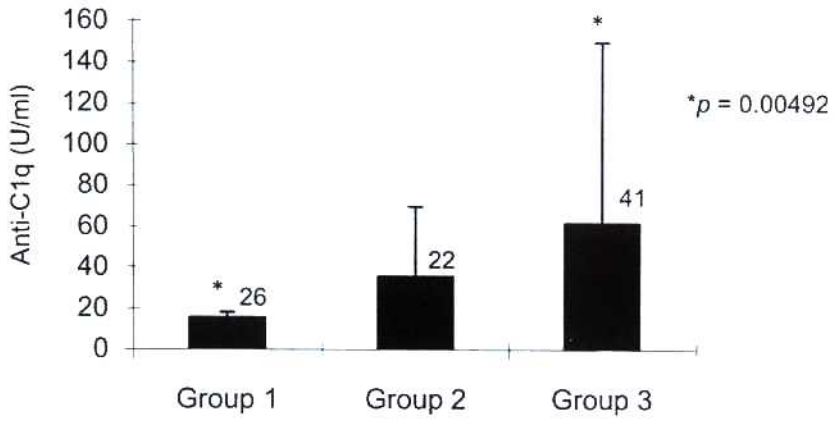

Fig. 2 Serum anti-C1q levels in each group (number of each group is shown above the bar).

Table 2 Laboratory parameters of SLE patients

	Group 2	Group 3	*p*-value
Numbers	22	41	
Albumin (g/dl)	4.25 ± 0.43	2.97± 0.82	< 0.001
C_3 (mg/dl)	65.87 ± 20.12	58.43 ± 24.97	0.453
Creatinine (mg/dl)	0.84 ± 0.13	1.15 ± 0.95	0.573
Anti-dsDNA (titers)	70.9 ± 146.0	101.2 ± 193.1	0.956
SLE-DAI* **score**	3.9 ± 3.8	10.5 ± 4.7	< 0.001

*Disease activity index, Group 2: without lupus nephritis, Group 3: with lupus nephritis

rum anti-C1q level was significantly correlated with the presence of C1q deposition in kidney tissue (66.038 ± 91.141 U/ml *vs* 16.652 ± 3.097 U/ml, $p < 0.01$; Wilcoxon Rank-Sum test) (Fig. 3). The serum anti-C1q were: 16.65 ± 3.08 U/ml in patients with no renal C1q staining, 109.26 ± 91.98 U/ml in patients with 1+ renal C1q staining, 67.92 ± 105.40 U/ml in patients with 2+ renal C1q staining, and 44.23 ± 66.75 U/ml with 3+ renal C1q staining. Though the serum anti-C1q level tended to bear a negative relationship with the staining intensity of renal C1q deposition, the correlation coefficient did not demonstrate a significant difference. Finally, among the patients of Group 3, no statistically significant correlation was observed between the titers of serum anti-C1q and the groups of different WHO classifications of lupus nephritis (data not shown).

DISCUSSION

Solid-phase C1q binding for the determination by an immune complex assay is based on a principle that initiation of classical complement pathway is started in case of binding of C1q to IgG- and/or IgM-containing immune complex.[8] Though both immune complex and anti-C1q antibody bind to C1q, their binding domains are different.[9] Further studies revealed that the C1q-binding domains of anti-C1q and immune complex were N-terminal (collagen-like region) and C-terminal (globular head region) end, respectively.[10,11] The purpose of the present study was to analyze to what extent the level of anti-C1q in peripheral blood correlated with the C1q deposition in renal tissue. If such a correlation could be demonstrated, it may provide further evidence for a pathogenic role of this antibody in the development of lupus nephritis.

The anti-C1q has been found to correlate strongly with the presence of proliferative lupus nephritis and an increase in its titers precedes the renal involvement of SLE.[4-7] In addition, this antibody has been recovered from renal tissues of lupus nephritis patient at higher concentrations than those in the serum.[12] In our series, 32 of 63 (50.8%) SLE sera contained anti-C1q and the anti-C1q was present in high titers in lupus nephritis patients. It indicates that the anti-C1q might play a potential role in the pathogenesis of lupus nephritis.[13]

One of the physiological functions of complement activation is the clearance of the circulating immune complexes. Both the circulating immune complex and *in situ* immune complex deposition are known to contribute to glomerulonephritis in SLE. The roles of complement in immune complex processing and of C1q in the clearance of apoptotic cells have been investigated.[14] In the present study, the titers of serum anti-C1q were higher in those patients with renal C1q deposition. This indicates that anti-C1q might influence the physiological role of C1q after binding to the immune complex,[15] such as in patients with hereditary C1q deficiency who carry the risk of developing autoimmune disease,[16,17] resulting in impairment of immune complex removal and systemic deposition. Furthermore, our data showed the serum anti-C1q level tended to bear a negative correlation with the intensity of the renal

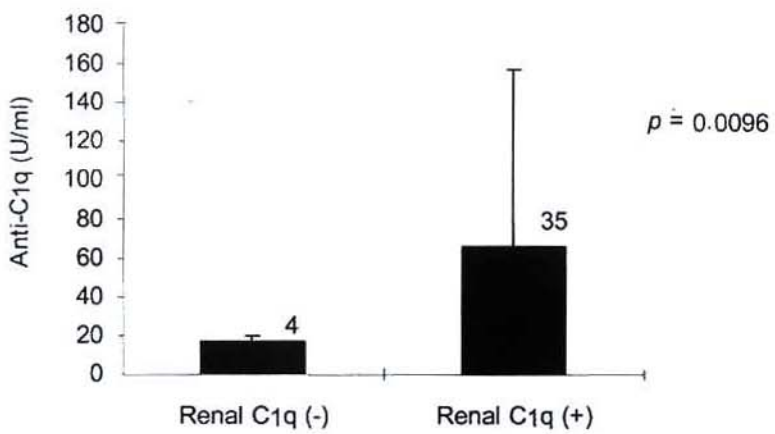

Fig. 3 Serum anti-C1q levels in patients with or without renal C1q staining (number of each group is shown above the bar).

C1q staining. It was suggested that circulating anti-C1q might bind to C1q already deposited in the kidneys of patients with SLE, leading to local or *in situ* immune complex formation and possibly development into glomerulonephritis.[18] Whether the consumption of serum anti-C1q by binding to the C1q-containing immune complexes then leading to the negative correlation between serum anti-C1q and renal C1q staining intensity needs further investigation.

In conclusion, our study suggests that the presence of high anti-C1q levels correlates with renal involvement in SLE patients. The interaction of renal C1q and serum anti-C1q might contribute to the development of lupus nephritis.

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