High levels of antibodies against C1q are associated with disease activity and nephritis but not with other organ manifestations in SLE patients

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Abstract

Objective

Serum concentration of antibodies to C1q (C1qAb) has been reported to be elevated in a high percentage of patients with systemic lupus erythematosus (SLE). The associations of high C1qAb levels with different clinical manifestations and the activity of the disease, however, are not definitely understood.

Methods

We measured the levels of IgG type C1qAb in the sera of 137 patients with SLE using an ELISA method.

Results

Serum concentrations of C1qAb were found to be higher ($p < 0.0001$) in SLE patients than in healthy controls. High titer (> 66 AU/ml) C1qAb was found in 40/137 (29.2%) SLE patients, and 4/192 (2.1%) healthy controls ($p < 0.0001$). A strong negative correlation ($R = -0.4$, $p < 0.0001$) between the age of the patients and the C1qAb titers could be detected. C1qAb levels in clinically active SLE patients significantly ($p < 0.0001$) exceeded those measured in the sera of patients in the inactive stage of the disease. A significant positive correlation was detected between C1qAb levels and the laboratory activity markers (anti-DNA, low C3 level) of the disease. We found a significant negative correlation between levels of C1qAb and a negative acute phase protein, alpha2-HS-glycoprotein. Renal involvement was present in 11/40 (27.5%) and 11/97 (11%) of the patients with high and low titers of C1qAb, respectively ($p = 0.038$). The prevalence of other organ manifestations was, however, the same in the patients with or without high titer C1qAb.

Conclusion

These findings indicate that C1qAb measurement is a useful method for detecting the activity of SLE and predicting renal manifestations, but not other organ involvement in the disease.

Key words
C1q, heat shock proteins, autoimmune disease, systemic lupus erythematosus.

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Introduction

Antibodies to C1q (C1qAb) are polyclonal IgG autoantibodies that bind to the collagen-like region of C1q, a subunit of C1, the first component of complement (1,2). Autoantibodies against C1q (C1qAb) were found to be present in various adult and pediatric renal diseases (3). C1qAb were found in 100% of patients with hypocomplementemic urticarial vascular disease (4), 88% of patients with membranoporoliferative glomerulonephritis, 76% of patients with Felty's syndrome, 34-59% of SLE patients, 32% of patients with rheumatoid vasculitis and 27% of patients with classic polyarteritis nodosa (3). In other autoimmune diseases, such as Sjögren's syndrome, ankylosing spondylitis, rheumatoid arthritis, systemic sclerosis, IgA nephropathy, membranous glomerulopathy or Wegener's granulomatosis the prevalence of C1q-Ab is equal to or only slightly higher than that in healthy individuals (4%) (3).

Most studies on the occurrence and clinical significance of the C1qAb have been performed in SLE. High C1qAb titers occurred in patients with active lupus nephritis (5). Increases in the levels of C1qAb may predict relapses of lupus nephritis (6, 7). According to recent studies by Trendelenburg *et al*. (8), nephritis does not occur in C1qAb negative SLE patients – that is, measurement of C1qAb has a 100% negative predictive value for lupus nephritis development. Ravelli *et al*. (9) and more recently Kumar *et al*. (10), however, did not find a correlation between either the presence or titer of C1qAb and any of the clinical manifestations, including nephritis.

In the present study we measured C1q-Ab in a large number of SLE patients and investigated the relation of high C1qAb titers to the clinical and laboratory signs of disease activity and organ manifestations of the disease.

Materials and methods

Patients and controls

A total of 137 patients (39.2 ± 14.5 (mean ± S.D.) years old, 18 males, 119 females) with SLE diagnosed according to the standard criteria (11) were tested. The patients were cared for at the 3rd Department of Internal Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary and at the 2nd Department of Internal Medicine, University Medical School, Pécs, Hungary between 1988 and 2000. Evaluation of disease activity was based on clinical and laboratory data: the presence of organ system involvement (arthritis, skin symptoms, serositis, recent onset of central nervous system symptoms), general symptoms (fever, subfebrility, weight loss) and laboratory findings (increased ESR, anti-dsDNA, decreased C3, proteinuria-hematuria) at the time of the investigation. SLEDAI scores (12) were also calculated. The presence of nephritis was defined by the persistent presence of proteinuria (> 0.5 g/24h) and/or hematuria (> 5 red blood cells/high power field) after stones, infection or other causes were excluded).

The controls consisted of 192 healthy blood donors (111 males, 81 females, 47.1 ± 9.8 years old).

Antigens

C1q isolated from human plasma by sequential chromatography on BioRex 70 and BioGelA5, as described by Tenner *et al*. (13), was used to coat ELISA plates for the C1qAb measurements.

C1q antibody measurement

Antibodies against the collagenous part of C1q were measured by the solid phase ELISA method of Siegert *et al*. (14). Briefly ELISA plates (Maxisorp, Denmark) were coated with C1q in bicarbonate buffer (3 g/well, pH 9.6), and incubated for 2 hours at 37°C or overnight at room temperature, and washed three times with PBS containing 0.05% Tween. 100 l/well sera diluted 1:30 and 1:60 in PBS containing 0.05% Tween, 1% delta-FCS, 1 M NaCl were added. The plates were incubated at 37°C for 1 hour and washed 3 times with PBS containing 0.05% Tween, after which monoclonal anti-human IgG antibody in PBS containing 0.05% Tween and 1% delta-FCS (HB-43DIG, 1:2500) was added, and the plates were incubated for 1 hour at 37° C and washed 3 times with PBS con-

taining 0.05% Tween. As a second antibody, anti-DIG HRP in PBS containing 0.05% Tween and 1% delta-FCS (1:5000) was added, and the plates were washed 3 times with PBS containing 0.05% Tween.

The reaction was developed in ABTS and H_2O_2 with30 min incubation times. The optical density was measured using a microplate reader at 415 nm. C1qAb were expressed as arbitrary units/ml (AU/ml) related to one standard serum. The mean titer + 2 SD in serum samples from 192 blood donors was regarded as the upper limit of normal.

Other laboratory procedures

The C3 concentration was determined by single radial immunodiffusion and serum concentrations of anti-DNA antibodies were determined by an indirect IFA assay (Biosystems, Barcelona, Spain). Serum AHSG levels were measured by rocket immunoelectrophoresis as described elsewhere (15).

Statistical analysis

Since the antibody titres measured in the different groups did not display a Gaussian distribution, non-parametric tests were used. Groups were compared using the Mann-Whitney U test. Categorical data were compared by Fisher's exact test. The degree of correlation between the levels of two antibodies was analyzed by the non-parametric Spearman's test. The age-adjusted relationship between C1qAb levels and disease activity was calculated by multiple logistic regression analysis. $P < 0.05$ was considered to be statistically significant.

Results

Occurrence of C1qAb in SLE patients

The levels of C1qAb were measured in the sera of 137 patients with SLE and in 192 healthy blood donors as controls. Significantly ($p < 0.0001$) higher serum concentrations of C1qAb were found in the SLE patients (126 ± 22.1 AU/ml; mean ± SEM) as compared to those measured in the healthy subjects (7.52 ± 2.14 AU/ml). High titers (exceeding 66 AU/ml, mean + 2 SD of the amounts measured in the healthy controls) of C1qAb were found in 40/137 (29.2%) and 4/192 (2.1%) of SLE patients and healthy controls, respectively ($p < 0.0001$). The relative risk of SLE patients to have C1qAb was 14.0 (95% CI: 5.1 to 38.3).

C1qAb and the age of SLE patients

A strong negative correlation (Spearman correlation coefficient -0.4, $p < 0.0001$) between the age of the patients and the C1qAb titers could be detected. When C1qAb in the lowest, medium and higher age tertiles were compared by the Kruskal-Wallis test, a highly significant ($p < 0.0001$) difference was obtained, the median (25 – 75% percentile) C1qAb titres being 43.08 (0 – 355.6), 30.46 (0 – 142.0), and 0 (0 – 22.5) U/ml, in the youngest, medium and eldest age groups, respectively. High (> 66 U/ml) C1qAb was found in 22/49 (45%), 13/41 (32%), and 5/47 (11%) patients, respectively.

Correlation between C1qAb, other laboratory parameters and disease activity in SLE patients

Taking into account the high occurrence rate of C1qAb in SLE patients, it seemed worthwhile to calculate the correlation between the levels of this autoantibody and some of the laboratory parameters characteristic for the activity of SLE. When patients with high levels of C1qAb (>66 AU/ml) and little or no (<66 AU/ml) C1qAb were compared (Fig. 1), significantly ($p < 0.0001$) lower C3 concentrations (Fig. 1A) and significantly ($p < 0.0001$) higher anti-DNA levels (Fig. 1B) were found in the patients with high C1qAb levels. The levels of the negative acute phase protein AHSG were found to be significantly ($p = 0.018$) lower in SLE patients with C1qAb than in those with no C1qAb (Fig. 1C).

Only 15% of the patients in remission, but 41% of the patients in the active stage had high titers of C1qAb (Table I). The median (interquartile range) of C1qAb was 0 (0 – 35.0) and 36.0 (0 – 262.5) U/ml, respectively in these two groups ($p < 0.0001$). Taking into account the strong age-dependency of the C1qAb levels, we calculated the age-adjusted relationship between the activity of the disease and C1qAb by using multiple logistic regression analysis. There was a significant ($p = 0.002$) correlation between disease activity and C1qAb even after adjustment for the age of the patients. SLEDAI scores were also determined. A positive correlation was found between C1q antibody levels and the SLEDAI scores ($r = 0.24$, $p = 0.006$).

Twenty-one serum-pairs obtained in relapse and in remission from the same SLE patients were available. The clinical activity of the disease was markedly characterized by high (219.2 ± 87.5 AU/ml) titers of C1qAb that significantly ($p = 0.0017$, Wilcoxon matched paired test) differed from those (24.56 ± 12.14 AU/ml) found in remission.

Study of the correlation between different organ manifestations and C1qAb levels in the SLE patients

We studied the possible association between C1qAb levels and the occurrence of organ manifestations at the time of blood sampling (TableII). Renal involvement (nephritis) was present at the time of blood sampling in 3/66 (4.5%) and 19/70 (27%) patients with inactive and active disease, respectively ($p = 0.0003$, Fisher's exact test); and in 11/93 (12%) of C1qAb negative and 11/36 (31%) of C1qAb positive patients ($p = 0.017$, Fisher's exact test). All but one C1q positive patient with renal involvement were in the clinically active stage of the disease. Kidney biopsies were performed in 23 patients. No association between C1qAb and the different histological types of SLE nephritis (diffuse proliferative, membranous or focal segmental) was observed. Diffuse proliferative glomerulonephritis was found in 3/6 patients with C1qAb and in 6/17 patients without C1qAb, but the difference was not significant.

Lymphadenopathy and pleuritis/pericarditis were observed more often in C1qAb positive than in C1qAb negative patients; the differences, however, were not statistically significant ($p = 0.06$ and $p = 0.051$, respectively). The titers of C1qAb showed no relationship to the occurrence of other organ involvement.

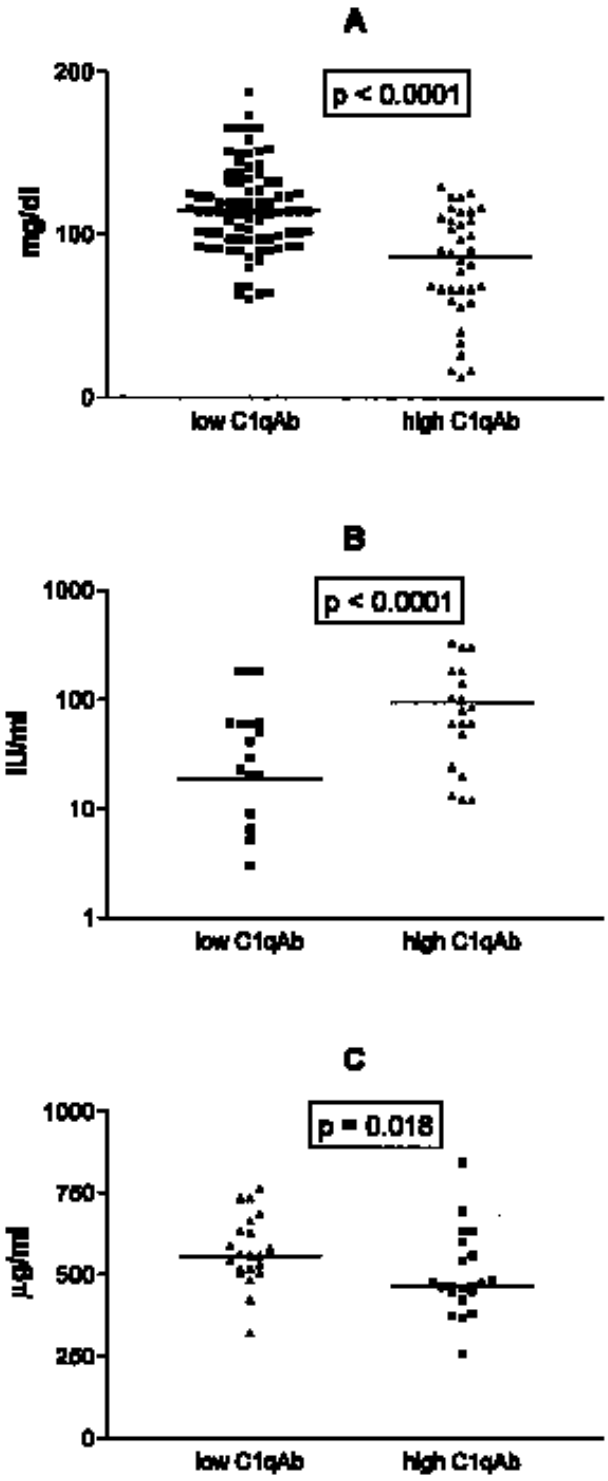

Fig. 1. Serum concentrations of C3 (**A**),anti-DNA antibody (**B**), and the negative acute phase protein AHSG (**C**) in SLE patients with no or low titer (less than 66 U/ml) C1qAb and high titer (more than 66 U/ml) C1qAb. Horizontal lines indicate median values.

Discussion

The high number of SLE patients tested allowed us to study the possible associations between C1qAb levels and several characteristics of the disease. Our present findings showing a strong correlation between high C1qAb titers and SLE activity are in agreement with many previous results. Our data also support the age-dependency of the levels of C1qAb in SLE patients. In agreement with a number of previous positive findings, we observed a correlation between the amounts of C1qAb and lupus nephritis, but not with other organ manifestations of the disease.

In accordance with previous observations of other authors (5, 9, 16), we found a clear-cut positive correlation between the presence of high titer C1qAb and SLE activity based on clinical and laboratory data. Almost half (29/70) of the SLE patients in the active stage, but only 10/66 of the patients in the inactive stage had high anti-C1q levels. Similar findings were found when serum pairs obtained in the active and inactive stages of the disease from the same patients were compared. C1qAb levels were in a positive correlation with the SLEDAI scores.

In addition to clinical activity, a highly significant positive correlation was found between C1qAb levels and different objective laboratory markers of SLE activity, such as low serum concentrations of C3 and high anti-dsDNA levels.

Similarly to our studies, low C3 levels (a sign of complement activation in SLE) were found to occur more frequently in C1qAb positive than in C1qAb negative patients in several previous studies (5,9,10,16). A strong positive correlation between C1qAb levels and antibodies against dsDNA was also reported by some authors (5, 9, 10).

We are the first to study the relationship between C1qAb and the negative acute phase protein AHSG in SLE. The two variables were negatively correlated to each other. Compared to healthy controls, low levels of serum AHSG were observed in a previous study that included 63 patients with SLE, and the extent of the decrease in AHSG was found to correlate with laboratory activity, including a statistically significant positive correlation with C3 (17).

Siegert *et al*. (18) reported an interesting observation, i.e. higher C1qAb titers in younger as compared to elder SLE patients. The results of the present study on a large cohort of SLE patients definitely confirm this finding. The prevalence of high titer C1qAb among 15 to 30-year-old SLE patients was four times higher than that among the SLE patients above 45 years of age.

Table I. Relationship between disease activity and C1qAb titers in SLE patients.

	C1qAb < 66 U/ml	C1qAb > 66 U/ml	Total
Patients with inactive SLE	56	10	66
Patients with active SLE	41	29	70
Total	97	39	136

p = 0.0011 (Fisher's exact test), odds ratio: 3.96 (95% CI: 1.74 - 9.03).

Table II. Association between the C1qAb levels and the presence of clinical manifestations at the time of blood sampling in 129 SLE patients.

	Patients with missing or low C1qAb (<66 U/ml)		Patients with high titer C1qAb (>67 U/ml)		
Clinical manifestation	Present	Absent	Present	Absent	P*
Nephritis	11 (12%)	82 (88%)	11 (31%)	25 (69%)	*0.017
CNS involvement	10 (11%)	83 (89%)	5 (14%)	31 (86%)	n.s.
Pulmonary manifestation	5 (5%)	88 (95%)	2 (6%)	34 (94%)	n.s.
Lymphadenopathy	7 (8%)	86 (92%)	7 (19%)	29 (81%)	n.s. (0.06)
Cardiac manifestation	4 (4%)	89 (96%)	2 (6%)	34 (94%)	n.s.
Pericarditis and/or pleuritis	2 (2%)	91 (98%)	4 (11%)	32 (89%)	n.s. (0.051)
Polyarthritis	28 (30%)	65 (70%)	14 (39%)	22 (61%)	n.s.
Skin rash/ exanthema	13 (14%)	80 (86%)	9 (25%)	27 (75%)	n.s.
Subfebrility or fever**	5 (5%)	88 (95%)	5 (14%)	31 (86%)	n.s.
Weight loss	3 (3%)	90 (97%)	2 (6%)	34 (94%)	n.s.
ESR > 25 mm/hour	21 (23%)	72 (77%)	13 (36%)	23 (64%)	n.s.
Leukocytopenia***	6 (6%)	87 (94%)	4 (11%)	32 (89%)	n.s.

* Fisher's exact test, ** > 38°C, *** < 4000 cell/µl, n.s.: not significant.

We observed high C1qAb levels more often in SLE patients with lupus nephritis than in patients without renal involvement. The C1qAb titers had no value in differentiating patients with other organ manifestations from patients without such organ involvement. In an earlier study, one of us (MD) observed that high titer C1qAb occur significantly more frequently in patients with SLE nephritis than in patients without renal manifestations (19), and in a prospective study found IgG type C1qAb to predict nephritis in SLE (18). Similar results were published by Haseley *et al.* (20). Recently Trendelenburg *et al* (8) reported a lack of occurrence of severe lupus nephritis among C1qAb negative patients that may endow this laboratory parameter with a 100% negative predictive value in the development of severe SLE nephritis.

In contrast to these findings, Ravelli *et al.* (9) and more recently Kumar *et al*. (10) did not observe in pediatric Italian SLE patients, and in adult SLE patients in India, respectively, a correlation between C1qAb levels and any of the clinical manifestations, including nephritis. Our present findings indicate that nephritis may develop in C1qAb negative SLE patients as well, but much less frequently than in C1qAb positive patients. The conflicting results of these studies may be due to the differing genetic backgrounds of the cohorts (21) or to differences in the age of onset of the disease. Furthermore, good surrogate marker(s) for the detection of lupus nephritis activity are lacking. C1qAb seems to be a useful marker for detecting continuously active kidney disease, but during therapy it is difficult to distinguish between cases whose renal disease is in remission and cases with still ongoing, active kidney inflammation. Additional studies (involving more kidney biopsies) will be required to provide more information on the usefulness of the different activity markers.

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