The Effect of High Salt Concentration on Detection of Serum Immune Complexes and Autoantibodies to C1q in Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To compare the binding of immune complexes and anti-C1q in systemic lupus erythematosus (SLE) sera to C1q and to the purified collagen-like region of C1q (CLR) in high and physiologic NaCl concentrations.

Methods. Sera from patients with SLE were tested for binding of IgG to C1q and to CLR in physiologic (0.15 M NaCl) or high (1.0 M NaCl) salt concentration. Sera were ultracentrifuged to separate monomeric IgG and high molecular weight IgG (immune complexes), and fractions were tested for binding to C1q and to CLR in the presence of physiologic or high salt concentrations.

Results. Decrease in binding of immune complexes to C1q in the presence of high salt ranged from 0 to 96.8%, with a median of 48.4%. Decrease of the binding of monomeric IgG to C1q in the presence of high salt ranged from 2.2 to 74.3%, with a median of 46.2%. Binding of monomeric autoantibodies to CLR also was decreased by high salt. Nevertheless, anti-C1q measured by binding to CLR in physiologic salt correlated highly with the binding to C1q in both physiologic (r = 0.978) and high salt (r = 0.983).

Conclusion. C1q-binding of immune complexes in SLE sera is not uniformly abrogated by high salt and binding of autoantibodies to CLR is variably reduced by high salt. Binding of IgG to C1q in high salt correlates with but is not equivalent to quantifying these autoantibodies by binding to CLR. (J Rheumatol 2002;29:84–9)

Key Indexing Terms:
COMPLEMENT 1Q AUTOANTIBODIES
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Systemic lupus erythematosus (SLE) has been considered a prototype of human diseases mediated by immune complexes. Deposition or formation of immune complexes in target organs, including renal glomeruli, are well established mechanisms of tissue injury in patients with SLE[1]. The binding of immune complexes to C1q, a protein of the first component of the complement system, has been used to detect and quantify immune complexes in serum of patients with SLE[2]. The C1q molecule contains 6 globular heads that bind immune complexes. The nonglobular regions of C1q polypeptide chains form collagen-like helices. One of the assays for immune complexes involves measurement of IgG that has bound to solid-phase C1q immobilized on a plastic surface. Most of the serum IgG from SLE patients that binds to solid-phase C1q, however, was shown to sediment in density gradient ultracentrifugation at 6.6 Svedberg units (S), indistinguishable from the sedimentation of monomeric IgG, and these molecules consist of autoantibodies to the collagen-like region of C1q (CLR)[3-6]. Several independent investigations have established the high prevalence of autoantibodies to C1q in patients with proliferative lupus glomerulonephritis[5,7-9]. In addition, increasing levels of these autoantibodies were shown to herald a flare of glomerulonephritis in patients with SLE[10,11]. Finally, antibodies to the CLR of C1q were shown to be deposited and concentrated, as compared to serum, in renal glomeruli of patients with SLE[12]. All these findings support the idea that antibodies to C1q contribute significantly to renal injury in patients with SLE.

To measure antibodies to C1q, investigators have quantified the binding of IgG to intact C1q in the presence of high NaCl (e.g., 1.0 M NaCl), rather than using purified CLR as the antigen[7,10,11,13-17]. This simplified assay was based on the finding that high salt concentrations abrogated the binding of aggregated human IgG, as a surrogate for immune complexes, to C1q[3,4,6]. While the influence of high salt on binding of aggregated IgG to C1q has been studied, little is known about the binding of immune complexes in lupus sera in the presence of high NaCl. We therefore compared the binding of anti-

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C1q and of immune complexes to intact C1q and to CLR in the presence of 0.15 M or 1.0 M NaCl.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from patients with SLE at the University of Washington Medical Center. Thirteen sera, stored in aliquots at –20°C, were kindly provided by Dr. E.J. Lewis (Chicago, IL) for the study correlating lupus renal disease and C1q binding IgG levels[5].

Preparation of C1q and CLR of C1q. Human C1q was purchased from Cytotech (San Diego, CA, USA). C1q was further purified with affinity chromatography, using agarose beads coupled with $F(ab')_2$ fragments of goat antibodies to human IgG, to remove small amounts of IgG in these preparations. The concentration of C1q was measured by absorbance at 280 nm[18].

CLR was prepared as described[4,19], using pepsin digestion of C1q in 0.1 M acetate buffer, pH 4.45, at 37°C for 20 h. After digestion, it was purified by gel filtration on Sephacryl S-300 (Pharmacia Fine Chemicals, Piscataway, NJ, USA) with Tris-HCl buffer, pH 7.6. The CLR was free of contaminating intact C1q, IgG, and IgM, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with Western blotting under both reducing and nonreducing conditions.

Enzyme linked immunosorbent assay. The ELISA procedures to detect IgG binding to C1q and CLR were as described[9], except the method was modified for the use of the more sensitive chemiluminescence technology. Opaque, black Fluoro Nunc Maxi-Sorp microtiter plate wells (Nunc, Naperville, IL, USA) were coated with 50 µl of C1q or CLR solution at a concentration of 10 µg/ml in phosphate buffered saline (PBS; 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2) and incubated overnight at 4°C. The wells were then washed 3 times with PBS containing 0.05% Tween 20 (PBS-Tween). Subsequently, 100 µl of blocking buffer, consisting of PBS with 2% nonfat milk, was added and incubated for 1.5 h at room temperature, followed by washing 3 times with PBS-Tween. Test sera were diluted 1:100 using PBS-Tween with 2% nonfat milk, and 100 µl of the diluted sera were placed into each well and incubated overnight at 4°C. After washing 3 times with PBS-Tween, 100 µl of peroxidase conjugated $F(ab')_2$ fragments of goat antibodies specific for the Fc fragment of human IgG (Jackson Immunoresearch, West Grove, PA, USA), diluted 1:10,000 in PBS-Tween with 2% nonfat milk, was added to each well. Plates were incubated 3 h at room temperature, then washed 5 times with PBS-Tween. Super Signal chemiluminescence substrate (Pierce, Rockford, IL, USA) was added (100 µl per well), incubated for 5 min, and the relative light units quantified with a luminometer (Packard Lumicount, Meriden, CT, USA). For assays in high salt concentration, the serum was diluted 1:100 in PBS-Tween with 2% nonfat milk, made 1.0 M with NaCl.

Assays were performed in duplicate. Each result is reported as the mean relative light units of duplicate assays. With each test specimen, an uncoated, blocked well was used to detect nonspecific binding to the microtiter wells. The relative light units from the blank well were subtracted from relative light units detected for the same serum with coated wells.

Other methods. Sucrose density gradient (SDG) ultracentrifugation with analysis of gradient fractions was performed as described, with fractions collected from the bottom of each SDG tube and using the described method for preventing loss of immune complexes[5,20]. Aliquots of sera were diluted 1:5 in PBS and 200 µl of the diluted sera were applied to the gradients. To determine the C1q and CLR binding activity within individual SDG fractions, a 500 µl aliquot from each fraction was diluted 1:2, and 100 µl were added to C1q and CLR coated wells and a blank well. The assays for binding to C1q or to CLR were performed as described above.

The graphs of binding of monomeric IgG to C1q or to CLR in SLE sera after SDG ultracentrifugation had a Gaussian or near-Gaussian distribution, with the 6.6 S peak consistently at approximately 80% of the SDG volume. A shoulder of faster sedimenting IgG was frequently present just above 60% of the SDG volume, most likely representing IgG dimers. IgG sedimenting faster and detected in fractions below 60% of the SDG volume was considered to be IgG contained within immune complexes. The relative light units measured in the immunoassays of the fractions below 60% of the SDG volume minus the blank relative light units were summed to calculate the fraction of IgG that was of higher size than the monomeric IgG (designated > 6.6 S), and the sum of relative light units from the immunoassays of aliquots sedimenting slower than 60% of the SDG volume was considered to be monomeric IgG (designated 6.6 S).

Heat aggregated IgG was prepared by heating human Cohn Fraction II (ICN Biomedicals, Costa Mesa, CA, USA) at 2 mg/ml to 63°C for 10 min. Heat aggregated IgG was used within 1 day of preparation, after storage at 4°C.

Statistical analysis of the data was performed using Microsoft Excel software.

RESULTS

Binding to C1q and CLR in physiologic salt (0.15 M) and high salt (1.0 M NaCl) was measured in the same assay run in unfractionated sera from 25 different patients with SLE. The binding of serum IgG to CLR under standard conditions in physiologic salt (0.15 M NaCl) correlated highly with the binding to C1q in both 0.15 M NaCl (r = 0.978) and 1.0 M NaCl (r = 0.983) (Figure 1). As expected, binding of IgG to CLR in physiologic salt and high salt gave almost identical results (r = 0.992, data not shown). For unfractionated sera, the reduction in binding to intact C1q in high salt compared with physiologic salt ranged from 0 to 90.4%, with a median reduction of 43.4%. Ten sera revealed over 50% reduction in binding to C1q in high salt. The reduction in binding to CLR in 1.0 M NaCl, compared with 0.15 M NaCl, ranged from 0 to 68.2% with a median reduction of 21.9%. Only 2 sera showed greater than 50% reduction in binding to CLR, and those same sera also had over 50% reduction in binding to C1q in high salt. By a paired t test, high salt caused a significantly greater reduction in binding to C1q than to CLR (p = 0.03).

Experiments were performed in which wells coated with C1q or with CLR were incubated overnight with buffered 1.0 M NaCl, washed, and then the assays were performed as usual

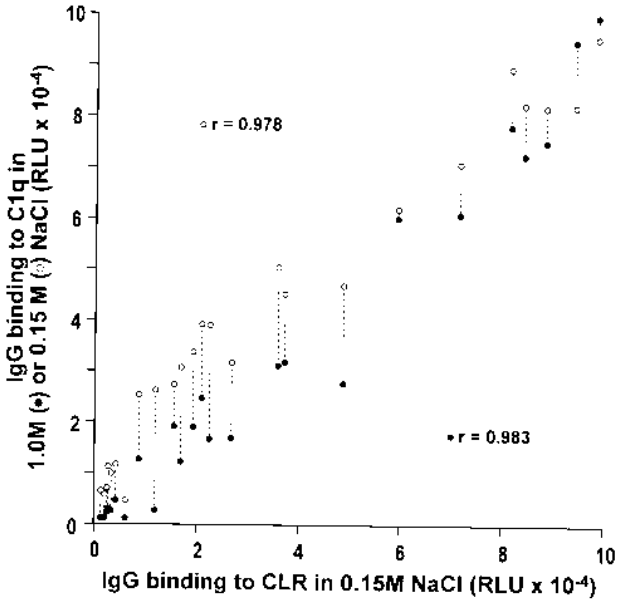

Figure 1. Correlation between IgG binding to CLR in 0.15 M NaCl with IgG binding to C1q in 1.0 M (●) or 0.15 M (○) NaCl. RLU: relative light units.

Table 1. SLE sera were separated by sucrose density gradient ultracentrifugation and fractions were analyzed for IgG binding to C1q and to CLR. The percent binding in greater than monomer IgG (> 6.6 S) fractions to C1q and CLR in 0.15 M NaCl are shown and the decrease of binding to C1q and to CLR in 1.0 M NaCl are shown both for the > 6.6 S fractions and the monomeric IgG fractions (6.6 S).

	% RLU in > 6.6 S in 0.15 M NaCl		% Decrease in Binding to Clq in 1.0 M NaCl		% Decrease in Binding to CLR in 1.0 M NaCl	
Serum	C1q	CLR	> 6.6 S	6.6 S	> 6.6 S	6.6 S
L1	39.1	8.4	96.8	74.3	45.0	57.9
L2	35.1	41.8	46.9	49.6	58.8	54.5
L3	20.1	3.9	88.8	34.0	21.8	23.1
L4	17.0	6.0	77.4	49.7	33.9	35.1
L5	16.0	10.4	53.3	12.3	15.5	11.5
L6	9.7	6.1	33.9	37.3	10.2	30.2
L7	8.0	7.3	41.6	7.7	21.9	16.7
L8	6.0	6.0	33.5	27.9	29.4	13.6
L9	5.6	1.8	71.1	53.0	39.8	29.2
L10	5.4	6.5	64.6	36.5	49.2	27.8
L11	5.1	6.9	48.4	49.7	37.5	42.4
L12	5.1	2.5	88.3	33.2	30.8	16.1
L13	2.5	2.2	15.8	46.2	0.2	30.9
L14	2.4	1.1	58.6	2.2	29.0	13.5
L15	2.4	No peak	0	49.7	NA	27.3
L16	2.4	4.1	60.9	46.9	36.9	19.3
L17	1.5	No peak	27.2	28.7	NA	20.9
L18	1.3	No peak	72.6	56.1	NA	81.5
L19*	No peak	No peak	NA	64.2	NA	72.8
L20*	No peak	No peak	NA	73.3	NA	71.5
L21	No peak	No peak	NA	22.8	NA	16.4
Aggregated IgG	94.4	No peak	97.4	NA	NA	NA

* The values for these specimens are the average of 2 separate sucrose density gradient ultracentrifugation experiments. NA: Percentage decrease calculation is not applicable, since there was no peak in the fractions measured in 0.15 M NaCl. CLR: collagen-like region. RLU: relative light units. S: Svedberg units.

in 0.15 M NaCl. Assays so performed were compared with wells that had been treated as usual, without preincubation with 1.0 M NaCl. Results of these sets of immunoassays were equivalent, indicating that incubation of the coated wells in the presence of 1.0 M NaCl did not irreversibly alter or remove the C1q or CLR from the coated wells. We therefore attributed reductions in binding that were observed in high salt to changes in the IgG interaction with C1q or CLR, rather than to changes in the amount of C1q or CLR in each well.

Sucrose density gradient (SDG) ultracentrifugation was performed on sera from 21 lupus cases, including 10 of the 25 described above, to determine the size of IgG that binds to C1q and to CLR. Data from analysis of SDG fractions for binding to C1q and CLR in the presence of physiologic salt (0.15 M NaCl) and high salt (1.0 M NaCl) from illustrative SLE cases (L3, L21) are shown (Figures 2 and 3). A median of 5.1% of the total C1q binding IgG in 0.15 M NaCl consisted of immune complexes (> 6.6 S), with a range of 0 to 39.1% (Table 1). In 16 sera, the > 6.6 S material made up less than 10% of total C1q binding IgG, and for 3 sera (L19, L20, L21) there was no detectable C1q binding IgG that was > 6.6 S. High salt concentration decreased the binding of IgG to C1q in most SDG fractions. For the > 6.6 S fractions, the decrease in IgG binding to C1q in the presence of high salt ranged from 0 to 96.8% with a median reduction of 48.4% (Table 1). For the 6.6 S fractions, the decrease in IgG binding to C1q in high salt ranged from 2.2 to 74.3%, with a median reduction of 46.2%. The binding of IgG to CLR in the 6.6 S fractions was also decreased by 1.0 M NaCl, with a range of 11.5% to 81.5% and a median reduction of 27.3% in high salt.

Unexpectedly, some IgG that bound to CLR sedimented at > 6.6 S. The fast sedimenting IgG that bound to CLR in 0.15 M NaCl ranged from 0 to 41.8% of the total CLR binding IgG, with a median value of 3.9%. For 6 sera, including the 3 for which there was no > 6.6 S IgG that bound to intact C1q, there was no detectable IgG that bound to CLR among the > 6.6 S fractions. The binding of IgG to CLR in the > 6.6 S fractions was decreased by 1.0 M NaCl to a variable degree, ranging from 0.2 to 58.8%, with a median of 30.8%.

The percentage decrease of monomeric IgG (6.6 S) binding to C1q caused by high salt was compared with the percentage decrease in C1q binding of fast sedimenting IgG (> 6.6 S) caused by high salt. There was no significant correlation between the 2 ($r = 0.21$, $p = 0.40$), nor was there a significant correlation between the salt dependent decrease in the binding to CLR in the monomeric versus the fast sedimenting

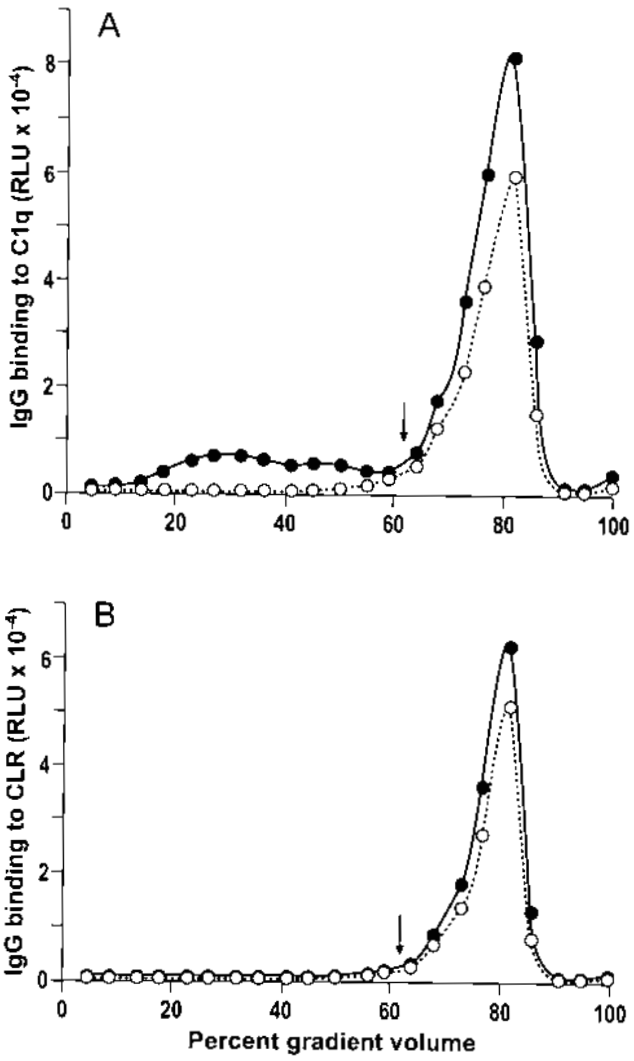

Figure 2. SDG separation of serum of patient L3. In panel A the fractions were analyzed for IgG binding to Clq, and in panel B for IgG binding to CLR. In both panels, solid circles (●—●) designate IgG binding in phosphate buffered 0.015 M NaCl and open circles (○—○) designate IgG binding in phosphate buffered 1.0 M NaCl. Vertical arrow indicates that fractions to the right were assigned to monomer IgG (6.6 S) and fractions to the left were assigned to polymer IgG (> 6.6 S). In this serum, the binding of immune complexes to C1q was largely abrogated in the presence of 1.0 M NaCl, but the binding of monomeric IgG to both C1q and to CLR was also reduced (see Table 1 for percentages). RLU: relative light units.

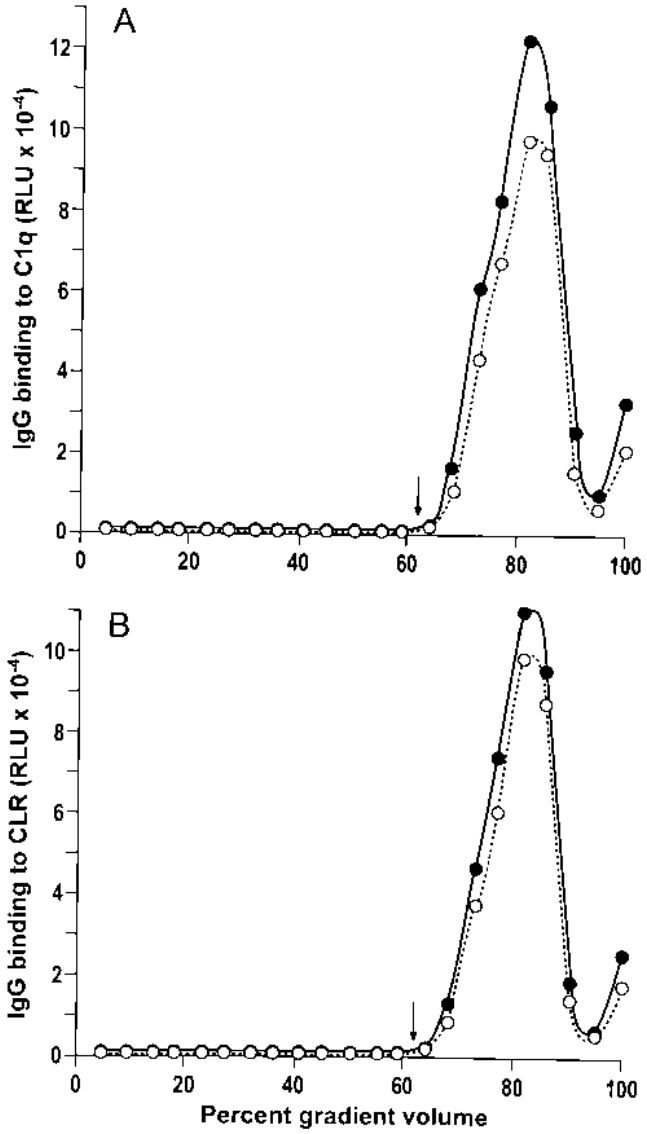

Figure 3. SDG separation of serum of patient L21. In panel A the fractions were analyzed for IgG binding to C1q and in panel B for IgG binding to CLR. See legend to Figure 2 for identification of the symbols. In this serum, the IgG binding to C1q and to CLR was confined to the monomeric IgG (6.6 S) and in both was somewhat reduced by the presence of 1.0 M NaCl (see Table 1 for percentages). RLU: relative light units.

IgG ($r = 0.49$, $p = 0.06$). It is to be expected that the reduction in binding caused by high salt in the unfractionated sera should correlate well with the sum of the reduction in the individual SDG fractions. These correlations hold true for both binding to C1q ($r = 0.83$, $p = 0.019$) and binding to CLR ($r = 0.97$, $p = 0.0003$).

Heat aggregated IgG was also fractionated by SDG ultracentrifugation and the fractions were tested for binding to C1q and CLR. As expected, almost all (94.4%) of the heat aggregated IgG that bound to C1q sedimented in the fractions > 6.6 S, and the binding of aggregated IgG to C1q was almost entirely abrogated (97.4% reduction) by 1.0 M NaCl (Figure 4). None of the heat aggregated IgG in either the > 6.6 S fractions or the 6.6 S fractions bound to CLR.

DISCUSSION

The composition of the C1q binding monomeric IgG in SLE sera was clarified in the late 1980s. Monomeric IgG from SLE sera was found to bind to solid-phase but not fluid-phase C1q, and it was shown that IgG and $F(ab')_2$ fragments of IgG from sera of patients with SLE bind to the CLR rather than to the globular heads of C1q. The binding of isolated monomeric autoantibodies to CLR was found to persist in 1.0 M NaCl, while the binding to C1q of heat aggregated human IgG, a sur-

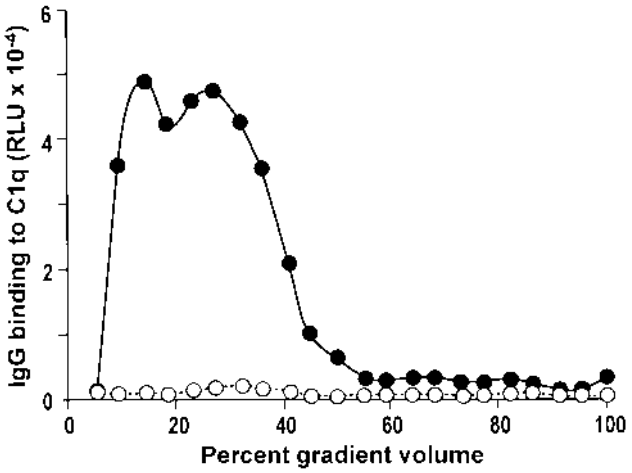

Figure 4. SDG separation of heat aggregated human IgG. The fractions were analyzed for IgG binding to C1q in the presence of phosphate buffered 0.15 M NaCl (●—●) and in the presence of phosphate buffered 1.0 M NaCl (○—-○). Binding of heat aggregated IgG to C1q is nearly completely abrogated by 1.0 M NaCl. RLU: relative light units.

rogate for immune complexes, was abrogated in the presence of 1.0 M NaCl[4,6]. Other investigators found that the binding of model immune complexes (prepared with rabbit antibodies) to C1q was decreased by > 90% in the presence of 0.75 M NaCl and abrogated totally by 1.2 M NaCl[16]. The same authors observed that the binding to C1q of one Type II cryoglobulin and one Type III cryoglobulin decreased by 90% in the presence of 0.75 M NaCl. Collectively, these observations suggested that immune complexes would not contribute to C1q binding in high salt, whereas binding of antibodies to C1q would be unaffected by high salt. Based on these findings, investigators have used binding of serum IgG to intact C1q in the presence of high salt (0.75–1.0 M NaCl) as a measure of autoantibodies to C1q, with the implicit assumption that binding of immune complexes to C1q is prevented by the high NaCl concentration. In published series of cases of SLE, the prevalence of anti-C1q measured using purified CLR as antigen has varied from 34 to 47% with antibody activity measured in 0.15 M NaCl[8,9,21], and was 28 to 35% when 0.75 M NaCl was used to test for binding to CLR[22,23]. Using high salt concentration during measurement of IgG binding to C1q, the prevalence of elevated results in sera from patients with SLE has been similar, with a range of 17 to 56%[7,10,11,13-16]. Because of substantial differences in patient selection in the different series, it is unclear the degree to which differences in detection techniques and in patient populations influenced the observed differences in the prevalence of C1q autoantibodies. Since high salt (0.75 M NaCl) has been shown to decrease binding of anti-C1q by 50 to 60% in serum from a patient with hypocomplementemic urticarial vasculitis[16], a condition known to be associated with anti-C1q[22], it is possible that measurement of anti-C1q in high salt could result in a lower percentage of positive results compared with measuring anti-C1q in 0.15 M NaCl in a test system using CLR. We are not aware of publications exploring in detail the salt dependent reduction in binding of IgG to CLR and C1q in sera of SLE patients.

In view of previous findings and approaches to detecting anti-C1q, we determined how the binding of IgG in lupus sera to C1q and CLR changed in the presence of high salt concentrations. If the assumptions of previous investigators are correct that binding to intact C1q in high salt is equivalent to measurement of anti-C1q by binding to CLR, then binding of fast sedimenting > 6.6 S IgG (immune complexes) to C1q should be uniformly and completely eliminated in high salt, and binding of IgG in 6.6 S SDG fractions to CLR and to C1q should be unaffected by high salt. In contrast to those predictions, sera differed in their responses to high salt. We found that there was incomplete and variable reduction of binding of > 6.6 S IgG to C1q in high salt, and 6.6 S IgG binding both to C1q and to CLR was variably affected by high salt. In spite of these observations, the measurements of anti-C1q by binding to CLR were highly correlated with results of binding to intact C1q in high salt. In addition, these measurements were highly correlated with C1q binding in physiological salt concentrations, reflecting that the majority of C1q binding IgG in SLE sera represented anti-C1q rather than immune complexes. There was poor correlation between the salt sensitivity of the monomeric (6.6 S) and of the immune complex (> 6.6 S) fractions from the sucrose density gradients in binding to C1q or to CLR.

Our data challenge the validity of the approach of previous investigators who considered measurement of binding of IgG to intact C1q in the presence of high salt concentrations as the equivalent of measuring anti-C1q by binding to CLR. On the other hand, testing for anti-C1q by binding to CLR in SLE sera correlates highly with solid-phase C1q binding assays when the C1q binding test is performed in normal or high salt concentrations. Therefore, conclusions concerning the clinical associations and significance of anti-C1q that were drawn from previous observations measuring IgG binding to C1q in high salt as a surrogate for measuring the binding to CLR are probably valid.

Unexpectedly, we also observed IgG binding to CLR in the > 6.6 S fast sedimenting fractions. The mechanism for the high molecular weight IgG binding to CLR is unknown. It is possible that anti-C1q is contained in some immune complexes. Previous studies, including those performed in our laboratory, would not have detected these possibilities, since only monomeric IgG binding to CLR was investigated. If immune complexes existed in serum with bound C1q, then antibodies to C1q could bind to these complexes. In the presence of 1.0 M NaCl the binding of C1q to immune complexes might be disrupted. This in turn would lead to dissociation of anti-C1q from C1q, since antibodies to C1q do not bind to free, fluid phase C1q. The free antibodies to C1q would then bind to the solid-phase C1q or to CLR in the test system. Alternatively, antibodies to C1q could bind to other serum proteins or protein

complexes, which would increase the rate of sedimentation. Studies by Menzel, *et al* raise the possibility that immune complexes containing fibronectin may bind to the triple-helical CLR of C1q, which would then bind antibodies to C1q[24]. These possibilities should be the focus of further investigation.

The binding of monomeric IgG fractions from SLE sera to C1q and to CLR represents autoantibodies to the CLR of C1q. As noted, the binding of these autoantibodies to the isolated CLR or to the CLR of intact C1q was variably decreased by the presence of 1.0 M NaCl. The epitopes on CLR that interact with antibodies to C1q are not known and may vary among patients with these autoantibodies. The binding of many antibodies to their antigens is not perturbed by the presence of 1.0 M NaCl. On the other hand, the interaction of some antibodies to double-stranded DNA in patients with SLE is known to decrease in the presence of high salt concentration[25]. The possibility of charge-charge interactions in the union of DNA and antibodies to DNA has been suggested as the cause of this observation. Similarly, charge-charge interactions may also exist in the binding of antibodies to C1q and therefore be diminished by the presence of high salt concentrations.

We observed that binding of immune complexes in sera from patients with SLE to intact C1q is incompletely eliminated by high salt concentrations. Further, the binding of anti-C1q both to intact C1q and to CLR can be reduced variably by high salt concentrations. Nevertheless, binding of IgG to intact C1q in high salt correlates with but is not the equivalent of direct measurement of anti-C1q by use of purified CLR of C1q. Future studies of anti-C1q ideally should employ purified CLR as the antigen, since binding of IgG to C1q in high salt is not always a reliable indicator of anti-C1q.

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REFERENCES

1. Theofilopoulos AN, Dixon FJ. The biology and detection of immune complexes. Adv Immunol 1979;28:89-220.
2. Wener MH. Tests for circulating immune complexes and autoantibodies to C1q. In: Rose NR, deMacario E, Folds JD, Lane HC, Nakamura RM, editors. Manual of clinical laboratory immunology. 5th ed. Washington: ASM Press; 1997.
3. Uwatoko S, Aotsuka S, Okawa M, et al. Characterization of C1q-binding IgG complexes in systemic lupus erythematosus. Clin Immunol Immunopathol 1984;30:104-16.
4. Uwatoko S, Aotsuka S, Okawa M, et al. C1q solid-phase radioimmunoassay: evidence for detection of antibody directed against the collagen-like region of C1q in sera from patients with systemic lupus erythematosus. Clin Exp Immunol 1987;69:98-106.
5. Wener MH, Mannik M, Schwartz MM, Lewis EJ. Relationship between renal pathology and the size of circulating immune complexes in patients with systemic lupus erythematosus. Medicine (Baltimore) 1987;66:85-97.
6. Uwatoko S, Mannik M. Low-molecular weight C1q-binding immunoglobulin G in patients with systemic lupus erythematosus consists of autoantibodies to the collagen-like region of C1q. J Clin Invest 1988;82:816-24.
7. Siegert C, Daha M, Westedt ML, van der Voort E, Breedveld F. IgG autoantibodies against C1q are correlated with nephritis, hypocomplementemia, and dsDNA antibodies in systemic lupus erythematosus. J Rheumatol 1991;18:230-4.
8. Menzel JE, Schezak O, Kolarz G, Gamerith F, Youngchaiyud U. A method to differentiate between anti-C1q antibodies and C1q-binding immune complexes using collagenase-digested solid phase C1q. J Immunol Methods 1991;138:165-71.
9. Wener MH, Uwatoko S, Mannik M. Antibodies to the collagen-like region of C1q in sera of patients with autoimmune rheumatic diseases. Arthritis Rheum 1989;32:544-51.
10. Siegert CEH, Daha MR, Tseng C, Coremans IEM, van Es LA, Breedveld FC. Predictive value of IgG autoantibodies against C1q for nephritis in systemic lupus erythematosus. Ann Rheum Dis 1993;52:851-6.
11. Coremans IEM, Spronk PE, Bootsma H, Daha MR, van der Voort E, Kater L, Breedveld FC, Kallenberg CGM. Changes in antibodies to C1q predict renal relapses in systemic lupus erythematosus. Am J Kidney Dis 1995;26:595-601.
12. Mannik M, Wener MH. Deposition of antibodies to the collagen-like region of C1q in renal glomeruli of patients with proliferative lupus glomerulonephritis. Arthritis Rheum 1997;40:1504-11.
13. Siegert C, Daha M, van der Voort E, Breedveld FC. IgG and IgA antibodies to the collagen-like region of C1q in rheumatoid vasculitis. Arthritis Rheum 1990;33:1646-54.
14. Cook AD, Rowley MJ, Wines BD, Mackay IR. Antibodies to the collagen-like region of C1q and type II collagen are independent non-cross-reactive populations in systemic lupus erythematosus and rheumatoid arthritis. J Autoimmun 1994;7:369-78.
15. Norsworthy P, Theodoridis E, Botto M, et al. Over-representation of the Fc gamma receptor type IIA R131/R131 genotype in caucasoid systemic lupus erythematosus patients with autoantibodies to C1q and glomerulonephritis. Arthritis Rheum 1999;42:1828-32.
16. Haseley AL, Wisnieski JJ, Denburg MR, et al. Antibodies to C1q in systemic lupus erythematosus: characteristics and relation to Fc RIIA alleles. Kidney Int 1997;52:1375-80.
17. Wener MH, Mannik M. C1q autoantibodies. In: Peter JB, Shoenfeld Y, editors. Autoantibodies. Amsterdam: Elsevier; 1996.
18. Reid KBM. C1q. Methods Enzymol 1982;82:319-24.
19. Reid KBM. Isolation by partial pepsin digestion of the three collagen-like regions present in subcomponent C1q of the first component of human complement. Biochem J 1976;155:5-17.
20. Wener MH, Mannik M. Selective losses of large immune complexes during density gradient ultracentrifugation and an approach for prevention of these losses. J Immunol Methods 1985;84:1-10.
21. Frémeaux-Bacchi V, Weiss L, Demouchy C, Blouin J, Kazatchkine MD. Autoantibodies to the collagen-like region of C1q are strongly associated with classical pathway-mediated hypocomplementemia in systemic lupus erythematosus. Lupus 1996;5:216-20.
22. Wisnieski JJ, Jones SM. IgG autoantibody to the collagen-like region of C1q in hypocomplementemic urticarial vasculitis syndrome, systemic lupus erythematosus, and six other musculoskeletal or rheumatic diseases. J Rheumatol 1992;19:884-8.
23. Wisnieski JJ, Jones SM. Comparison of autoantibodies to the collagen-like region of C1q in hypocomplementemic urticarial vasculitis syndrome and systemic lupus erythematosus. J Immunol 1992;148:1396-403.
24. Menzel EJ, Smolen JS, Liotta L, Reid KBM. Interaction of fibronectin with C1q and its collagen-like fragment (CLF). FEBS Lett 1981;129:188-92.
25. Smeenk RJT, Aarden LA, van Oss CJ. Comparison between dissociation and inhibition of association of DNA/anti-DNA complexes. Immunol Commun 1983;12:177-88.