Low-molecular weight C1q-binding Immunoglobulin G in Patients with Systemic Lupus Erythematosus Consists of Autoantibodies to the Collagen-like Region of C1q

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Abstract

The majority of C1q-binding IgG in sera of some patients with systemic lupus erythematosus (SLE) cosediments with monomeric IgG. This study was undertaken to provide definitive proof that the low-molecular weight C1q-binding IgG consists of autoantibodies to C1q. Monomeric C1q-binding IgG was isolated from five SLE plasmas by C1q affinity chromatography and gel filtration. All C1q-binding IgG preparations and their $F(ab')_2$ fragments bound to both C1q and the collagen-like region of C1q by an ELISA. To rule out the possibility that small DNA–antiDNA immune complexes caused this binding activity, Fab' fragments of the C1q-binding IgG preparations were digested with DNase I to degrade any DNA. The Fab' fragments continued to bind to C1q and its collagen-like region after this treatment. C1q-binding IgG was heterogenous on isoelectric focusing. Interaction of C1q-binding IgG with solid-phase C1q was retained in 1 M NaCl, whereas the binding of DNA or heat-aggregated IgG to solid-phase C1q was abrogated or markedly diminished. The association constant of C1q-binding IgG with solid-phase C1q was 2.7×10^7 M^{-1}.

We conclude that low-molecular weight C1q-binding IgG in the studied patients with SLE consists of autoantibodies to the collagen-like region of C1q.

Introduction

Systemic lupus erythematosus (SLE)[1] has been considered a prototype of human diseases mediated by immune complexes. Immune deposits in tissues may arise by local formation of immune complexes or by deposition of circulating immune complexes. To detect immune complexes in circulation, a number of biological assays have been designed and used to study patients with SLE (1). The ability of immune complexes to bind to C1q, a protein of the first component of the complement system, has been used to detect immune complexes. The C1q solid-phase assay (C1qSP) for immune complexes has been used extensively and has been suggested as a useful test for monitoring disease activity in patients with SLE (2, 3). Several investigations have indicated that some of the C1q-binding IgG in patients with SLE is of the same size as monomeric IgG (4–6). In patients with SLE, the presence of C1q-binding IgG that cosedimented with normal IgG was related to proliferative glomerular lesions, accompanied by mesangial and subendothelial glomerular immune deposits on electron microscopy (7). Two recent observations suggested that the C1q-binding IgG, which cosediments with normal IgG, consists of autoantibodies to C1q in patients with SLE. First, the $F(ab')_2$ fragments of IgG containing the C1q-binding material continued to bind to C1q (5). Second, this material bound to the collagen-like region of C1q (CLR) rather than to the globular regions of C1q that are known to bind immune complexes (8).

This investigation was undertaken to isolate by affinity chromatography the C1q-binding IgG from a small number of patients with SLE and to obtain definitive proof that these IgG molecules are autoantibodies to C1q. Studies were carried out to show that small immune complexes, particularly those containing a small segment of DNA and one molecule of antibody to DNA, were not present in the isolated material. Furthermore, the isolated IgG molecules were heterogeneous on isoelectric focusing (IEF) and their binding to C1q was not consistent with charge–charge interactions. Therefore, the presented data indicate that the monomeric C1q-binding IgG in patients with SLE consists of autoantibodies directed to the collagen-like region of C1q.

Methods

Preparation of C1q and CLR. C1q was purified from outdated human plasma with affinity chromatography on a human IgG agarose column by the method of Kolb et al. (9). CLR was prepared by pepsin digestion of C1q (30:1, wt/wt) at 37°C for 20 h and gel filtration on Sephacryl S-300 (Pharmacia Fine Chemicals, Piscataway, NJ) as described by Reid (10). The purity and functional activity of these preparations were confirmed as previously described (8). The concentrations of C1q and CLR were determined by absorbances at 280 nm ($E^{1\%}$/1 cm = 6.82) (11) and at 275 nm ($E^{1\%}$/1 cm = 2.1) (12), respectively.

C1qSP and CLR solid-phase assays. The solid-phase RIA were performed by a modification of the methods of Hay et al. (13) as previously described (8). In brief, C1q- or CLR-coated tubes were prepared by incubating 1 ml of C1q or CLR solution (5 μg/ml in 0.15 M Tris-HCl buffer, pH 7.6 [TB]) in polystyrene tubes (12 × 75 mm,

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1. *Abbreviations used in this paper:* AHG, heat-aggregated IgG; 1% BSA-TB, TB containing 1% BSA; CFII, human Cohn fraction II; CLR, collagen-like region of C1q; C1qSP, C1q solid-phase assay; dsDNA, double-stranded DNA; HVUS, hypocomplementemic vasculitis-urticaria syndrome; IEF, isoelectric focusing; PBS-E, 5 mM phosphate and 0.15 M NaCl, pH 7.4, containing 10 mM EDTA; SDG, sucrose-density gradient; SLE, systemic lupus erythematosus; TB, 0.15 M Tris-HCl buffer, pH 7.6; TBS, 15 mM Tris-HCl buffer and 0.15 M NaCl, pH 7.6.

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Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) for 2 h at 37°C and for 18 h at room temperature. Unreacted sites on the tubes were then covered with BSA by incubating the tubes with TB containing 1% BSA (1% BSA-TB). 1 ml of the test samples was added to the tubes and incubated for 2 h at 37°C. The amount of IgG bound to the tubes was determined by measuring ^{125}I-labeled F(ab')$_2$ fragments of anti-IgG retained on the tubes. Binding of IgM to solid-phase C1q was assayed using ^{125}I-labeled F(ab')$_2$ fragments of goat antibodies to human Fcμ (Jackson Immuno Research Laboratories, Inc., Avondale, PA).

The ELISA were performed as follows. Flat-bottomed microtiter plates (Immunoplate II; Nunc, Roskilde, Denmark) were coated by overnight incubation with C1q or CLR using 50 μl/well at 20 μg/ml in TB. After washing three times with Tris-buffered saline (TBS, 15 mM Tris-HCl buffer and 0.15 M NaCl, pH 7.6), the wells were filled with 100 μl of 1% BSA-TB and incubated for 1 h at room temperature. After aspiration, samples were added at 50 μl/well in 1% BSA-TB and incubated overnight at 4°C. After being washed with TBS containing 0.1% BSA and 0.1% Triton X-100 (washing buffer), horseradish peroxidase-conjugated F(ab')$_2$ fragments of goat antibodies to human Fcγ or F(ab')$_{2\gamma}$ (Cappel Laboratories, Malvern, PA), diluted 1:2,000 in 1% BSA-TB were added and incubated for 4 h at room temperature. After a final wash, the peroxidase substrate 2,2'-azino-di-[3-ethyl-benzthiazoline sulfonate] and H_2O_2 were added and absorbance at 405 nm was detected on a plate reading spectrophotometer (Bio-Tek Instruments, Inc., Burlington, VT).

C1q bound to Latex beads (Latex 0.81; Difco Laboratories, Detroit, MI) was prepared for the inhibition study (described in Results) by incubating C1q with Latex at 4°C overnight and centrifuging for 15 min at 3,000 rpm and washing the Latex with 1% BSA-TB three times. The amount of C1q bound to Latex was quantitated using ^{125}I-labeled C1q.

Isolation of C1q-binding IgG. A C1q affinity column (1.0 × 12.7 cm, containing 2.3 mg C1q/ml of Sepharose CL-4B [Pharmacia Fine Chemicals]) was prepared using the protein coupling procedure previously described (14). 90–200-ml aliquots of the sera or plasmas containing 10 mM EDTA were applied to the C1q affinity column, equilibrated with PBS-E (5 mM phosphate and 0.15 M NaCl, pH 7.4 containing 10 mM EDTA), and run at the flow rate of 4 ml/h. The column was washed with PBS-E until the absorbance of the effluent was < 0.05 at 280 nm. The bound proteins were eluted with 1 M sodium thiocyanate and 0.04 M Tris-HCl, pH 7.6. The eluates were dialyzed against PBS-E, concentrated by ultrafiltration with membranes (YM30; Amicon Corp., Danvers, MA) and divided into several aliquots. Each aliquot was applied to a 1.6 × 90 cm Sephadex G-200 column, equilibrated with PBS-E, and operated at the flow rate of 8.4 ml/h. The fractions were collected into four pools (I–IV), concentrated by ultrafiltration, and stored at −70°C. C1q-binding IgG was obtained as pool III, consisting of fractions corresponding to monomeric IgG.

F(ab')$_2$ fragments of C1q-binding IgG were prepared by digestion with pepsin (Worthington Diagnostics Div., Freehold, NJ) at a 1:10 pepsin/protein weight ratio for 20 h at 37°C. The digestion mixtures were applied to an HPLC (Waters Associates, Millipore Corp., Milford, MA) gel filtration column (Superose 6; Pharmacia Fine Chemicals). The material eluted at the same time as control F(ab')$_2$ fragments and no peak corresponding to undigested IgG was detected. Fab' fragments were obtained from the F(ab')$_2$ fragments by reduction and alkylation of F(ab')$_2$ fragments (15). Analysis of the F(ab')$_2$ and Fab' preparations in SDS-PAGE in the presence of 10^{-4} M iodoacetamide showed no intact IgG in either preparations and confirmed the reduction and alkylation of Fab'. The digestion of the Fab' preparation with pancreatic DNase I (Sigma Chemical Co., St. Louis, MO) to destroy any DNA that might be present was done as described by Emlen et al. (15).

IEF and immunoblotting. IEF was carried out with Pharmalytes, pI range 3–10 (Pharmacia Fine Chemicals) as previously described (16). Nonelectric transfer of proteins from IEF gels to nitrocellulose paper was performed by the method of Reinhart and Malamud (17). Enzyme immunodetection on nitrocellulose paper was performed essentially as described by Natori et al. (18). Briefly, after the transfer, the paper was placed in the washing buffer. After 5 min incubation at room temperature, the paper was incubated for 2 h at room temperature with horseradish peroxidase-conjugated goat antibodies to human Fcγ or to HSA (Cappel Laboratories), diluted 1:1,000 and 1:100 in 1% BSA-TB, respectively, and then washed three times. Color was developed by incubating the paper with substrate solution (0.05 M Tris-HCl, pH 7.4, 0.2 M NaCl, 0.06% 4-chloro-naphthol, and 0.01% H_2O_2) for 5–10 min at room temperature.

HPLC gel filtration under dissociating condition. 200 μl of pool III from patient M.J. was applied to a Superose 6 column equilibrated with PBS containing 6 M urea and 10^{-4} M iodoacetamide. The flow rate was 0.35 ml/min, and 0.53 ml fractions were collected. The fractions were dialyzed against PBS containing 10^{-4} M iodoacetamide. As control, an aliquot of the sample was also fractionated without 6 M urea on the same column. Fractions from both experiments were analyzed for C1q-binding.

Other methods. Monomeric IgG, heat-aggregated IgG (AHG), HSA, rabbit antibodies to HSA, and monoclonal IgM were prepared as previously described (14, 19, 20). Purified polyclonal human IgM (Behring Diagnostics, American Hoechst Corp., San Diego, CA), human Cohn fraction II (CF II) (Miles Scientific Div., Miles Laboratories, Inc., Naperville, IL) and antisera to human IgG, IgM, C1q, fibronectin, fibrinogen, and whole serum (Behring Diagnostics) were purchased. ^{125}I-double-stranded DNA (dsDNA) was prepared by previously described methods (15) and generously provided by Dr. Carol Horgan (University of Washington).

Proteins except C1q were labeled with ^{125}I by the iodine monochloride method (21). Radioiodination of C1q was performed in lactoperoxidase-catalyzed reactions as described by Heusser et al. (22). For SDS-PAGE analysis, preformed 4–30% polyacrylamide gradient gels (PAA 4/30; Pharmacia Fine Chemicals) were used. Quantification of IgG and IgM was performed using a sandwich immunoradiometric assay (19). Sucrose-density gradient (SDG) ultracentrifugation was performed as previously described (19). The association constant for the C1q-binding IgG interaction with solid-phase C1q was determined by Scatchard analysis as previously described (23). IgM rheumatoid factor was measured by an RIA as described by Wernick et al. (24).

Sera and plasmas. Plasmas were obtained from five SLE patients (S.D., G.S., J.B., M.J., and K.M.) who underwent plasmapheresis for therapeutic reasons determined by their attending physicians. Three plasmas (those of S.D., G.S., and J.B.) were converted to sera by addition of $CaCl_2$ to a final concentration of 10 mM. On SDG ultracentrifugation of these sera and plasmas, the predominant peak of C1q-binding activity cosedimented with monomeric IgG. All patients satisfied American Rheumatism Association criteria for the classification of SLE (25). A normal plasma sample was obtained from a subject (A.J.) who underwent plasmapheresis during pregnancy due to Rh incompatibility. The specimens were stored at −20°C until used. The selection of all plasma samples was based solely on availability and no other criteria were used.

Statistical analysis. The statistical differences were analyzed by paired *t* test.

Results

Isolation of C1q-binding IgG. C1q affinity column chromatography was used as the first step to purify C1q-binding IgG from SLE sera or plasmas. 90–200 ml of individual specimens, containing 10 mM EDTA, were applied to the C1q affinity column equilibrated with PBS-E. The elution of C1q affinity column with 1 M sodium thiocyanate and 0.04 M Tris-HCl, pH 7.6 revealed a single peak (Fig. 1). The C1q affinity column was overloaded with each specimen since 23–63% of C1q-binding IgG of the applied specimens remained in the fall-

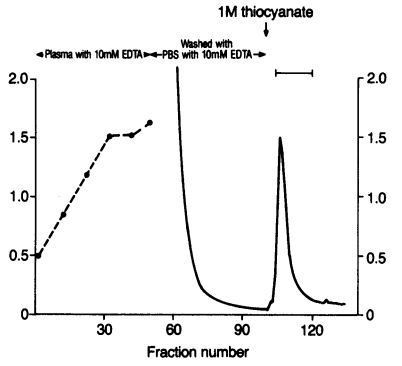
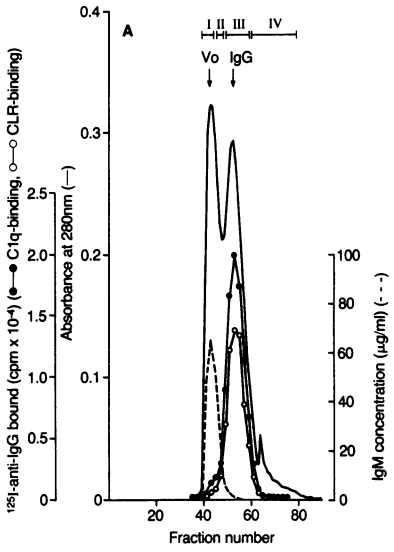


Figure 1. C1q-Sepharose CL-4B affinity chromatography of an SLE plasma. 100 ml SLE plasma (from patient M.J.) containing 10 mM EDTA was applied to a 1.0 × 12.7 cm C1q-Sepharose CL-4B column, equilibrated with PBS-E. The flow rate of the column was 4 ml/h and two fractions were collected per hour. After being washed with PBS-E, the column was eluted with 1 M sodium thiocyanate and 0.04 M Tris-HCl, pH 7.6. C1q-binding activity (^{125}I-anti-IgG bound, cpm × 10^{-3}) of the fall-through fractions is shown by the dotted line, indicating that the column was overloaded. ——, absorbance at 280 nm. The eluted peak, designated with a bar, was processed for gel filtration.



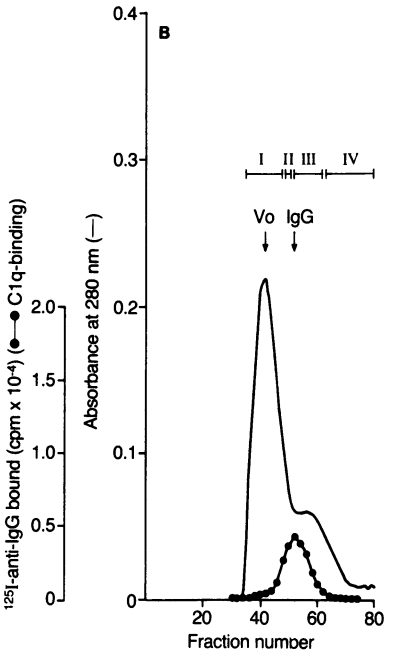


Figure 2. Sephadex G-200 gel filtration of the eluates from the C1q-Sepharose CL-4B affinity column. Eluates were dialyzed against PBS-E, concentrated by ultrafiltration, and applied to a 1.6 × 90 cm Sephadex G-200 column, equilibrated with PBS-E. The flow rate was 8.4 ml/h, and 2.1-ml fractions were collected. Absorbance at 280 nm (——), C1q-binding IgG (— ● —), CLR-binding IgG (— o —), and concentration of IgM (- - -) are shown. Monomeric IgG is contained in pool III. (*A*) Pattern for patient M.J. (*B*) Pattern for K.M.

through fractions. These calculations were based on the concentrations of C1q-binding IgG and volumes of applied materials and fall-through fractions. Furthermore, the C1q-binding IgG was decreased in initial fall-through fractions, and in later fractions the concentration of this material was the same as in the applied plasma. More than 85% of C1q-binding IgG removed from the applied sera or plasmas were recovered in the eluates. 1 M sodium thiocyanate and 0.04 M Tris-HCl, pH 7.6 was used for eluting the proteins bound to the C1q affinity column, because in pilot studies with SLE sera, 95.1% of IgG bound to C1q-coated polystyrene tubes was eluted from the tubes by 1 M thiocyanate, whereas 83.1% of the bound IgG was eluted by 0.1 M glycine-HCl, 0.15 M NaCl, pH 2.5.

As the second step of purification, gel filtration on a Sephadex G-200 column was performed to obtain monomeric C1q-binding IgG. On Sephadex G-200 gel filtration, the eluates from the C1q affinity column were resolved into two major peaks that eluted respectively in the void volume and the elution volume at which known monomeric IgG is recovered (Fig. 2). The C1q-binding IgG was centered in the second peak of protein in three specimens, and in two others the second peak of protein was not prominent. In all five specimens, however, the C1q-binding IgG peaked in the fractions corresponding to the elution volume of monomeric IgG. Four pools were created by combining the fractions of the first peak (pool I) between the first and second peaks (pool II), the second peak (pool III), and the proteins eluting after the second peak (pool IV), and concentrated. The final yields of C1q-binding IgG in pool III for the five specimens from patients with SLE were 3.5–13.0 μg/ml of serum or plasma applied to the C1q affinity column (Table I). C1q-binding IgG was also isolated from the plasma of a normal subject (A.J.), using the identical purification procedure and yielding pool III, 3.4 μg/ml of plasma (Table I).

IgM was detected in the first peak of the gel filtration pattern. This IgM bound to C1q comparable to purified polyclonal or monoclonal IgM. By a sensitive RIA, IgM rheumatoid factor activity was not present in the excluded peak of gel filtration.

SDS-PAGE, IEF, and immunological analysis. Purity of C1q-binding IgG in pool III from all specimens was investigated by double immunodiffusion analyses in agarose, immunoelectrophoresis, and SDS-PAGE. Pool III showed a single major band corresponding to IgG on SDS-PAGE under nonreducing conditions, together with two other faint bands with apparent molecular weights of 140,000 and 67,000 (Fig. 3, lane *B*). Two bands corresponding to gamma and light polypeptide chains were visualized upon reduction with 2-mercaptoethanol (Fig. 3, lane *D*). Double immunodiffusion and immunoelectrophoresis of pool III revealed a single precipitin line with antibodies to human IgG or antibodies to normal human serum, and no precipitin lines were seen when tested against antibodies to IgM or antibodies to HSA. ^{125}I-labeled pool III showed a single symmetrical peak in the 7S region on SDG ultracentrifugation.

SDS-PAGE analysis of pool I under nonreducing conditions exhibited several bands with apparent molecular weights larger than that of IgG, the major band of which corresponded to IgM (Fig. 3, lane *A*). Several bands other than mu and light

Table I. Yield of C1q-binding IgG from Plasma of Patients with SLE

Patient	C1q-binding activity in serum or plasma*	Amount of isolated C1q-binding IgG
	μg AHG equivalent/ml	*μg/ml of serum or plasma*
M.J.	64.0	13.0
S.D.	7.5	4.5
G.S.	26.2	6.8
K.M.	8.3	3.5
J.B.	47.0	10.7
A.J. (normal)	5.4	3.4

* C1q-binding activity in serum or plasma was tested by ELISA and standardized using AHG.

polypeptide chains were present under reducing conditions (Fig. 3, lane *C*). Double immunodiffusion analysis of pool I showed precipitin lines with antibodies to IgM, fibrinogen, and fibronectin.

The electrophoretic heterogeneity of C1q-binding IgG was investigated to determine if charge–charge interactions might be involved in binding of anionic IgG to the relatively cationic C1q. IEF, followed by protein staining of pool III, showed a polydispersed pattern characteristic of polyclonal IgG (Fig. 4). The same results were obtained when IEF was followed by immunoblotting assay with peroxidase-conjugated antibodies to human Fcγ (data not shown). By protein staining of the IEF gels, a distinct anionic band was present in all preparations of pool III and was more evident in pool IV, as illustrated with J.B. pools (Fig. 4). This anionic band was identified as HSA by immunoblotting assay, using peroxidase-conjugated antibodies to HSA for detection.

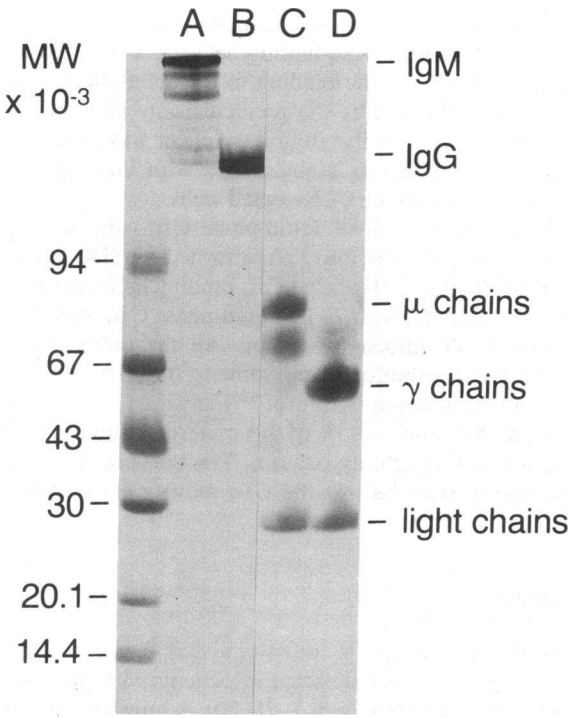


Figure 3. SDS-PAGE analysis of pool I and III from one patient (M.J.). 4–30% gradient SDS-polyacrylamide gels were used in the presence or absence of 2-mercaptoethanol. Lane *A*, nonreduced pool I; *B*, nonreduced pool III; *C*, reduced pool I; and *D*, reduced pool III.

Binding characteristics of C1q-binding IgG to solid-phase C1q or CLR. Pool III from all specimens was tested by ELISA for binding to C1q and CLR. All preparations of pool III bound to solid-phase C1q and CLR as detected by ELISA (Fig. 5, Table II). $F(ab')_2$ preparations of pool III retained the binding activity to solid-phase C1q and CLR as detected with antibodies to $F(ab')_{2\gamma}$. Nearly all the values for binding of $F(ab')_2$ fragments to C1q or to CLR were higher than the binding of intact molecules, but this difference did not reach statistical significance. The increased values are attributed to the presence of antibodies to the pepsin agglutinator site in horseradish peroxidase-conjugated $F(ab')_2$ fragments of goat antibodies to human $F(ab')_{2\gamma}$. These antibodies bind to the $F(ab')_2$ fragments, but not to the intact IgG molecules (26). The conversion of IgG molecules to $F(ab')_2$ fragments was nearly complete, since very little binding was detected with peroxidase-conjugated anti-human Fcγ.

To rule out the possible presence of immune complexes consisting of a small segment of DNA and antibodies to DNA, digestion with DNase I was used. Previous studies had shown that when antibodies to DNA were bound to DNA by monogamous bivalent binding, ~ 40 bp of DNA were protected from degradation by DNase and that conversion of the $F(ab')_2$ fragments to Fab′ fragments of the antiDNA molecules allowed degradation of the DNA by DNase I (15). Therefore, the $F(ab')_2$ fragments of pool III were first converted to Fab′ fragments by reduction and alkylation of disulfide bonds and then treated with DNase I. The binding of Fab′ fragments to C1q and to CLR was decreased, as compared with the binding of $F(ab')_2$ fragments (Table II). This decrease in binding may have resulted from decrease of the valence of the antibody molecules when converted to Fab′ fragments. The treatment of the Fab′ fragments with DNase, however, did not decrease their binding to C1q or to CLR. AHG bound to solid-phase C1q, but not to solid-phase CLR. Neither normal human monomeric IgG nor its $F(ab')_2$ and Fab′ fragments, all prepared from CFII, showed any binding to solid-phase C1q or CLR. In comparison, the IgG of pool III and $F(ab')_2$ and Fab′ fragments prepared from a normal plasma showed binding to C1q and to CLR. The yield of this material from normal plasma was less than that from most SLE plasmas.

To investigate if other unknown low-molecular weight antigens in small (e.g., Ag_1Ab_1) immune complexes could be responsible for the C1q-binding activity, pool III from patient M.J. was fractionated on a Superose 6 column in the presence or absence of 6 M urea. From both experiments, the fractions were assayed for C1q-binding activity. Superimposable C1q-binding IgG distributions were found in both studies, corresponding to monomeric IgG (data not shown).

The association constant of C1q-binding IgG with solid-phase C1q was $2.7 \times 10^7\ M^{-1}$, as determined by Scatchard plot analysis using ^{125}I-labeled pool III from patient M.J.

The isoelectric focusing patterns, as shown above, indicated that charge–charge interactions between C1q and the pool III IgG isolates were unlikely because the isolated IgG preparations showed electrophoretic heterogeneity. Charge–

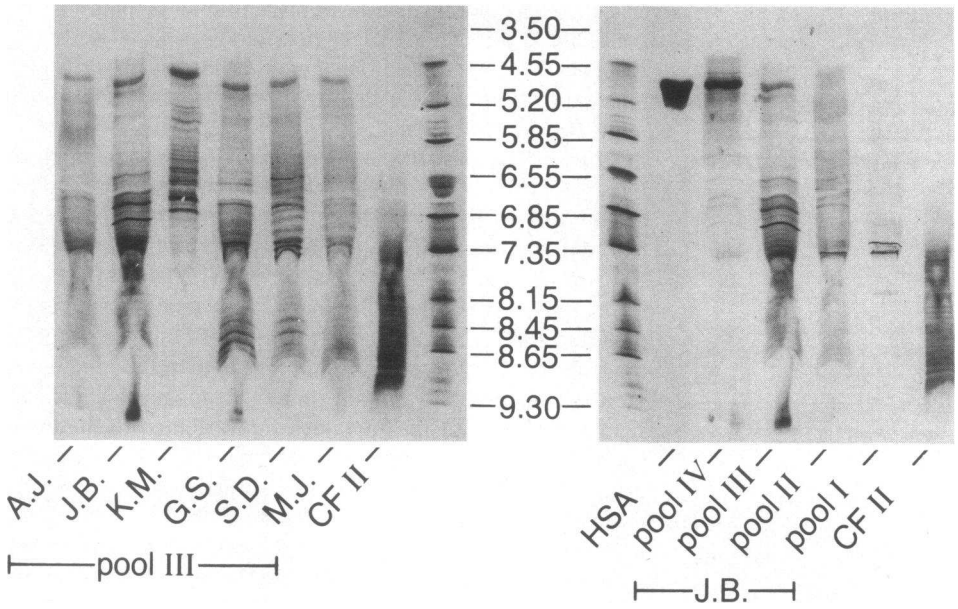


Figure 4. IEF patterns of purified C1q-binding IgG on the left and gel filtration pools from patient J.B. on the right. All preparations of pool III showed a polydispersed pattern. The anionic band seen in all preparations of pool III was more evident in pool IV, as seen in J.B. pools, and was identified as HSA.

charge interactions, however, can arise from a local charge effect of a sequence of cationic amino acids in a molecule that has a total neutral charge on isoelectric focusing, as illustrated by the presence of multiple lysines in the carboxy-terminal portion of platelet factor IV (27). Therefore, the binding of pool III IgG from one patient (M.J.) to C1q was examined under varying salt concentrations and compared with the binding of AHG and DNA to C1q. Pool III (M.J.), AHG, and ^{125}I-dsDNA were incubated with the solid-phase C1q in 5 mM Tris-HCl buffer, pH 7.6 containing various concentrations of NaCl. The binding of ^{125}I-dsDNA and AHG to solid-phase C1q was almost completely inhibited at the NaCl concentration of 0.25 and 1.0 M, respectively (Fig. 6). In comparison, the reactivity of pool III C1q-binding IgG with solid-phase C1q persisted at an NaCl concentration of 1.0 M.

Interaction of C1q-binding IgG with liquid-phase C1q. A previous study showed that the binding activity in SLE sera to the solid-phase C1q was not inhibited by the preincubation of the sera with liquid-phase C1q, suggesting that C1q-binding IgG is not bound to endogenous C1q (6). The inhibitory effect of liquid-phase C1q was investigated in this study using the purified C1q-binding IgG. The preincubation of 1 μg C1q-binding IgG with excess liquid-phase C1q (0–10 μg) had a limited inhibitory effect on binding of C1q-binding IgG to solid-phase C1q; ~ 0.1 μg of C1q was bound to individual wells, as estimated by binding of ^{125}I-labeled C1q. In comparison, Latex-bound C1q (0–5 μg) caused effective inhibition. This inhibition, however, was not complete for reasons that are not known. When the C1q-binding IgG was converted to Fab′ fragments, however, the binding to the solid-phase C1q was significantly inhibited by the preincubation with liquid-phase C1q (Fig. 7). A similar inhibitory effect was observed when C1q-binding IgG was preincubated with liquid-phase CLR and then added to the CLR-coated wells.

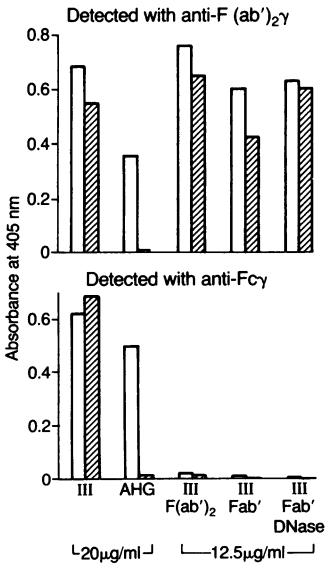


Figure 5. ELISA for binding of IgG or of IgG fragments of pool III (patient M.J.) to C1q- (□) or CLR- (▨) coated wells. The binding of pool III to solid-phase C1q or CLR persisted after pepsin digestion as detected with antibodies to $F(ab')_{2\gamma}$ (*top*). The completion of digestion was determined with antibodies to Fc_γ (*bottom*). To rule out binding mediated by small DNA fragments, the Fab′ fragments were treated with DNase I.

The limited interaction of liquid-phase C1q with the isolated C1q-binding IgG was also demonstrated by SDG ultracentrifugation. 0.39 μg ^{125}I-labeled C1q-binding IgG was incubated at 4°C overnight with 20 μg liquid-phase C1q and then submitted to SDG ultracentrifugation. All the radioactivity remained in the distribution of monomeric IgG. This finding did not result from denaturation of ^{125}I-labeled C1q-binding IgG by iodination, since > 95% of this material bound to C1q when applied to C1q affinity column. The binding of ^{125}I-labeled normal human IgG to the C1q affinity column was < 5%.

Discussion

C1q-binding IgG, which is indistinguishable in size from monomeric IgG, has been detected in patients with SLE with C1qSP by several workers (3–5, 7, 28–30). A number of possibilities have been suggested for the nature of this C1q-binding IgG. First, an in vivo alteration of the Fc region of IgG, which would then enhance the binding of the Fc region to C1q, has been proposed (29, 30). This mechanism has also been sug-

Table II. ELISA for Binding of Pool III IgG, F(ab')$_2$ Fragments, Fab' Fragments, or DNase-treated Fab' Fragments to C1q- or CLR-Coated Wells

Patient	C1q-binding activity				CLR-binding activity			
	III	III F(ab')$_2$	III Fab'	III Fab' + DNase	III	III F(ab')$_2$	III Fab'	III Fab' + DNase
M.J.	0.684*	0.760	0.598	0.631	0.548	0.651	0.421	0.599
	0.620*	0.020	0.009	0.007	0.687	0.013	0.001	0.000
S.D.	0.646	0.669	0.476	0.401	0.546	0.543	0.166	0.110
	0.653	0.033	0.037	0.025	0.616	0.023	0.007	0.014
G.S.	0.764	0.783	0.624	0.675	0.645	0.733	0.554	0.623
	0.658	0.019	0.008	0.011	0.598	0.019	0.003	0.008
K.M.	0.604	0.975	0.459	0.418	0.612	1.109	0.347	0.331
	0.701	0.040	0.026	0.032	0.454	0.033	0.018	0.018
J.B.	0.555	0.919	0.424	0.499	0.551	0.950	0.520	0.567
	0.529	0.023	0.012	0.010	0.777	0.020	0.006	0.012
Mean±SD	0.651±0.080	0.821±0.124‡	0.516±0.089§	0.525±0.124‖	0.580±0.045	0.797±0.230‡	0.402±0.155§	0.446±0.221‖
	0.632±0.064	0.027±0.009	0.018±0.013	0.017±0.011	0.626±0.119	0.022±0.007	0.007±0.007	0.010±0.007
A.J. (normal)	0.419	0.561	0.151	0.140	0.507	0.812	0.119	0.138
	0.717	0.031	0.025	0.031	0.457	0.021	0.009	0.017

* Values are optical absorbance at 405 nm. The upper and lower lines for each patient represent the data obtained with anti-F(ab')$_{2\gamma}$ and anti-Fcγ, respectively. ‡ Not significantly different as compared with the corresponding values of pool III. § $P < 0.05$ as compared with the corresponding values of F(ab')$_2$. ‖ Not significantly different from the corresponding values of Fab' before treatment with DNase.

gested for the low-molecular weight (7S) C1q-precipitins, encountered in patients with hypocomplementemic vasculitis-urticaria syndrome (HVUS) (30, 31). If altered Fc regions of selected IgG molecules were to account for the observed C1q-binding IgG, then this activity should be lost upon pepsin digestion of the isolated molecules and the isolated Fc fragments might continue to bind to C1q. Second, Agnello et al. have suggested that DNA–antiDNA immune complexes, consisting of one antibody and one short fragment of DNA, could be responsible for this C1q-binding activity (28). This suggestion was attractive because monomers of IgG react poorly in C1qSP, but DNA that independently binds to C1q could enhance the binding of these small complexes to C1q. If small DNA–antiDNA complexes were to account for the observed C1q-binding IgG, then this activity might persist after pepsin digestion, depending on the relative contributions of the Fc regions or the bound DNA fragment. If the activity is retained after pepsin digestion, then conversion of the F(ab')$_2$ fragments to Fab' fragments and digestion with DNase would abrogate the binding to C1q. Third, theoretically, other small antigen–antibody complexes could account for the low-molecular weight C1q binding. Fourth, anionic IgG molecules could bind to the relatively cationic C1q entirely by charge–charge interactions. This property would reside mainly in the F(ab')$_2$ fragments, because of the presence of the variable regions in the IgG molecule. Finally, another possibility is that the C1q-binding IgG represents antibodies directed against C1q (5).

In this study, C1q-binding IgG was purified by C1q affinity chromatography and gel filtration. Monomeric C1q-binding IgG was obtained as the second peak on gel filtration of the eluates from the C1q affinity column. Monomeric IgG eluted from the C1q affinity column bound avidly to the C1q affinity column when reapplied to it.

The binding of the F(ab')$_2$ fragments, prepared from C1q-

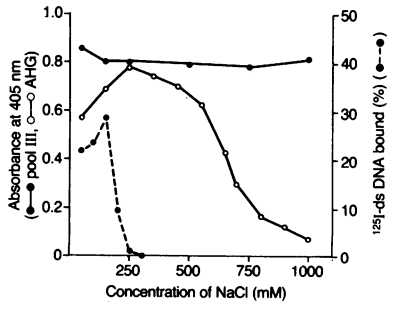


Figure 6. Effect of sodium chloride concentration on the binding of pool III IgG, AHG, and ^{125}I-DNA to solid-phase C1q. Pool III (M.J., 20 μg/ml), AHG (20 μg/ml) and ^{125}I-ds-DNA (5 ng/ml) were incubated with solid-phase C1q in 5 mM Tris-HCl buffer, pH 7.6, containing various concentrations of NaCl. Pool III binding (— ● —) was not disrupted by 1 M NaCl, but binding of AHG (— ○ —) was diminished by increasing salt concentration. The binding of dsDNA to C1q (– – ● – –), which is thought to be mediated by charge–charge interactions, was disrupted completely by 250 mM NaCl.

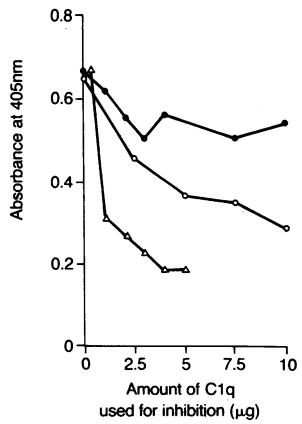


Figure 7. Inhibition of the binding of pool III (M.J.) IgG to the solid-phase C1q by liquid-phase C1q. Pool III (M.J., 1 μg) (— ● —) and its Fab' (0.625 μg) (— ○ —) were preincubated with various amounts of liquid-phase C1q (0–10 μg) at 4°C overnight and then added to the C1q-coated wells. Progressive inhibition of only Fab' fragments is evident. In comparison, when pool III (M.J., 1 μg) was preincubated with Latex-bound C1q (0–5 μg) (— △ —), effective inhibition was found.

binding IgG, to C1q or CLR was clearly not due to DNA–antiDNA immune complexes, which would bind to C1q via DNA. The DNase digestion did not decrease the binding activity of Fab' preparations to solid-phase C1q or CLR. The binding of dsDNA to C1q also decreased markedly by raising the ionic strength, which is consistent with the results obtained by van Schravendijk and Dwek (32). In comparison, the binding activity of C1q-binding IgG was not decreased by even 1 M NaCl. Furthermore, it has been suggested that the globular region of C1q, rather than CLR, is involved in DNA binding (32). The presence of small C1q-binding immune complexes was ruled out by the gel filtration experiment in 6 M urea. If immune complexes had been present, they must have contained a small antigen molecule and one antibody molecule, because at neutral pH, the isolated C1q-binding material possessed the size characteristics of monomeric IgG. Therefore, gel filtration in 6 M urea should have dissociated and separated from IgG a small antigen molecule. The results thus strongly argue that small immune complexes with an unknown antigen were not present.

The purified C1q-binding IgG did not consist of anionic IgG. Furthermore, studies with increasing concentrations of added salt ruled out charge–charge interactions between C1q and the isolated IgG.

The presented results thus show that the C1q-binding IgG in sera of patients with SLE consists of antibodies to C1q, directed against the CLR. Previous investigators had concluded that the observed phenomenon was not a result of antibodies to C1q. Agnello et al. detected 7S C1q-precipitins in SLE sera by double immunodiffusion assay (33). They dismissed the possibility of the presence of antibodies to C1q because, in one carefully studied patient, the binding of the 7S material to C1q was lost when the IgG was reduced and alkylated (33). In our studies, however, the C1q-binding activity persisted after reduction and alkylation and after conversion to $F(ab')_2$ fragments (5). Marder et al. purified 7S C1q-precipitins in SLE by using C1q-coated polystyrene beads, elution with 20 mM citrate buffer, pH 3.2, followed by Staphylococcal protein A affinity chromatography (30). They characterized the isolated 7S C1q-precipitins as a monomeric polyclonal IgG. On the basis of their earlier work, these authors suggested that the binding of IgG to C1q occurred by the Fc fragment. In that earlier study, the authors showed that 7S C1q-precipitins in HVUS lost the C1q-binding activity after pepsin digestion (31). The reasons for the loss of C1q-precipitating activity by these previous investigations are not entirely clear. The test system obviously was less sensitive than the C1q-binding assay used in this report. The methods were different and the previously studied specimens were obtained from patients with HVUS and not from patients with SLE.

C1q-binding IgG was also obtained in a small quantity from the plasma of an normal subject (A.J.). The Fab' fragments of C1q-binding IgG from A.J., however, showed a markedly reduced binding activity to C1q and CLR, as compared with those of the Fab' preparations of SLE patients. This finding suggests that antibodies to C1q also can occur in normal persons, but they may differ from those found in patients with SLE. The prevalence of antibodies to CLR among normal persons, however, has not been determined.

Previous reports indicated that the C1q-precipitins and C1q-binding IgG possessed preferential binding to C1q bound to a solid-phase (6, 29, 30), presumably resulting from exposure of antigenic determinants not present on liquid-phase C1q (30). Alternatively, preferential binding of antibodies to C1q in solid-phase may arise as a result of multivalent interaction with the C1q adherent to a surface (29). The presented results supported both possibilities. Radiolabeled C1q-binding IgG did not form complexes in liquid-phase, as determined by SDG ultracentrifugation. In addition, a large excess of liquid-phase C1q did not significantly inhibit the binding of antibodies to C1q to the solid-phase C1q or CLR, whereas Latex-bound C1q was an effective inhibitor. All of these findings support the notion that new antigenic determinants are exposed on C1q molecules when they become attached to a solid surface. On the other hand, when the antibodies to C1q were converted to univalent Fab' fragments, liquid-phase C1q was able to inhibit binding of these molecules to solid-phase C1q, albeit not very effectively. In vivo, the binding of the described antibodies to circulating C1q may be inhibited by the presence of C1r and C1s, which also bind to the CLR of the C1q molecule.

The IgM in the excluded protein peak on gel filtration bound to C1q in solid-phase comparable to pooled, normal human IgM and to a Waldenström's macroglobulin. Previous studies have shown that IgM molecules bind to C1q better than IgG molecules (34). Therefore, the available information does not argue for the presence of IgM antibodies to C1q. The reasons for the presence of fibrinogen and fibronectin in this fraction have not been examined. Interestingly, despite careful washing of the C1q columns before elution, small amounts of HSA were present in the eluate. This HSA was isolated from one pool III by affinity chromatography and did not differ from normal HSA on SDS-PAGE and on IEF. Furthermore, purified HSA did not bind to the C1q column. The binding of HSA to IgM or IgA by intermolecular disulfide bonds was excluded by showing that when the isolations were carried out in the presence of 10^{-4} M iodoacetamide, the HSA remained in monomeric distribution. This procedure had previously been used to prevent cleavage of intermolecular disulfide bonds between HSA and IgA or IgM (35).

Antibodies to C1q can contribute to the pathogenesis of glomerulonephritis in patients with SLE by several mechanisms. Wener et al. found that the presence of large amounts of C1q-binding 6.6S IgG in serum is associated with proliferative lupus nephritis and that there is a significant negative correlation between the presence of this C1q-binding IgG and subepithelial electron-dense deposits (7). Immune complexes that have deposited in the subendothelial area and have bound C1q can be stabilized further by antibodies to C1q and therefore remain in the subendothelial area. Greisman et al. have reported that in patients with SLE the presence of C1q in circulating immune complexes correlates with the presence of renal disease (36). C1q may bind to immune complexes in circulation. Alternatively, immune complexes may form in circulation between antibodies to C1q and C1q molecules that have exposed antigenic determinants for these antibodies. Interestingly, deposits of C1q are seen more frequently in lupus nephritis than in other glomerulonephropathies (37, 38). Antibodies to C1q may not be confined to patients with SLE since Strife et al. have recently demonstrated that C1q-binding IgG in serum from patients with membranoproliferative glomerulonephritis most likely represents antibodies to a cryptic anti-

gen revealed when C1q is bound to a solid surface (39). Further studies should clarify the contributions of antibodies to C1q to the pathogenesis of lupus nephritis.

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