

Interaction of Human C1q with IgG and IgM: Revisited[†]

Mihaela G. Gadjeva,^{*,‡} Marieta M. Rouseva,^{§,||} Alexandra S. Zlatarova,^{+,@} Kenneth B. M. Reid,[#] Uday Kishore,⁺ and Mihaela S. Kojouharova[‡]

Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, Department of Medical Microbiology and Immunology, Aarhus University, Bartholin Building, Wilhelm Meyers Alle, Building 240, Aarhus, Denmark 8000, Department of Biochemistry, Sofia University "St. Kliment Ohridski", 8 Dragan Tzankov Strasse, Sofia, Bulgaria 1164, Medical Research Council Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K., and Laboratory of Human Immunology, Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge, West London, U.K.

Received June 16, 2008; Revised Manuscript Received September 10, 2008

ABSTRACT: The first step of activation of the classical complement pathway involves the binding of the globular C1q domain (gC1q) to the antigen-bound IgG or IgM. To improve our understanding of the mechanism of interaction of gC1q with IgG and IgM, we compared the immunoglobulin binding properties of single-residue mutants of individual globular modules of A and C chains. We found that Lys^{A200} and Lys^{C170} are significant for binding with both immunoglobulins. In addition, two C1q-specific scFv antibodies known as potent inhibitors of C1q–IgG and –IgM interactions were used in the epitope mapping analysis. A set of important residues, which participate in the C1q epitopes for scFv, were identified: Lys^{C170} for the scFv3(V) epitope and Arg^{B108} and Arg^{B109} for the scFv10(V) epitope. The ability of scFv3(V) and scFv10(V) to bind preformed C1q–IgG or C1q–IgM complexes differed: scFv3(V) retained its ability to bind C1q, while scFv10(V) lost it. Given the different locations of the epitopes and the varying abilities of both antibodies to bind C1q–IgG and C1q–IgM complexes, we found that residues from the apical surface of C1q [where the scFv3(V) epitope was located] were involved in the initial recognition of IgG and IgM, while Arg^{B108} and Arg^{B109} are able to interact during the initial recognition as well as during the final binding of immunoglobulins. The reported results provide the first experimental evidence supporting the notion that apical and equatorial surfaces of gC1q have consecutive involvement following the gC1q reorientation during the interaction with specific C1q ligands.

C1q is the first subcomponent of the classical complement pathway and a charge-pattern recognition molecule of the innate immunity. It initiates the classical pathway by binding IgG and IgM associated with immune complexes, which results in the formation of the terminal membrane attack complex (MAC) (1, 2). In addition to binding IgG and IgM, C1q recognizes a great variety of ligands, which are highly diverse in their overall structure and charge pattern. Bacterial

porins, pentraxins such as C-reactive protein (CRP),¹ serum amyloid P component (SAP), and long pentraxin 3 (PTX3), apoptotic cells, viral envelope proteins, and lipopolysaccharides are some examples. C1q binding to its diverse ligands results in a wide range of immunologically important events, including inflammation, clearance of apoptotic cells, microbial phagocytosis, viral neutralization, cell adhesion, and modulation of adaptive immunity (3–5). C1q is also a crucial link between primordial innate immunity and the humoral wing of adaptive immunity.

The C1q molecule has a bouquet-of-tulips-like structure composed of six C-terminal heterotrimeric globular head domains (gC1q) and an N-terminal triple-helical collagen region (CLR). Each gC1q domain is composed of C-terminal portions of A (ghA), B (ghB), and C (ghC) chains (6–8). The strong ability of the gC1q domain to engage such a wide array of ligands is supported by the modular organization of the individual globular head regions. Individually expressed recombinant modules of gC1q domain have been shown to have differential target binding properties, although in the native C1q molecule they exist as a heterotrimer (9–11). The notion of the modular nature of the gC1q domain has been reaffirmed by the crystal structure, determined at 1.9 Å resolution (12) that has revealed a compact, spherical heterotrimer with a pseudo-3-fold symmetry with a well-exposed Ca²⁺ ion, located near the apex of the molecule.

[†] This investigation was supported partially by the Bulgarian National Scientific Foundation (Grant K501 to M.S.K.). M.G.G. and M.M.R. are funded by MRC, Aarhus University, Denmark. U.K. acknowledges a BRIEF award from Brunel University.

* To whom correspondence should be addressed: Brigham and Women's Hospital, Channing Laboratory, Harvard Medical School, Boston, MA 02115. Fax: (617) 525-2510. E-mail: mgadjeva@rics.bwh.harvard.edu.

[‡] Harvard Medical School.

[§] Aarhus University.

^{||} Present address: Department of Medical Biochemistry, University of Wales, College of Medicine, Cardiff, U.K.

⁺ Sofia University "St. Kliment Ohridski".

[@] Present address: Cytonet Sofia-Tissue Bank, Sofia 1000, Bulgaria.

[#] University of Oxford.

⁺ Brunel University.

¹ Abbreviations: gC1q, globular head region of C1q; CLR, collagen-like region of C1q; MBP, maltose binding protein; ghA, ghB, and ghC, recombinant forms of the carboxy-terminal gC1q region of the A, B, and C chains, respectively; WT, wild type; HAIgG, human aggregated IgG; CRP, C-reactive protein; PTX3, long pentraxin 3; scFv, single-chain antibody.

The three modules within gC1q show clear differences in their electrostatic surface potentials (12). Two of the modules (ghA and ghC) show a combination of basic and acidic residues scattered on their external surface, whereas ghB shows a predominance of positive charges. Within the C1q heterotrimer, ghB has an equatorial position, which allows the module to be most accessible for interactions, especially with negatively charged target molecules. Thus, the modular organization of the heterotrimeric assembly together with different surface charge patterns and the spatial orientation of individual modules enables gC1q to interact with a diverse range of ligands (3–5).

The interactions of C1q with two of its most important ligands, IgG and IgM, have been studied extensively. It is widely accepted that they are predominantly ionic in nature and, thus, sensitive to salt concentrations (13). In addition, Ca^{2+} is necessary for reaching the maximum capacity of binding (12, 14). The C1q binding site of IgG (the best examined C1q ligand) is a species-specific motif consisting of Glu³¹⁸, Lys³²⁰, and Lys³²² residues in murine IgG2b (15) and Asp²⁷⁰, Lys³²², Pro³²⁹, and Pro³³¹ in human IgG1 (16). The gC1q binding site on IgM is considered to reside within the C μ 3 and probably C μ 4 domains (17, 18). Two motifs [Asp³⁵⁶, Asp⁴¹⁷, Glu⁴¹⁸, and His⁴²⁰ (19) and Asp⁴³²-X-Pro⁴³⁴-X-Pro⁴³⁶ (20)] within the mouse C μ 3 domain have been reported as putative C1q binding sites on IgM. The corresponding IgG binding site on C1q was intensively investigated during the past decade using different approaches: chemical modifications (21), epitope mapping (22), mutational analysis (11, 23, 24), biophysical methods (25), and molecular modeling (12). In general, a leading role of the ghB module in C1q–IgG interaction has recently been established, whereas the contributions of ghA and ghC are still debatable. The crystal structure of the human heterotrimeric gC1q domain (12) provided new insight into the organization of the gC1q domain. It revealed that the ghB module has a predominantly positively charged outer surface composed of a number of arginine residues and is the most accessible of the three globular head modules (ghA, ghB, and ghC) due to its equatorial position. Molecular modeling based on the crystal structure of C1q has predicted that the IgG binding site on C1q is located on the exposed side surface of ghB and is represented by a motif consisting of Arg^{B101}, Arg^{B109}, Arg^{B114}, Arg^{B129}, and Glu^{B162}. The significance of the last four residues as well as of several others (Arg^{B108}, Arg^{B163}, His^{B117}, Lys^{B136}, and Tyr^{B175}), also residing within ghB, has been independently demonstrated by mutational analysis (23, 24, 26). The IgM binding site on the gC1q domain is less clear, and no molecular modeling study involving the C1q–IgM interaction is available at present. The analysis of the interactions of individual recombinant ghA, ghB, and ghC modules with IgM led to the speculation that all three modules of the gC1q domain are involved in IgM binding (10, 22). Mutational analysis revealed that three of the residues from ghB (Arg^{B108}, Arg^{B109}, and Tyr^{B175}) seem to be involved to a different extent in interactions with both immunoglobulins, suggesting that the two binding sites overlap within the ghB module (24). A similar conclusion emerged from the study aimed at localizing IgG and IgM binding sites using C1q-specific single-chain antibodies (22). Notably, it was found that scFv10(V), a ghB-specific

antibody, potentially inhibited the interaction of C1q with both IgG and IgM.

This study addresses the questions of (i) whether amino acid residues from the apical surface of the gC1q domain, especially from chains A and C, are involved in interactions with both IgG and IgM and (ii) how gC1q can interact with IgG via the apical and equatorial surfaces simultaneously. Since the role of His^{C101} and Lys^{C170} from the C1q apical area in IgG binding has already been established (10), it was important to examine their significance in the gC1q–IgM interaction. Furthermore, the contribution of another well-exposed residue from the A chain (Lys^{A200}) to the interaction of C1q with IgG and IgM was investigated. Our results indicate that lysine residues from ghA (Lys^{A200}) and ghC (Lys^{C170}), residing within the apical area of the gC1q domain, participate differently in the interaction with IgM and IgG. To map more precisely the proper location of the IgG and IgM binding site(s) on C1q, we studied the epitopes of two C1q-specific single-chain antibodies [scFv3(V) and scFv10(V)]. These antibodies, which are effective inhibitors of interactions of C1q with IgG and IgM, bind epitopes with different locations.

MATERIALS AND METHODS

Purified Proteins and Antibodies. C1q was isolated from human plasma using affinity (IgG-Sepharose) and Mono-S columns (27). The purity of C1q was assessed by SDS–PAGE on a 15% (w/v) polyacrylamide gel under reducing conditions (28) where it appeared as three bands, corresponding to the A, B, and C chains of 34, 32, and 27 kDa, respectively. C1q-deficient serum, human IgG and IgM, mouse monoclonal antibody ME9A10, goat anti-rabbit IgG–horseradish peroxidase (HRP) conjugate, rabbit anti-mouse IgG–HRP conjugate, mouse anti-MBP antibodies, and *o*-phenylenediamine dihydrochloride (OPD) were purchased from Sigma-Aldrich. Aggregated human IgG (HAIGG) was obtained as follows. Human IgG (18–20 mg/mL) in water was heated to 63 °C for 30 min; the insoluble aggregates were removed by centrifugation (14000g) for 20 min, and the soluble immune complexes were precipitated with PEG 6000 (final concentration of 3.5%) to eliminate the free IgG molecules. After centrifugation (3000g) for an additional 30 min, the pellet was washed and dissolved in PBS to a final concentration of 10 mg/mL. Rabbit anti-human C1q polyclonal antibodies were purchased from DAKO. Antibodies specific for the collagen-like region (CLR) of C1q were obtained as follows. First, the CLR of C1q was isolated from purified C1q by pepsin digestion as described in ref 29 and immobilized on CNBr-activated Sepharose; then, the antibodies specific for the CLR of C1q were isolated from rabbit anti-human C1q polyclonal antibodies by affinity chromatography on the resulting matrix as described previously (30).

Single-Chain Antibodies. Clones secreting C1q-specific single-chain antibodies scFv3(V) and scFv10(V) were generated from large human Vaughan (V) library and kindly provided by G. Winter (MRC Center for Protein Engineering, Cambridge, U.K.). Recombinant scFv antibodies were purified using metal (Ni) affinity chromatography (31).

Site-Directed Mutagenesis and Cloning of Single-Residue Substitution Mutants of ghA, ghB, and ghC. A number of single-residue mutants of the recombinant globular modules

of the C1q A, B, and C chains, which have been generated recently, were used (11, 23, 24). The mutant Lys^{A200}Glu was generated by site-directed mutagenesis using an overlapping PCR approach (32). The PCR products incorporating the new mutation were digested with *Xba*I and *Hind*III and subcloned into the pMal-c2 expression vector (New England Biolabs, Beverly, MA). The following custom-made primers (Invitrogen, Paisley, U.K.) for Lys^{A200}Glu were used to generate the mutation: FP primer, CCAACAAGGGTCACATTTAC-CAGGGCT; and RP primer, CTCAGAGCCCTGGTAAAT-GTGACCCTTGTGG. The sequence of the mutant was confirmed by automated DNA sequencing (ABI Prism 3100 analyzer; Applied Biosystems) using bacteriophage M13 and MBP-specific male primers.

The mutant Lys^{A200}Glu was used to study the contribution of Lys^{A200} to IgG and IgM binding. Mutants Lys^{C170}Glu and His^{C101}Ala were used to determine the role of Lys^{C170} and His^{C101} in IgM binding. Mutants Arg^{A162}Ala/Glu, Lys^{A200}Glu, His^{C101}Ala, Arg^{C156}Ala/Glu, and Lys^{C170}Glu were used for characterization of the epitope for scFv3(V), and mutants Arg^{B108}Glu, Arg^{B109}Glu, Arg^{B114}Ala/Glu/Gln, His^{B117}Ala/Asp, Arg^{B129}Ala/Glu, Arg^{B163}Ala/Glu, and Tyr^{B175}Leu were used for characterization of the epitope for scFv10(V).

Intracellular Expression and Purification of the Wild Types and Substitution Mutants of Recombinant ghA, ghB, and ghC Modules. The recombinant globular head regions of the wild-type (WT) A chain (ghA, residues 88–223), B chain (ghB, residues 90–226), and C chain (ghC, residues 87–217) and their corresponding mutants were expressed in *Escherichia coli* as fusion proteins linked to MBP and purified using amylose resin-based affinity chromatography (10). The expressed proteins were additionally purified by ion-exchange chromatography on Q-Sepharose (23). Mutant Lys^{A200}Glu was tested using an ELISA for its ability to bind polyclonal human C1q-specific antibodies and anti-MBP monoclonal antibodies.

ELISA for Detection of the Interaction of Recombinant WT ghA and WT ghC and Their Substitution Mutants with HAIgG or IgM. The microtiter wells were coated overnight at 4 °C with either HAIgG or IgM in sodium carbonate buffer (pH 9.6), washed with phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20 (PBST), and then blocked for 2 h at 37 °C with PBS containing 2% (w/v) BSA. Next, different concentrations of recombinant WT ghA or WT ghC or their corresponding mutants were added to the wells and incubated for 2 h at 37 °C. After being washed with PBST, the wells were incubated with mouse anti-MBP antibodies for 1 h at 37 °C, and the amount of bound recombinant protein was detected with a rabbit anti-mouse IgG–HRP conjugate (2 h at 37 °C). The color was developed using OPD as a substrate system. MBP was used as a negative control protein. The data are given as the means of three experiments ± the standard deviation (SD).

Competitive ELISA. The inhibitory effects of mutant proteins on the interactions between recombinant globular fragments ghA (and ghC) and HAIgG or IgM were examined as follows. ELISA plates were coated with WT ghA or WT ghC, then blocked, and washed. A fixed amount of HAIgG (or IgM) and different amounts of mutants were added to the wells. The amount of bound HAIgG or IgM was detected with a goat anti-human IgG–HRP conjugate or a goat anti-human IgM–HRP conjugate, respectively. Alternatively, a

competitive ELISA was set up to test the inhibitory effect of mutants on C1q–HAIgG or C1q–IgM interactions. ELISA plates coated with HAIgG or IgM were incubated with C1q mixed with recombinant proteins for 2 h at room temperature. Then the amount of bound C1q was detected with rabbit polyclonal antibodies, specific for the CLR of human C1q and anti-rabbit IgG–HRP conjugate. The inhibition of C1q binding, reached by the WT ghA or WT ghC at a concentration 100 µg/mL, was considered to be 100%, and the inhibitory activity of the mutants was compared to this value. The data included are the means ± SD of triplicate measurements.

Inhibition of C1q-Dependent Hemolysis by WT ghA or WT ghC or Their Mutants. Sheep red blood cells (SRBC), sensitized with hemolysin-derived IgG (EA_{IgG}) or IgM (EA_{IgM}) (10⁷ cells per 100 µL), were prepared in DGVB²⁺ [dextrose gelatin veronal-buffered saline containing 0.15 mM CaCl₂, 0.5 mM MgCl₂, 2.5% (w/v) glucose, and 0.1% (w/v) gelatin (pH 7.4)]. Reconstitution of C1q deficient serum (1/40 dilution in DGVB²⁺) with 1 µg/mL purified human C1q was sufficient to lyse >90% of the sensitized SRBC. This concentration of human C1q was subsequently used in the assay described below. To determine if the pretreatment of EA_{IgG} or EA_{IgM} with recombinant modules or mutant proteins could protect EA_{IgG} and EA_{IgM} from C1q-mediated hemolysis, aliquots of 100 µL of EA_{IgG} or EA_{IgM} cells (10⁷ cells) were preincubated for 1 h at 37 °C with different amounts of recombinant modules or their mutants (1.25, 2.5, 5, and 10 µg per tube) in DGVB²⁺. Cells were centrifuged, and the pellet was washed and resuspended in 100 µL of DGVB²⁺. Each aliquot of pretreated EA_{IgG} (or EA_{IgM}) cells was added to a mixture composed of 1 µg of C1q in 10 µL of DGVB²⁺ buffer, 2.5 µL of C1q-deficient serum, and 87.5 µL of DGVB²⁺. Following incubation for 1 h at 37 °C, the reaction was stopped by transferring the tubes on ice and adding 600 µL of ice-cold DGVB²⁺. The unlysed cells were pelleted by centrifugation, and the OD₄₁₂ of 100 µL aliquots of the supernatant was red. Total hemolysis (100%) was estimated as the amount of hemoglobin released upon cell lysis with water. The C1q-dependent hemolytic activity was represented as a percentage of the total hemolysis. MBP was used as a negative control protein.

ELISA for Binding of C1q-Specific ScFv Antibodies to Individual C1q Globular Head Modules and Their Mutants. Microtiter wells were coated overnight at 4 °C with the recombinant proteins (WT or their corresponding mutants) at 10 µg/mL in sodium carbonate buffer (pH 9.6), washed, blocked, and then incubated (37 °C for 2 h) with different concentrations of scFv3(V) or scFv10(V). After three rounds of washing with PBST, the amount of bound scFv was probed with a mouse monoclonal ME9A10 antibody (2 h at 37 °C), followed by the rabbit anti-mouse IgG–HRP conjugate. The color was developed using OPD as a substrate. OD₄₉₂ was measured. MBP was used as a negative control protein. The data are given as the means of three experiments ± SD.

ELISA for Detecting the Interaction of scFv3(V) and scFv10(V) with HAIgG- and IgM-Bound Human C1q. The ELISA for the interaction of HAIgG- or IgM-captured C1q with C1q-specific scFv antibodies were performed as described previously (33). Briefly, microtiter wells were coated with C1q, washed and blocked, and allowed to interact with

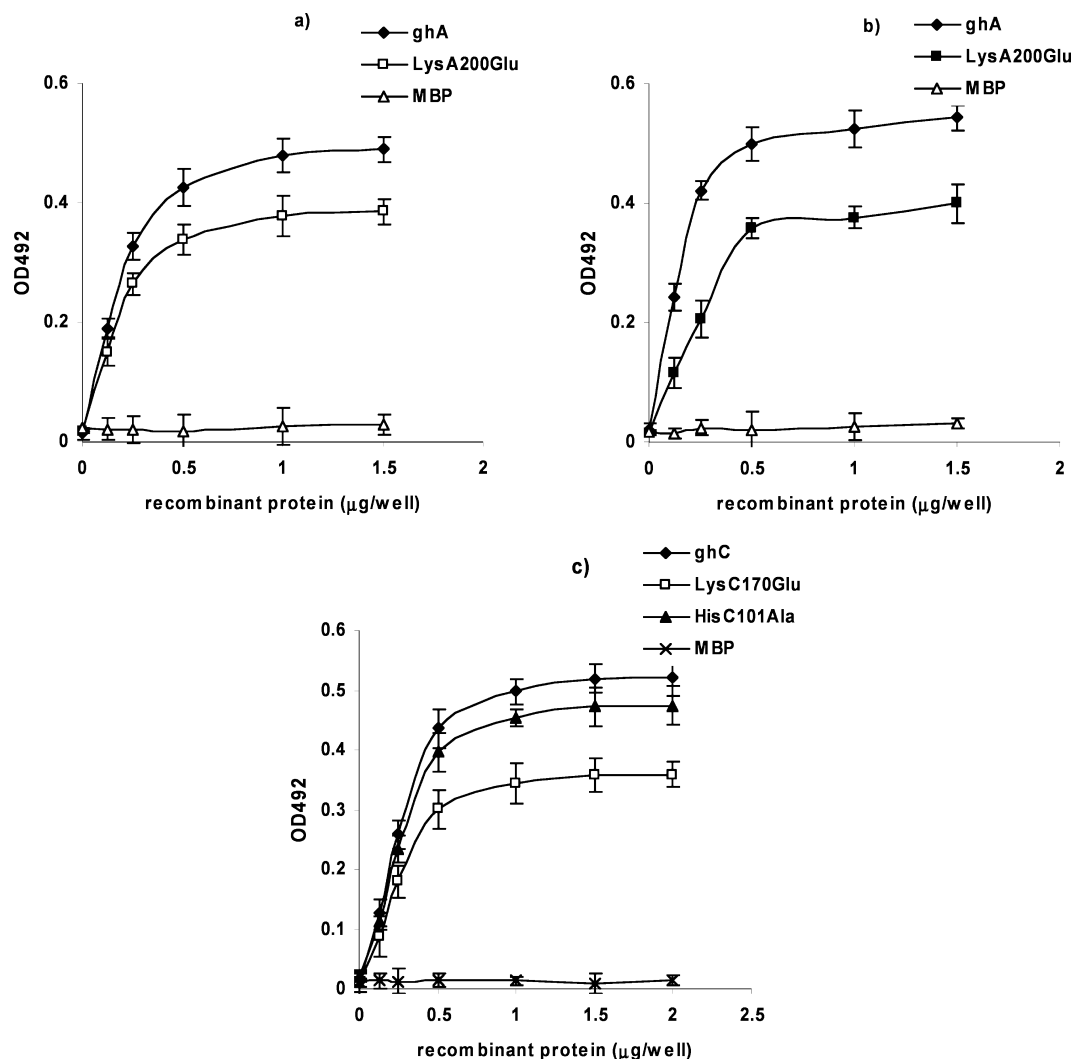


FIGURE 1: HAIgG and IgM binding of WT ghA and WT ghC and their corresponding mutants Lys^{A200}Glu, His^{C101}Ala, and Lys^{C170}Glu. (a) Direct ELISA for HAIgG binding of ghA or the Lys^{A200}Glu mutant. WT ghA and the Lys^{A200}Glu mutant were incubated at different concentrations (0.125–1.5 μg/well) with HAIgG-coated microtiter wells (1 μg/well). (b) Direct ELISA for IgM binding of ghA and Lys^{A200}Glu. Recombinant globular head proteins (0.125–1.5 μg/well) were incubated with IgM-coated microtiter wells (2 μg/well). (c) Direct ELISA for IgM binding of ghC or the His^{C101}Ala or Lys^{C170}Glu mutant. Different concentrations of WT ghC and mutants (0.125–1.5 μg/well) were incubated with IgM-coated wells (2 μg/well). Mouse anti-MBP antibodies and rabbit anti-mouse IgG–HRP conjugate probed the amount of bound recombinant protein. MBP was used as a negative control protein. The data are shown as means ± SD of triplicate measurements.

either IgM or HAIgG for 2 h at 37 °C. After the wells had been washed, scFv antibodies were added and incubated for 2 h at 37 °C. The amount of bound scFv was detected as described above. The value for scFv binding to C1q alone was considered to be 100%. The data are given as the means of three experiments ± SD. MBP was used as a negative control protein.

RESULTS

Bacterial Expression and Purification of Substitution Mutants of ghA, ghB, and ghC. To examine the contribution of single amino acid residues from the gC1q domain to the recognition of C1q ligands, a number of mutants of individual recombinant gC1q modules were generated, using PCR-based site-directed mutagenesis. All the mutants were expressed and purified as previously described (10, 11, 23, 24). The purified proteins reacted immunologically with human C1q-specific antisera (data not shown), indicating that no significant changes in their antigenic structure appeared due to the single residue replacement.

Specificity-Determining Residues in the Globular Fragments of A and C Chains. To understand if residues residing within the apical surface of the gC1q domain and belonging to ghA and ghC were involved in the interactions with IgG and IgM, we examined the HAIgG and IgM binding ability of WT ghA and WT ghC and their corresponding substitution mutants Lys^{A200}Glu, His^{C101}Ala, and Lys^{C170}Glu. The binding curves of WT ghA and mutant Lys^{A200}Glu with immunoglobulins are shown in Figure 1a,b. In all cases, the binding was dose-dependent. With an increase in the amounts of WT or mutant recombinant proteins added to the ELISA wells, an increased level of binding was detected. The ligand binding activity of the mutant was significantly lower than that of WT ghA, reaching ~80% for HAIgG binding and ~72.5% for IgM binding. To estimate the inhibitory ability of the Lys^{A200}Glu mutant, two types of competitive ELISAs were used. First, different concentrations of WT ghA or mutant Lys^{A200}Glu were allowed to compete with a fixed amount of C1q for binding solid phase HAIgG or IgM. Mutant Arg^{A200}Glu was a less potent inhibitor than ghA.

Table 1: Inhibitory Potentials of Mutant Forms of ghA and ghC on C1q-HAIgG and C1q-IgM Interactions^a

inhibitor (10 $\mu\text{g}/\text{well}$)	inhibition of C1q-HAIgG binding (%)	inhibition of C1q-IgM binding (%)
ghA	100 \pm 0.5	100 \pm 1.2
Lys ^{A200} Glu	78.4 \pm 2.3	71.1 \pm 2.4
ghC	100 \pm 2.2	100 \pm 1.1
His ^{C101} Ala	97.1 \pm 1.9	91.3 \pm 2.6
Lys ^{C170} Glu	80.1 \pm 2.4	67.4 \pm 2.9

^a C1q (1.5 $\mu\text{g}/\text{well}$) and recombinant proteins (10 $\mu\text{g}/\text{well}$) were co-incubated with ELISA plates coated with HAIgG (1 $\mu\text{g}/\text{well}$) and IgM (2 $\mu\text{g}/\text{well}$), respectively. The polyclonal anti-CLR antibodies detected bound C1q. The inhibition of C1q binding, measured with WT ghA or WT ghC, was considered to be 100%.

When compared to that of WT ghA, the inhibition by the mutant reached \sim 78.4% for the C1q-HAIgG and \sim 71.1% for the C1q-IgM interaction (Table 1). Similar results were obtained in the alternative type of competitive ELISA, where Lys^{A200}Glu was allowed to compete with WT ghA for binding of HAIgG (or IgM) (data not shown).

Like Lys^{A200}, His^{C101} and Lys^{C170} are located in the apical area of C1q. To determine their importance for binding of IgM, the two mutants His^{C101}Ala and Lys^{C170}Glu were compared to WT ghC (Figure 1c). ghC and the mutants bound IgM in a dose-dependent manner. The substitution of His^{C101} with Ala did not lead to any significant changes in IgM binding activity (10% reduction). Unlike His^{C101}Ala, the substitution of Lys^{C170} with Glu resulted in significantly weaker IgM binding ($<$ 68%). The inhibitory potential of the two mutants on the interaction of immunoglobulins with C1q or WT ghC was tested in a competitive ELISA. The obtained results correlate well with the data from the direct binding assays (see Table 1). The significance of Lys^{A200}Glu, His^{C101}Ala, and Lys^{C170}Glu substitutions was also examined with a C1q-dependent hemolytic assay. In comparison to \sim 68% inhibition of C1q-dependent hemolysis of EA_{IgG} by WT ghA at the maximum concentration used (10 μg), mutant Lys^{A200}Glu was the weaker inhibitor (\sim 48%) (Figure 2a). For EA_{IgM} cells, the inhibitory activity of WT ghA was found to be \sim 51%, as compared to \sim 18% inhibition of hemolysis by Lys^{A200}Glu (Figure 2b). MBP did not interfere with C1q-dependent hemolysis. When WT ghC and mutants His^{C101}Ala and Lys^{C170}Glu were tested in a hemolytic assay for EA_{IgM} cells, the inhibition of hemolysis by WT ghC and His^{C101}Ala was almost equal and reached up to \sim 50%, while mutant Lys^{C170}Glu was a weaker inhibitor with less than 19% inhibition (Figure 2c).

Binding of scFv3(V) to ghA and ghC and Their Corresponding Mutants. Since it has been shown that scFv3(V) binds individual recombinant globular fragments of A and C chains (22), the effect of point mutations within ghA and ghC on scFv3(V) binding was analyzed. WT ghA and mutants Arg^{A162}Ala and Lys^{A200}Glu (Figure 3a) or WT ghC and mutants His^{C101}Ala, Arg^{C156}Ala, and Lys^{C170}Glu (Figure 3b) were tested for scFv3(V) binding. It was found that only the replacement of Lys^{C170} with Glu led to a significant reduction in the antigenicity of WT ghC (up to \sim 23%).

Binding of scFv10(V) to ghB and Its Corresponding Mutants. scFv10(V) has previously been identified as a B chain-specific antibody (22). To narrow down the location of its epitope within the B chain, the binding of scFv10(V) with WT ghB and mutants Arg^{B108}Glu, Arg^{B109}Glu, Arg^{B114}Glu/

Ala, His^{B117}Ala/Asp, Arg^{B129}Glu/Ala, Arg^{B163}Glu/Ala, and Tyr^{B175}Leu was tested (Figure 3c). A significant reduction in the level of scFv10(V) binding was found for mutant Arg^{B108}Glu (\sim 35%) and even more remarkably for Arg^{B109}Glu (\sim 46%). The other mutations caused small (11–14% for Arg^{B129} and Arg^{B114}) or negligible reduction ($<$ 10% for His^{B117}, Arg^{B163}, and Tyr^{B175}) in the level of antibody binding.

Binding of scFv3(V) and scFv10(V) to the Complexes of C1q with HAIgG and IgM. We compared the binding abilities of these single-chain antibodies with respect to preformed C1q-HAIgG and C1q-IgM complexes. When scFv10(V) was incubated with C1q captured with immunoglobulins, a significant reduction in its level of binding to C1q was observed (Figure 4). This reduction reached up to 88% for IgM-captured C1q and was even more significant (\sim 95%) for HAIgG-captured C1q. The obtained results suggest that the epitope of scFv10(V) became hindered after the interaction of the ligand (IgG or IgM) with C1q. A different effect was observed when scFv3(V) interacted with the C1q-HAIgG or C1q-IgM complex: scFv3(V) bound almost equally well to HAIgG-captured C1q and C1q alone, whereas its level of binding to IgM-captured C1q was significantly reduced (\sim 42%), indicating that the epitope of scFv3(V) is not involved in the final formation of, at least, the C1q-HAIgG complex.

A summary of the characteristic binding features of scFv3(V) and scFv10(V), based on data reported here and previously described (22, 33, 34), is presented in Table 2.

DISCUSSION

In this study, we have attempted to provide first experimental evidence supporting the notion that the interaction of C1q with the majority of its negatively charged targets is a two-step process, which includes an initial binding of residues residing within the gC1q apical area, Ca²⁺-facilitated rotation of gC1q, and subsequent final binding by residues located within the exposed side surface of the B chain.

We have investigated, for the first time, the contribution of Lys^{A200}Glu to binding of both HAIgG and IgM, and the contribution of His^{C101}Ala and Lys^{C170}Glu to IgM binding. The investigated residues are located within the C1q apical surface; they are well-exposed to the solvent and belong to the highly variable residues within the gC1q domain as revealed when ghA, ghB, and ghC sequences were aligned with the other gC1q-containing C1q family members using ConSurf (5). It is expected that such residues are functionally critical for their ability to form binding sites on the gC1q domain. According to the crystal structure, molecular modeling of the C1q-IgG complex, bioinformatics, and *in silico* mathematical calculations of electric moments (12, 14), it has been predicted that Lys^{A200} is important for CRP binding and that His^{C101} participates in the interaction with HTLV-1 (12, 5). Recently, it was shown that Lys^{C170} is involved in binding of IgG1 (11). The mutational analysis reported here revealed that both Lys^{A200} and Lys^{C170} are differentially important for binding IgG or IgM, while His^{C101} does not participate in binding of either immunoglobulin. The contribution of Lys^{A200} and Lys^{C170} is more significant for IgM binding than for IgG binding. The substitution of Lys^{A200} with Glu resulted in a reduction in the level of

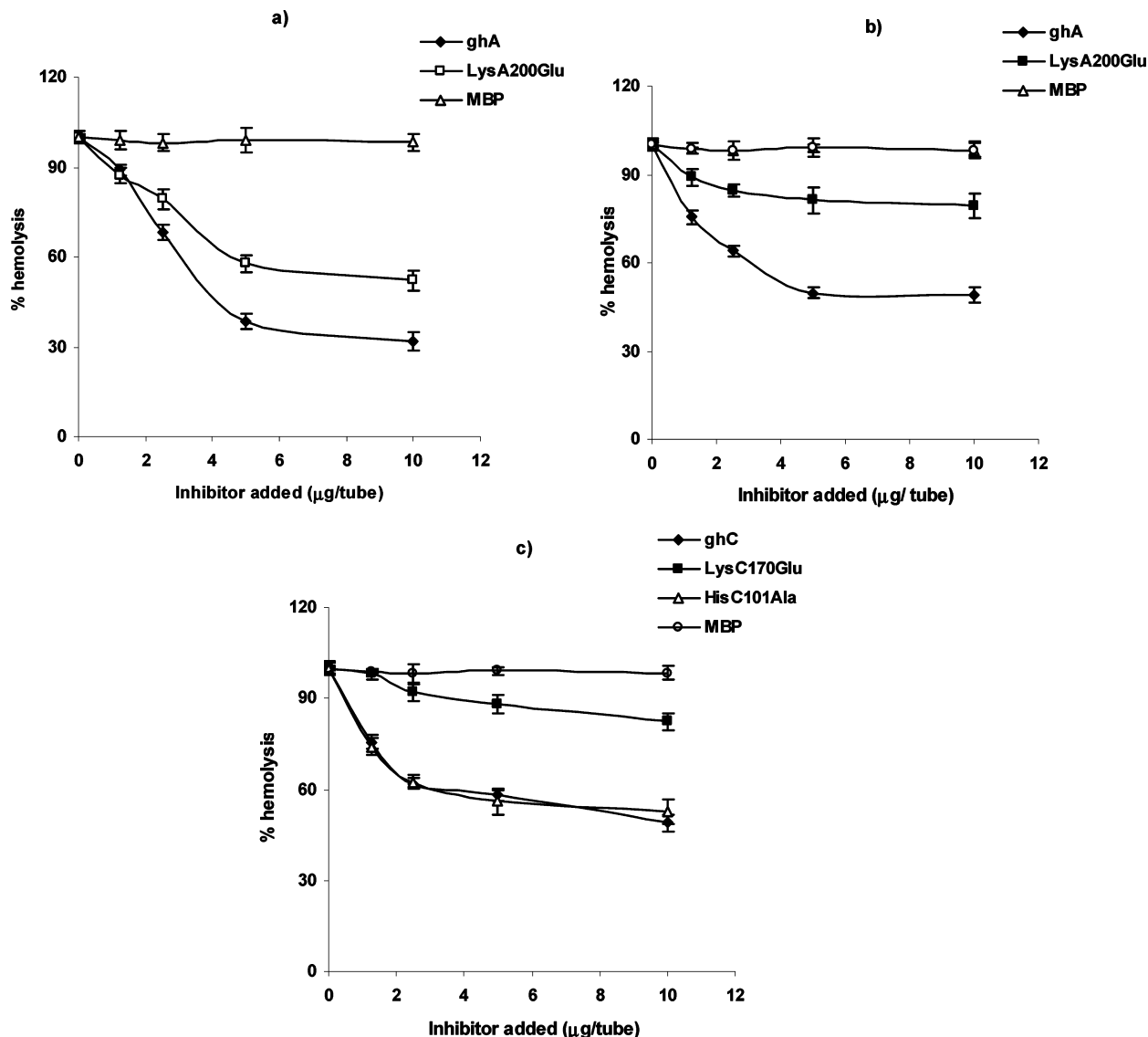


FIGURE 2: Inhibition of C1q-dependent hemolysis of SRBC sensitized with IgG (a) or IgM (b and c) by WT ghA, WT ghC, or their corresponding mutants. SRBCs (1×10^7 cells in $100 \mu\text{L}$ of DGVB^{2+}) sensitized with (a) IgG (EA_{IgG}) or (b and c) IgM (EA_{IgM}) were pretreated with various concentrations of WT ghA, $\text{Lys}^{\text{A200}}\text{Glu}$, WT ghC, $\text{His}^{\text{C101}}\text{Ala}$, or $\text{Lys}^{\text{C170}}\text{Glu}$ for 1 h at 37°C . After reconstitution of C1q-deficient serum, the reaction was stopped, nonlysed cells were pelleted by centrifugation, and OD_{412} values of the supernatants were read. Total hemolysis (100%) was taken as the amount of hemoglobin released upon cell lysis with water. The C1q-dependent hemolytic activity was expressed as a percentage of the total hemolysis. The means of three experiments are shown.

binding of $\sim 20\%$ for IgG and $\sim 27\%$ for IgM. The substitution of Lys^{C170} with Glu lead to a reduction in the level of binding of $\sim 31\%$ for IgM (this study) and $\sim 21\%$ for IgG (11). Taken together, the data reported here and recently (11) demonstrated that three residues from the apical area of C1q and located within all three C1q chains, namely, Lys^{A200} from the A chain, Tyr^{B175} from the B chain, and Lys^{C170} from the C chain, take part in the interaction with immunoglobulins. These results correlate well with analysis of the IgG binding activity of individually expressed recombinant globular fragments of C1q (9, 10) and biophysical analysis (25) but do not correlate with the molecular model of the C1q–IgG complex, postulating that only residues from the B chain, located within the side surface of gC1q domain, are engaged in the binding of IgG (12).

Recently, it was found (14) that the exposed Ca^{2+} within the gC1q heterotrimer influences primarily the target recognition properties of C1q toward IgG, IgM, CRP, and PTX3. At pH 7.4, the loss of Ca^{2+} leads to changes in the direction

of the electric moment from the gC1q apical surface toward the equatorial side of the B chain, perpendicular to the molecular axis. Thus, two surfaces (the apical and the side exposed surface of ghB) can be defined with potential importance for target recognition and binding. It can be proposed that during the interactions of C1q with target molecules, Ca^{2+} is attracted by negatively charged C1q binding sites of the target, which in turn leads to reorientation of gC1q around Arg^{B108} and Arg^{B109} (14). The localization of Arg^{B108} and Arg^{B109} at the apical surface of C1q as well as at the side surface of the B chain allows the rotation around them without separating C1q from the target. Therefore, these two residues should have special importance for the interactions of the gC1q domain with its ligands. The finding that three residues (Lys^{A200} , Tyr^{B175} , and Lys^{C170}) from any of the three chains are involved in interaction with IgG and IgM led us to the assumption that they are initially involved in the interaction with the target. Subsequent molecular rotation around Arg^{B108} and Arg^{B109} may lead to

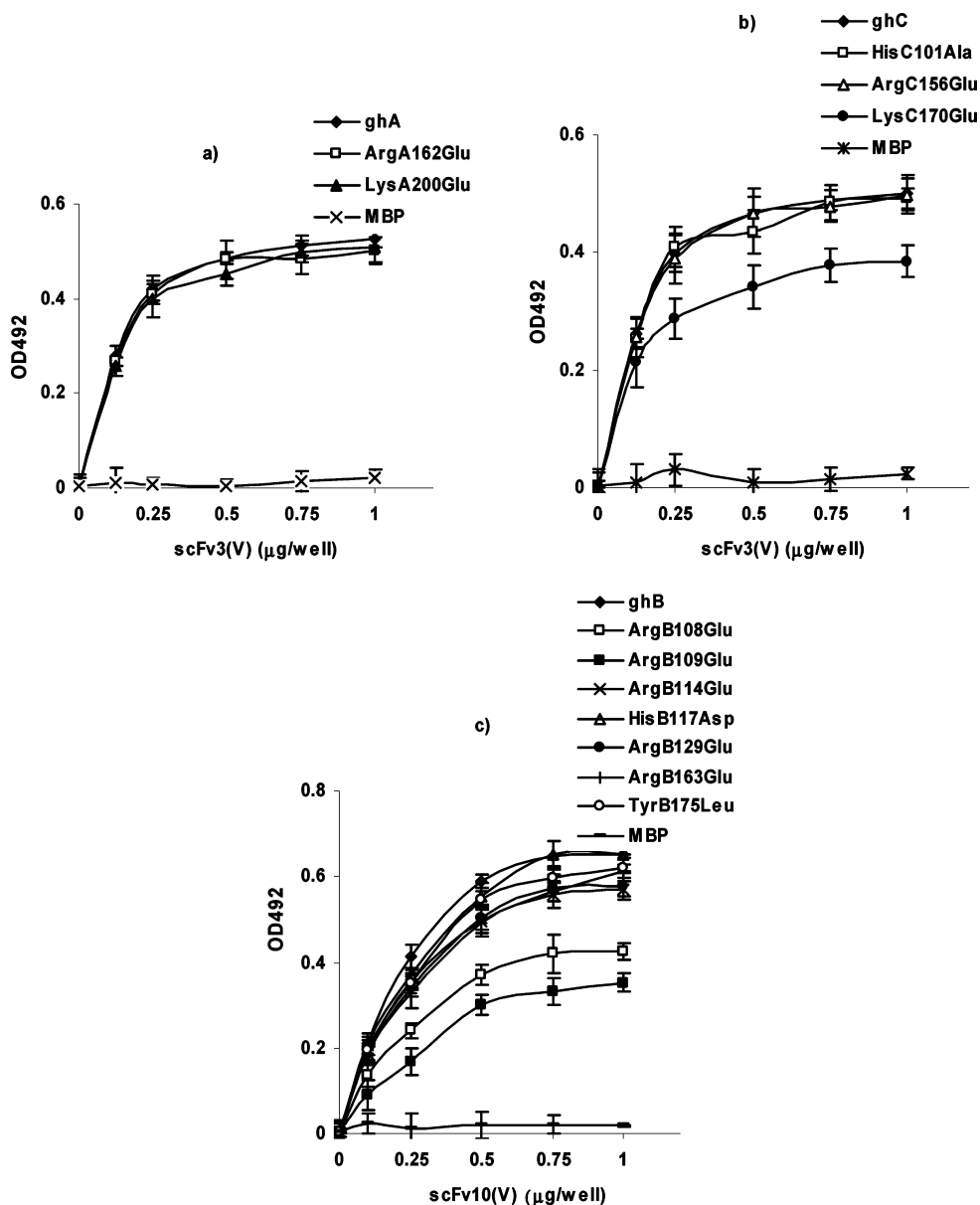


FIGURE 3: Interaction of scFv3(V) and scFv10(V) with wild-type ghA, ghB, and ghC and their corresponding mutants. (a) scFv3(V) binding to ghA or mutant Arg^{A162}Glu or Lys^{A200}Glu was analyzed with an ELISA. Microtiter wells were coated with ghA or Lys^{A200}Glu at a density of 1 μg/well and incubated with scFv3(V) at different concentrations (0.125–1.0 μg/well). (b) scFv3(V) binding to ghC or mutant His^{C101}Ala, Arg^{C156}Glu, or Lys^{C170}Glu was examined with an ELISA. WT ghC or mutants were coated at a density of 1 μg/well and incubated with scFv3(V) at different concentrations. (c) scFv10(V) binding to ghB or mutant Arg^{B108}Glu, Arg^{B109}Glu, Arg^{B114}Glu, Arg^{B129}Glu, Arg^{B163}Glu, and Tyr^{B175}Leu was examined with an ELISA. Microtiter wells were coated with WT ghB or mutants at a density of 1 μg/well and incubated with scFv10(V) at a density of 0.125–1.0 μg/well. The amount of bound recombinant scFv was probed with mouse monoclonal antibody ME9A10 and rabbit anti-mouse IgG–HRP conjugate. The color was developed using OPD as a substrate. OD₄₉₂ was measured. MBP was used as a negative control protein. The data are given as the means of three experiments ± SD.

disruption of few bonds, while other bonds can be formed with the residues residing within the equatorial side surface of the B chain (14). If this is the case in the C1q–IgG interaction, it appears that the molecular modeling of the C1q–IgG complex (12) deals with the final complex between these two molecules, and not with the initial stage of their interaction.

Since it has been difficult to generate recombinant forms of ghA, ghB, and ghC held together as one structure to fully represent native conditions, we expressed individual recombinant globular head regions of C1q, and we generated point mutations within each of them. The interpretation of the results from mutational analysis reported here and recently (11, 23, 24, 26) is based on the finding that the wild types

of the individually expressed recombinant gC1q modules were functionally active (9, 10). However, the possibility that some of the residues important for target interaction of individual C1q heads could be inaccessible within the native C1q molecule cannot be ruled out.

To validate conclusions derived from mutational analysis, we used a very important tool, two gC1q-specific antibodies [scFv3(V) and scFv10(V)], previously reported as potent inhibitors of C1q–IgG and C1q–IgM interactions (22). The binding characteristics (see Table 2) of these antibodies indicated that scFv3(V) binds two individually expressed gC1q modules, ghA and ghC, while scFv10(V) binds ghB only. Analysis of the interactions of scFv3(V) with the mutants of ghA and ghC indicated that its epitope is located

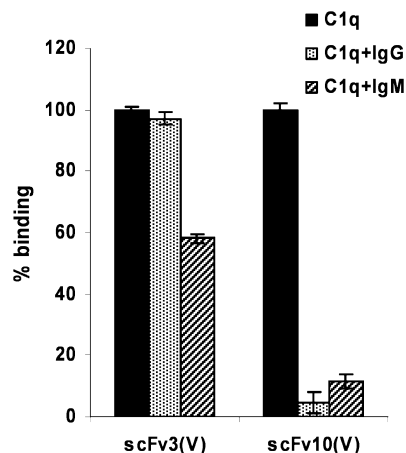


FIGURE 4: Interaction of scFv3(V) and scFv10(V) with HAIgG- or IgM-captured C1q. HAIgG (1 μ g/well) or IgM (2 μ g/well) was allowed to interact with C1q-coated microtiter wells (1 μ g/well) for 2 h at 37 °C, washed, and blocked. Wells were incubated with scFv antibody at a density of 1 μ g/well for 2 h at 37 °C. The amount of bound scFv was detected as in Figure 3.

Table 2: Summary of the Features of Human C1q-Specific Recombinant scFv3(V)^a and scFv10(V)^b Antibodies (based on this study and refs 22, 33, and 34)

characteristic binding feature of scFv	clone secreting C1q-specific scFv	
	scFv3(V)	scFv10(V)
C1q binding (%)	100	100
reduction of C1q–HAIgG complex binding (%)	2.8	95.5
reduction of C1q–IgM complex binding (%)	42	88.6
globular domain binding	+	+
collagen domain binding	–	–
C1q, Western blot	–	–
inhibition of hemolysis	+	+
ghA binding	+	–
ghB binding	–	+
ghC binding	+	–

^a Lys^{C170} is an important constituent of the scFv3(V) epitope.

^b Arg^{B108} and Arg^{B109} are essential components of the scFv10(V) epitope.

within the apical surface of the gC1q domain with participation and the important contribution of Lys^{C170}. It is still unclear whether scFv3(V) binds a shared epitope formed by the participation of residues from both ghA and ghC, or similar epitopes located within the A and C chains of C1q. However, when scFv3(V) was allowed to interact with the preformed C1q–HAIgG complex, no significant changes in C1q binding were found. This finding is very important since the epitope for scFv3(V) is located within the gC1q apical surface, and not within the side-exposed part of ghB. Therefore, we can conclude that this epitope is only initially engaged in the interaction between C1q and IgG and does not participate in the formation of the final complex of C1q and HAIgG. Once the latter is formed, the epitope for scFv3(V) becomes available for interaction, and scFv3(V) binds C1q even after formation of the C1q–HAIgG complex.

Using a range of single-residue mutants of ghB, we identified the location and main components of the epitope for scFv10(V). This epitope resides within the overlapping area between the apical surface of C1q and the exposed surface of the B chain, and Arg^{B108} and Arg^{B109} are its main constituents. When the ability of scFv10(V) to bind the

C1q–HAIgG complex was examined, it was found that the scFv10(V)–C1q interaction is almost completely blocked. This result supports the idea that the interaction of C1q with IgG is a process developing in phases. During the first phase, residues located within the gC1q apical surface and residing within the three chains are involved. After a Ca²⁺-dependent reorientation of the gC1q domain occurs, residues from only the side part of the ghB module participate in the formation of the C1q–IgG complex. Since Arg^{B108} and Arg^{B109} are located on the border between both surfaces, and the rotation around them could occur without tearing C1q from the target, these residues are able to interact during the initial recognition as well as during the final binding.

The mutational analysis revealed that the IgM binding site on gC1q appears to involve all three chains (10). In the absence of any modeling study on the interaction of C1q with IgM, this is an interesting step forward. Mutational analysis (26) has identified several residues from the ghB module (Arg^{B108}, Arg^{B109}, and Tyr^{B175}) as being important for this interaction. Residues that were previously identified as important for IgG binding (Arg^{B114}, His^{B117}, Arg^{B129}, and Arg^{B163}) do not participate in the formation of the IgM binding site on C1q. The significant contribution of two lysines (Lys^{A200} and Lys^{C170}) reported here supports the suggestion that residues from the apical surface of the gC1q domain residing within ghA, ghB, and ghC are involved in binding of IgM. It seems that C1q initially interacts with both IgG and IgM via the differential involvement of the same residues (namely, Lys^{A200}, Tyr^{B175}, and Lys^{C170}). When scFv3(V) and scFv10(V) were tested for their ability to bind the preformed C1q–IgM complex, it was found that the level of scFv3(V) binding was significantly reduced (42%), and reduction of the scFv10(V) binding ability is even more dramatic (88.6%). These results are more complicated to explain because scFv3(V) is not a chain-specific antibody and we cannot determine whether it interacts with one epitope on the apical area of C1q or binds similar antigenic determinants. Furthermore, IgM has more than one C1q binding site, and only some of them could be engaged in the interaction with C1q. We also have to take into consideration the fact that the geometry of IgM does not allow binding similar to that of IgG. Whatever the reason for the reduction of the level of scFv3(V) binding after the formation of the C1q–IgM complex, this result indicates that IgG and IgM binding sites differ despite the fact that during the initial interaction C1q recognizes IgM and IgG via the same area, and probably via the same residues. This finding does not rule out the proposed rotation of C1q around the Arg^{B108}–Arg^{B109} axis and the involvement of residues different from or in addition to those engaged in the initial interaction with IgM. The localization of these residues may be in the proximity of the epitope for scFv3(V).

In conclusion, the interaction of C1q with immunoglobulins appears to proceed initially via differential involvement of residues residing within the apical area of gC1q, and final binding via residues from the exposed side part of ghB (for IgG), or residues located near the epitope for scFv10(V) (for IgM) (Figure 5). The findings that scFv3(V) and scFv10(V) differentially bind complexes between C1q and both immunoglobulins (IgG or IgM), taken together with the different locations of their epitopes (within the C1q apical surface or within the Arg^{B108}–Arg^{B109} area), provide hints for solving

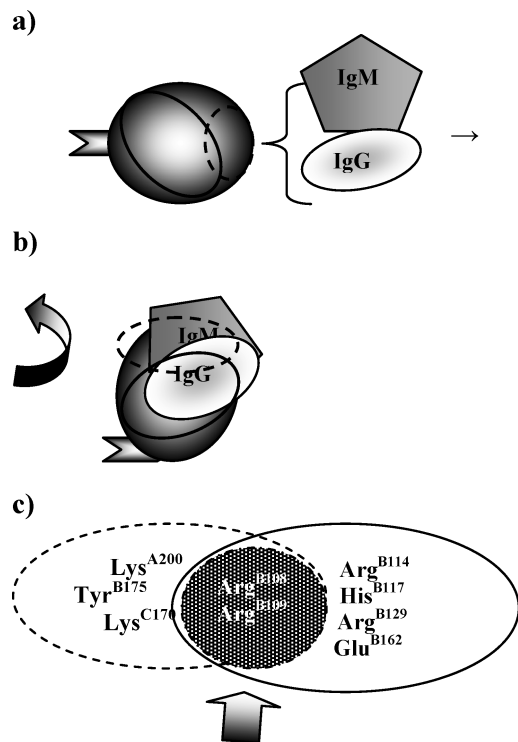


FIGURE 5: Schematic representation of the proposed model of the interaction of gC1q with IgG and IgM. (a) Initial recognition of IgG and IgM via residues located within the apical surface of gC1q. (b) Final binding via residues from the exposed side surface of the B chain (for IgG binding) or residues from the apical surface of gC1q located near the Arg^{B108}-Arg^{B109} area (for IgM). (c) Specificity-determining residues in gC1q are sorted on the basis of their position within the apical and side surfaces of gC1q. The proposed overlapping region of IgG and IgM binding sites on C1q is indicated with an arrow. The apical surface of gC1q is indicated with a dashed line; the side-exposed surface of the globular fragment of the B chain is underlined with a solid line, and the proposed site of rotation around Arg^{B108} and Arg^{B109} is shown with a hatched line.

the puzzle of how it is possible that residues from the top of the C1q molecule and residues from the side surface of the B chain are involved in the interaction of C1q with specific ligands. Furthermore, the reported results highlight a possible mechanism for activation of C1r. It has been proposed (35) that the signal that triggers C1q activation in C1 is a mechanical stress transmitted through the C1q-C1r-C1s interface from the C1q stem to the C1r catalytic region when C1q binds targets. The presented data support the suggestion that the Ca²⁺-facilitated rotation of the gC1q fragment after binding to negatively charged target causes this very mechanical stress, which in turn induces the necessary conformational changes in the CLR of C1q required for the formation of active C1r.

ACKNOWLEDGMENT

We thank Prof. G. Winter for providing clones secreting C1q-specific scFv antibodies. M.S.K. and M.G.G. are grateful to Prof. K. B. M. Reid (University of Oxford) for long-term collaboration in research in the field of complement. We are grateful to Prof. J. Jensenius (University of Aarhus) for his generous support and discussions.

REFERENCES

1. Arlaud, G. J., Gaboriaud, C., Thielens, N. M., Budayova-Spano, M., Rossi, V., and Fontecilla-Camps, J. C. (2002) Structural biology of the C1 complex of complement unveils the mechanisms of its activation and proteolytic activity. *Mol. Immunol.* 39, 383-394.
2. Arlaud, G. J., Gaboriaud, C., Thielens, N. M., Rossi, V., Bersch, B., Hernandez, J. F., and Fontecilla-Camps, J. C. (2001) Structural biology of C1: Dissection of a complex molecular machinery. *Immunol. Rev.* 180, 136-145.
3. Kishore, U., Kojouharova, M. S., and Reid, K. B. (2002) Recent progress in the understanding of the structure-function relationships of the globular head regions of C1q. *Immunobiology* 205, 355-364.
4. Kishore, U., Ghai, R., Greenhough, T. J., Shrive, A. K., Bonifati, D. M., Gadjeva, M. G., Waters, P., Kojouharova, M. S., Chakraborty, T., and Agrawal, A. (2004) Structural and functional anatomy of the globular domain of complement protein C1q. *Immunol. Lett.* 95, 113-128.
5. Ghai, R., Waters, P., Roumenina, L. T., Gadjeva, M. G., Kojouharova, M. S., Reid, K. B., Sim, R. B., and Kishore, U. (2007) C1q and its growing family. *Immunobiology* 212, 253-266.
6. Reid, K. B., and Porter, R. R. (1976) Subunit composition and structure of subcomponent C1q of the first component of human complement. *Biochem. J.* 155, 19-23.
7. Sellar, G. C., Blake, D. J., and Reid, K. B. (1991) Characterization and organization of the genes encoding the A-, B- and C-chains of human complement subcomponent C1q. The complete derived amino acid sequence of human C1q. *Biochem. J.* 274, 481-490.
8. Kishore, U., and Reid, K. B. (2000) C1q: Structure, function, and receptors. *Immunopharmacology* 49, 159-170.
9. Kojouharova, M. S., Panchev, I. D., Tchorbadjeva, M. I., Reid, K. B., and Hoppe, H. J. (1998) Differential binding of IgG and of a HIV gp41 peptide by the B chain and A chain globular head sequences of C1q, respectively. *J. Immunol.* 161, 4325-4331.
10. Kishore, U., Gupta, S. K., Perdikoulis, M. V., Kojouharova, M. S., Urban, B. C., and Reid, K. B. (2003) Modular organization of the carboxyl-terminal, globular head region of human C1q A, B, and C chains. *J. Immunol.* 171, 812-820.
11. Roumenina, L. T., Rouseva, M., Zlatarova, A., Kolev, M., Ghai, R., Olova, N., Gadjeva, M. G., Agrawal, A., Mantovani, A., Bottazzi, B., Reid, K. B., Kishore, U., and Kojouharova, M. S. (2006) Interaction of C1q with IgG1, C-reactive protein and pentraxin 3: Mutational analyses using recombinant globular regions of C1q A, B and C chains. *Biochemistry* 45, 4093-4104.
12. Gaboriaud, C., Juanhuix, J., Gruez, A., Lacroix, M., Darnault, C., Pignol, D., Verger, D., Fontecilla-Camps, J. C., and Arlaud, G. J. (2003) The crystal structure of the globular head of complement protein C1q provides a basis for its versatile recognition properties. *J. Biol. Chem.* 278, 46974-46982.
13. Poon, P. H., Phillips, M. L., and Schumaker, V. N. (1985) Immunoglobulin M possesses two binding sites for complement subcomponent C1q, and soluble 1:1 and 2:1 complexes are formed in solution at reduced ionic strength. *J. Biol. Chem.* 260, 9357-9365.
14. Roumenina, L. T., Kantardjiev, A. A., Atanasov, B. P., Waters, P., Gadjeva, M. G., Reid, K. B., Mantovani, A., Kishore, U., and Kojouharova, M. S. (2005) Role of Ca²⁺ in the electrostatic stability and the functional activity of the globular domain of human C1q. *Biochemistry* 44, 14097-14109.
15. Duncan, A. R., and Winter, G. (1988) The binding site for C1q on IgG. *Nature* 332, 738-740.
16. Idusogie, E. E., Presta, L. G., Gazzano-Santoro, H., Totpal, K., Wong, P. Y., Ultsch, M., Meng, Y. G., and Mulkerrin, M. G. (2000) Mapping of the C1q binding site on Rituxan, a chimeric antibody with a human IgG1 Fc. *J. Immunol.* 164, 4178-4184.
17. Perkins, S. J., Nealis, A. S., Sutton, B. J., and Feinstein, A. (1991) Solution structure of human and mouse immunoglobulin M by synchrotron X-ray scattering and molecular graphics modelling. A possible mechanism for complement activation. *J. Mol. Biol.* 221, 1345-1366.
18. Shulman, M. J., Collins, C., Pennell, M., and Hozumi, N. (1987) Complement activation by IgM: Evidence for the importance of the third constant domain of the μ heavy chain. *Eur. J. Immunol.* 17, 549-554.
19. Arya, S., Chen, F., Spycher, S., Isenman, D. E., Shulman, M. J., and Painter, R. H. (1994) Mapping of amino acid residues in the C mu 3 domain of mouse IgM important in macromolecular assembly and complement-dependent cytotoxicity. *J. Immunol.* 152, 1206-1212.
20. Taylor, B., Wright, J. F., Arya, S., Isenman, D. E., Shulman, M. J., and Painter, R. H. (1994) C1q binding properties of monomer and

- polymer forms of mouse IgM mu-chain variants. Pro544Gly and Pro434Ala. *J. Immunol.* 153, 5303–5313.
21. Marques, G., Anton, L. C., Barrio, E., Sanchez, A., Ruiz, S., Gavilanes, F., and Vivanco, F. (1993) Arginine residues of the globular regions of human C1q involved in the interaction with immunoglobulin G. *J. Biol. Chem.* 268, 10393–10402.
 22. Kojouharova, M. S., Tsacheva, I., Tchordadjieva, M., Reid, K. B. M., and Kishore, U. (2003) Localization of the ligand binding sites on the human C1q globular region using recombinant globular head fragments and single-chain antibodies. *Biochim. Biophys. Acta* 1652, 64–74.
 23. Kojouharova, M. S., Gadjeva, M. G., Tsacheva, I. G., Zlatarova, A. S., Roumenina, L. T., Tchordadjieva, M. I., Atanasov, B. P., Waters, P., Urban, B. C., Sim, R. B., Reid, K. B., and Kishore, U. (2004) Mutational analyses of the recombinant globular regions of human C1q A, B, and C chains suggest an essential role for arginine and histidine residues in the C1q-IgG interaction. *J. Immunol.* 172, 4351–4358.
 24. Zlatarova, A. S., Rouseva, M., Roumenina, L. T., Gadjeva, M. G., Kolev, M., Dobrev, I., Olova, N., Ghai, R., Jensenius, J. C., Reid, K. B. M., Kishore, U., and Kojouharova, M. S. (2006) Existence of different but overlapping IgG- and IgM-binding sites on the globular domain of human C1q. *Biochemistry* 45, 9979–9988.
 25. Tischenko, V. M., Zav'yalova, G. A., Bliznyukov, O. P., and Zav'yalov, V. P. (2004) Thermodynamic, conformational and functional properties of the human C1q globular heads in the intact C1q molecule in solution. *Mol. Immunol.* 20, 1223–1236.
 26. Zlatarova, A. S., Roumenina, L. T., Rouseva, M., Gadjeva, M. G., Kishore, U., and Kojouharova, M. S. (2005) Glu162 of the B-chain of human complement component C1q possesses a key role in IgG recognition. *Dokl. Bulg. Acad. Nauk.* 58, 215–220.
 27. Stemmer, F., and Loos, M. (1984) Purification and characterization of human, guinea pig and mouse C1q by fast protein liquid chromatography (FPLC). *J. Immunol. Methods* 74, 9–16.
 28. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–688.
 29. Reid, K. B. (1976) Isolation, by partial pepsin digestion, of the three collagen-like regions present in subcomponent C1q of the first component of human complement. *Biochem. J.* 155, 5–17.
 30. Zhelezarova, E., Rajkova, A., Getova, T., and Kojouharova, M. S. (1992) Isolation of monospecific antibodies to the collagen-like region of C1q. *C. R. Acad. Bulg. Sci.* 45, 115–118.
 31. Marks, J. D., Hoogenboom, H. R., Bonnert, T. P., McCafferty, J., Griffiths, A. D., and Winter, G. (1991) By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J. Mol. Biol.* 222, 581–597.
 32. Ausbel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. S., Smith, J. A., and Struhl, K. (1998) Mutagenesis of cloned DNA. In *Current Protocols in Molecular Biology*, Vol. 10.20.1, Wiley, New York.
 33. Kontermann, R. E., Wing, M. G., and Winter, G. (1997) Complement recruitment using bispecific diabodies. *Nat. Biotechnol.* 15, 629–631.
 34. Tsacheva, I. G., Panchev, I., and Kojouharova, M. S. (2002) Characterization of C1q specific single chain antibodies, generated by phage display technology. *Biotechnol. Biotechnol. Equip.* 2, 48–46.
 35. Gaboriaud, C., Thielens, N. M., Gregory, L., Rossi, V., Fontecilla-Capms, J. C., and Arlaud, G. J. (2004) Structure and activation of the C1q complex of complement: Unraveling the puzzle. *Trends Immunol.* 25, 369–373.

BI801131H