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# Modular Organization of the Carboxyl-Terminal, Globular Head Region of Human C1q A, B, and C Chains<sup>1</sup>

Uday Kishore,<sup>2\*†</sup> Sanjeev K. Gupta,\* Michael V. Perdikoulis,\* Mihaela S. Kojouharova,<sup>‡</sup> Britta C. Urban,<sup>†</sup> and Kenneth B. M. Reid\*

The first step in the activation of the classical complement pathway, by immune complexes, involves the binding of the globular heads of C1q to the Fc regions of aggregated IgG or IgM. Located C-terminal to the collagen region, each globular head is composed of the C-terminal halves of one A (ghA), one B (ghB), and one C chain (ghC). To dissect their structural and functional autonomy, we have expressed ghA, ghB, and ghC in *Escherichia coli* as soluble proteins linked to maltose-binding protein (MBP). The affinity-purified fusion proteins (MBP-ghA, -ghB, and -ghC) bound differentially to heat-aggregated IgG and IgM, and also to three known C1q-binding peptides, derived from HIV-1, HTLV-I, and  $\beta$ -amyloid. In the ELISAs, the MBP-ghA bound to heat-aggregated IgG and IgM as well as to the HIV-1 gp41 peptide; the MBP-ghB bound preferentially to IgG rather than IgM, in addition to binding  $\beta$ -amyloid peptide, whereas the MBP-ghC showed a preference for IgM and the HTLV-I gp21 peptide. Both MBP-ghA and MBP-ghB also inhibited C1q-dependent hemolysis of IgG- and IgM-sensitized sheep erythrocytes. However, for IgM-coated erythrocytes, MBP-ghC was a better inhibitor of C1q than MBP-ghB. The recombinant forms of ghA, ghB, and ghC also bound specifically to apoptotic PBMCs. We conclude that the C1q globular head region is likely to have a modular organization, being composed of three structurally and functionally independent modules, which retains multivalency in the form of a heterotrimer. The heterotrimeric organization thus offers functional flexibility and versatility to the whole C1q molecule. *The Journal of Immunology*, 2003, 171: 812–820.

The human C1q molecule (460 kDa), the first subcomponent of the classical complement pathway (CCP),<sup>3</sup> is composed of 18 polypeptide chains (6A, 6B, and 6C). The A chain (223 residues), B chain (226 residues), and C chain (217 residues) each have a short (three to nine residues) N-terminal region (containing a half-cystine residue involved in interchain disulfide bond formation), followed by an  $\sim$ 81 residue-long collagen-like region (CLR) and an  $\sim$ 135 residue-long C-terminal globular head region (gC1q) (1). Of the four conserved cysteine residues in each chain at positions 4, 135, 154, and 171, (as per the B chain numbering), the cysteine residue at position 4 is involved in the interchain disulfide bridge, yielding the A-B and C-C subunits. The other three cysteine residues are considered to yield one

intrachain disulfide bond and one free thiol group per gC1q domain. The interchain disulfide bonding yields 6A-B dimer subunits and 3C-C dimer subunits (2). The CLR sequence in the A and B chains of an A-B subunit, together with the equivalent CLR sequence in one of the C chains present in a C-C subunit, form a triple-helical structural unit of the composition ABC-CBA, which is held together by both covalent and noncovalent bonds. Three of these structural units are then considered to associate, via strong noncovalent bonds in the fibril-like central portion, to yield the hexameric C1q molecule (2, 3).

The gC1q modules are also found in a variety of noncomplement proteins including human type VIII (4) and type X collagen (5), precerebellin (6), chipmunk hibernation proteins (7), human endothelial cell protein, multimerin (8), a serum protein, Acrp-30/adiponectin (9), saccular collagen (10), and EMILIN found in elastin-rich tissue (11). The gC1q modules appear to form either a homotrimer (type X collagen, multimerin, Acrp-30, precerebellin, and saccular collagen; Refs. 12–15) or a heterotrimer (C1q and hibernation protein, which have three types of chain and type VIII collagen, which has two  $\alpha_1$  and one  $\alpha_2$  chains; Refs. 15–16). The crystal structure of a recombinant gC1q trimer of mouse Acrp-30 has revealed a structural and evolutionary link between TNF and gC1q-containing proteins (and hence recognition of a C1q/TNF superfamily). In the Acrp-30 structure, the asymmetric trimer of  $\beta$ -sandwich protomers, each of which has a 10-strand jelly-roll folding topology (14, 17), interacts through a cluster of hydrophobic interactions near the base whereas the trimer interface near the apex is largely hydrophilic. These features, associated with the primary structure, are conserved within the C1q/TNF superfamily trimers as are the conserved core  $\beta$  strand of gC1q domains. The heterotrimeric organization of the gC1q is considered to be maintained due to the presence of structural patches within the C-terminal region of each chain (only 30% sequence identity), which probably interact specifically during C1q biosynthesis (2).

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<sup>3</sup> Abbreviations used in this paper: CCP, classical complement pathway; CLR, collagen-like region of C1q; gC1q, globular head region found in C1q; IC, immune complex; ghA, C-terminal globular region of C1q A chain; ghB, C-terminal globular region of C1q B chain; ghC, C-terminal globular region of C1q C chain; HTLV-I, human T lymphotropic virus type I; MBP, maltose-binding protein; FP, forward primer; RP, reverse primer; AP, alkaline phosphatase; DGVB, dextrose gelatin Veronal buffer; VBS, Veronal-buffered saline; OPD, *o*-phenylenediamine; PI, propidium iodide; SLE, systemic lupus erythematosus.

Approximately 27% of the residues, which are conserved between the A, B, and C chains of human gC1q, including three cysteine and several hydrophobic and neutral residues, form the gC1q scaffold and impart upon it a largely  $\beta$ -sheet structure (18).

Several CCP-activating ligands, including immune complexes (IC), bind via the gC1q domain. Binding of C1q to IC (containing IgG or IgM), is considered to induce a conformational change in the CLR, which leads to the autoactivation of C1r that, in turn, activates C1s. Human C1q shows only weak binding to the Fc regions of nonaggregated IgG (functional affinity constant,  $K = 4 \times 10^3$  to  $5 \times 10^4 \text{ M}^{-1}$ ) (19–21). Upon presentation of multiple, closely spaced Fc regions (as found in IC), the strength of binding of the hexameric C1q to IgG increases dramatically ( $K = 10^7$  to  $10^8 \text{ M}^{-1}$ ) (22). The gC1q recognizes the C $\gamma$ 2 domain of IgG or the C $\mu$ 3 domain of IgM through ionic interactions. Three charged residues—Glu<sup>318</sup>, Lys<sup>320</sup>, and Lys<sup>322</sup> in the C $\gamma$ 2 domain (which are highly conserved in different IgG isotypes) and Asp<sup>356</sup>, Asp<sup>417</sup>, Glu<sup>418</sup>, and His<sup>420</sup> in the C $\mu$ 3—are considered central to gC1q-Ig interaction (23–24). However, the role and contributions of the C-terminal globular regions of C1q A (ghA), B (ghB), and C (ghC) chains in binding to IgG, IgM, and other non-Ig activators of the CCP have not been precisely defined.

We have expressed the ghA, ghB, and ghC as soluble fusion proteins and examined their interactions with heat-aggregated IgG and IgM, three known C1q-binding peptides derived from HIV-1 gp41, human T lymphotropic virus type I (HTLV-I) gp21 and  $\beta$ -amyloid (A $\beta$ 1-42), and apoptotic cells. Our results appear to suggest a modular organization of the gC1q, consistent with the idea that the globular region of each chain may fold and function with a certain degree of autonomy. It is likely that ghA, ghB, and ghC have evolved as functionally specialized domains or modules, which together as a heterotrimer, confer versatility and flexibility to an intact C1q molecule.

## Materials and Methods

### Human C1q and synthetic peptides

Human C1q was purified from human serum as described previously (25). The purity of C1q was assessed by SDS-PAGE (15% w/v) under reducing conditions where it appeared as three bands, corresponding to the A, B, and C chains of 34, 32, and 27 kDa, respectively. The HIV-1 gp41 peptide 601-613 (GIWGCSGKLICTT) was kindly provided by Dr. G. J. Arlaud (Institute de Biologie Structurale Jean-Pierre Ebel, Grenoble, France). The HTLV-I gp21 peptide 400-429 (CRFPNITNSHVPILQERPLENRV LTGWGL) was purchased from Mimotopes (Clayton, Victoria, Australia). The  $\beta$ -amyloid peptide, A $\beta$ 1-42 (DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVVIA), was purchased from American Peptide Company (Sunnyvale, CA).

### Construction of vectors for the intracellular expression of the globular head region of C1q A, B, and C chains as fusion proteins

The plasmid pMal-c, which codes for maltose-binding protein (MBP) under the P<sub>tac</sub> promoter, was used for protein expression (New England Biolabs, Beverly, MA). The following primers were used to PCR-amplify the DNA sequences corresponding to ghA, ghB, and ghC: ghA forward primer (FP) 5'-GCCCGAGGCCTAAGGACCCGAGGC-3' and ghA reverse primer (RP) 5'-GCCCGGCTGCAGCTAGGCAGATGGGAAGATG-3'; ghB FP 5'-GCCCGGAGGCCTAAGGCCACCCAGAAAATC-3' and ghB RP 5'-GCCCGGCTGCAGCTAGGCCTCCATATCTGG-3'; ghC FP 5'-GCCCGGAGGCCTAAGCAGAAATCCAGTC-3' and ghC RP 5'-GCCCGGCTGCAGCTAGTCGGGAAGAGCAG-3'. Plasmid pMal-c was double-digested with *StuI* and *PstI* and each PCR product was cloned as a *StuI-PstI* fragment. The expressed polypeptide sequence corresponded to residues 88-223 of ghA, 90-226 of ghB, and 87-217 of ghC. The recombinant vectors, containing the ghA, ghB, and ghC sequences linked to MBP, were designated pKBM-A, pKBM-B, and pKBM-C, respectively.

### Expression and purification of ghA, ghB, and ghC

The recombinant fusion proteins, MBP-ghA, -ghB, and -ghC, were expressed using *Escherichia coli* BL21 transformed with pKBM-A, pKBM-B, and pKBM-C, respectively. The bacterial cells were grown in 1 L Luria-Bertani medium containing ampicillin (100  $\mu\text{g/ml}$ ) to  $A_{600} = 0.5$  at 37°C, induced with 0.4 mM isopropyl  $\beta$ -D-thiogalactoside for 3 h, and centrifuged (3,000  $\times g$ , 4°C, 15 min). The cell pellet (~2.5 g) was suspended in 50 ml of lysis buffer (20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.2% v/v Tween 20, 1 mM EGTA, 1 mM EDTA, and 5% v/v glycerol) containing lysozyme (100  $\mu\text{g/ml}$ ) and PMSF (0.1 mM), and incubated over ice for 30 min. The cell lysate was then sonicated at 60 Hz for 30 s with an interval of 1 min (15 cycles) to disrupt the cells and shear the bacterial chromosomal DNA. After centrifugation (16,000  $\times g$  for 30 min), the supernatant was collected and diluted 5-fold using buffer I (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.2% v/v Tween 20, 1 mM EDTA, and 5% v/v glycerol) and passed through an amylose resin column (50 ml). The column was washed first with buffer I (150 ml), followed by buffer II (250 ml of buffer I without Tween 20). Each fusion protein was then eluted with 100 ml of buffer II containing 10 mM maltose.

To further remove minor contaminating proteins and DNA, the fusion protein was applied to a Q-Sepharose ion exchange column. The bound fraction was extensively washed with column buffer containing 20 mM Tris-HCl, pH 8.0, 100 mM NaCl to remove MBP-bound maltose. The fusion protein was subsequently eluted using a 0.1–1 M NaCl gradient. To examine their oligomeric forms, the fusion proteins were individually loaded over a Superose 12 gel filtration column equilibrated with buffer containing 20 mM Tris-HCl, 100 mM NaCl, and 1 mM EDTA, pH 7.4. When required, fusion proteins were dialyzed against factor Xa buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 2 mM CaCl<sub>2</sub>, 5% v/v glycerol), and cleaved with factor Xa (New England Biolabs; 1 U per 50  $\mu\text{g}$  of the fusion protein) at 4°C.

### Biotinylation of proteins

Proteins were biotinylated using an EZ-link NHS-LC biotinylation kit (Pierce, Rockford, IL). The concentration of *N*-hydroxysuccinimido-biotin used was 25-fold molar excess to the protein solution in PBS, pH 7.2 (protein concentration: 0.4 mg/ml for C1q, 0.22 mg/ml gC1q, 0.25 mg/ml for CLR, 0.9 mg/ml for ghA, 1.2 mg/ml for ghB, 1.7 mg/ml for ghC, and 1 mg/ml for MBP). The reaction was conducted at room temperature for 3 h followed by extensive dialysis against appropriate buffers to remove free biotin. The biotinylation efficiency was determined using an HABA assay (Pierce) and found to be 72% for C1q, 65% for gC1q, 68% for CLR, and between 75 and 81% for MBP and MBP-containing globular head fragments of each chain as fusion proteins. The hemolytic activity of human C1q was not altered significantly following biotinylation (<8% compared with unlabeled C1q at the same concentration). The biotinylation of recombinant proteins did not alter their ability to compete with unlabeled native human C1q in the hemolytic assays.

### Binding specificities of MBP-ghA, -ghB, or -ghC for heat-aggregated IgG and IgM

C1q, MBP-ghA, -ghB, -ghC, and MBP (0.125, 0.25, 0.5, 0.75, and 1  $\mu\text{g}$  in 100  $\mu\text{l}$  per well for IgG binding; 0.25, 0.5, and 1  $\mu\text{g}$  in 100  $\mu\text{l}$  per well for IgM binding) in carbonate buffer, pH 9.6, were coated to the microtiter wells overnight at 4°C. After blocking with PBS containing 2% w/v BSA for 2 h and a subsequent three rounds of washing, the wells were incubated with heat-aggregated IgG (10  $\mu\text{g/ml}$ ) or IgM (20  $\mu\text{g/ml}$ ) in TBS-NTC (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% w/v Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 0.05% v/v Tween 20 and 5 mM CaCl<sub>2</sub>) at 37°C. Following a 2-h incubation, the plates were washed and either goat anti-human IgG or IgM conjugated to alkaline phosphatase (AP) (1/10,000 dilution) was added to the respective wells. After 2 h, the color was developed using *p*-nitrophenyl phosphate and A<sub>405</sub> was measured.

### Inhibition of C1q-dependent hemolysis by MBP-ghA, -ghB, and -ghC

The hemolytic assays were conducted as previously described (26, 27). SRBC (EA), sensitized with either IgG (EA<sub>IgG</sub>) or IgM (EA<sub>IgM</sub>), were prepared in dextrose gelatin Veronal buffer (DGVB<sup>2+</sup>) (DGVB containing 2.5 mM sodium barbital, 71 mM NaCl, 0.15 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, 2.5% w/v glucose, 0.1% w/v gelatin, pH 7.4). The addition of human C1q (1  $\mu\text{g}$ ) to C1q-deficient serum (1/40 dilution in DGVB<sup>2+</sup>) was sufficient to lyse > 95% EA cells (EA<sub>IgG</sub> or EA<sub>IgM</sub>). This concentration of human C1q was subsequently used to examine whether the pretreatment of EA<sub>IgG</sub> or EA<sub>IgM</sub> with MBP-ghA, -ghB, or -ghC protected SRBC from C1q-mediated hemolysis.



The EA<sub>IgG</sub> or EA<sub>IgM</sub> ( $10^7$  cells per 100  $\mu$ l) were pretreated with a range of concentrations of MBP-ghA, -ghB, or -ghC (0.75, 2.5, 5, and 10  $\mu$ g each) for 1 h at 37°C. Cells were centrifuged and the pellet was washed and resuspended in 100  $\mu$ l of DGVB<sup>2+</sup>. Each aliquot of pretreated EA cells was added to a mixture, composed of 1  $\mu$ g of C1q in 10  $\mu$ l of buffer, 2.5  $\mu$ l of C1q-deficient serum, and 87.5  $\mu$ l of DGVB<sup>2+</sup>. Following a 1-h incubation at 37°C, the reaction was stopped by transferring the tubes to ice and adding 600  $\mu$ l of ice-cold DGVB<sup>2+</sup>. The unlyzed cells were pelleted by centrifugation and the A<sub>412</sub> of the supernatant was read. Total hemolysis (100%) was assessed as the amount of hemoglobin released upon cell lysis with water. The C1q-dependent hemolytic activity was expressed as percentage of total hemolysis. MBP was used as a negative control protein.

#### Interaction between MBP-ghA, -ghB, or -ghC with HIV-1 gp41 peptide 601-613

A synthetic peptide, corresponding to residues 601-613 of the HIV-1 gp41 (1  $\mu$ g in 100  $\mu$ l per well) was coated to microtiter wells in the carbonate buffer overnight at 4°C and subsequently blocked with Veronal-buffered saline (VBS) (5 mM sodium barbital, pH 7.4, 150 mM NaCl, 0.15 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> and 1% w/v BSA) for 1 h. After washing with VBS containing 0.1% v/v Tween 20, various amounts of C1q, MBP-ghA, -ghB, -ghC, or MBP were added in 100  $\mu$ l of TBS-NTC buffer and incubated for 4 h. After washing with VBS containing 0.1% v/v Tween 20, rabbit anti-human C1q polyclonal IgG (1/2500 dilution in VBS) was added to the wells and the amount of bound proteins were probed using goat anti-rabbit IgG-HRP conjugate, followed by addition of *o*-phenylenediamine (OPD) as substrate. In an alternative ELISA, immobilized peptides were probed with biotinylated C1q, MBP-ghA, -ghB, or -ghC using streptavidin-HRP conjugate.

In the competitive ELISA, biotinylated C1q (1  $\mu$ g in 100  $\mu$ l per well) was coincubated with different concentrations of C1q, MBP-ghA, -ghB, -ghC, or MBP (1, 2, 3, 4, and 5  $\mu$ g in 100  $\mu$ l per well) before being added to the wells previously coated with the peptide. Following a 3-h incubation at room temperature, the amount of bound C1q was detected using streptavidin-HRP conjugate.

#### Detection of interaction between MBP-ghA, -ghB, or -ghC with HTLV-I gp21 peptide 400-429

The HTLV-I gp21 peptide 400-429 (1  $\mu$ g in 100  $\mu$ l per well) was coated in carbonate buffer at 4°C overnight and probed with different concentrations of biotinylated C1q (1–5  $\mu$ g in 100  $\mu$ l per well), MBP-ghA, -ghB, or -ghC (2, 4, 6, and 8  $\mu$ g each in 100  $\mu$ l per well). The microtiter wells were blocked with 1% w/v BSA (37°C for 1 h) and washed with PBST (PBS + 0.1% v/v Tween 20) three times. Following incubation with streptavidin-HRP conjugate (1/1000 dilution, 37°C for 2 h), the color was developed using OPD as substrate and read at A<sub>492</sub>.

For competitive inhibition studies, the microtiter wells were coated with the peptide 400-429 (1  $\mu$ g in 100  $\mu$ l) and blocked. Different concentrations of nonbiotinylated C1q, MBP-ghA, -ghB, and -ghC (2, 4, 6, and 8  $\mu$ g in 100  $\mu$ l per well) were then allowed to compete with biotinylated C1q (2.5  $\mu$ g in 100  $\mu$ l per well) before being added to each well (37°C for 2 h). The color was developed using the same substrate, and the absorbance was read at A<sub>492</sub>.

#### ELISA for the detection of interaction between MBP-ghA, -ghB, or -ghC with A $\beta$ 1-42

Direct binding ELISA experiments were performed by coating 1  $\mu$ g of A $\beta$ 1-42 peptide in 100  $\mu$ l per well in carbonate buffer at 4°C overnight and probed with different concentrations of biotinylated C1q (1–3  $\mu$ g/well), MBP-ghA, -ghB, or -ghC (0.5, 1.0, 1.5, 2.0, and 2.5  $\mu$ g/well). The microtiter wells were blocked with 1% w/v BSA (37°C for 1 h) and washed with PBST three times. Following incubation with streptavidin-HRP conjugate (1/1000 dilution, 37°C for 2 h), the color was developed using OPD as substrate and read at A<sub>492</sub>.

For competitive inhibition studies, different concentrations of nonbiotinylated C1q, MBP-ghA, -ghB, and -ghC (0.5–2.5  $\mu$ g in 100  $\mu$ l per well) were added to the peptide-coated (1  $\mu$ g) wells and incubated (37°C for 2 h) following coincubation with biotinylated C1q (1  $\mu$ g in 100  $\mu$ l). After incubation at 37°C for 2 h and washing with PBST, the streptavidin-HRP conjugate (1/2000) and the OPD system were used to develop color.

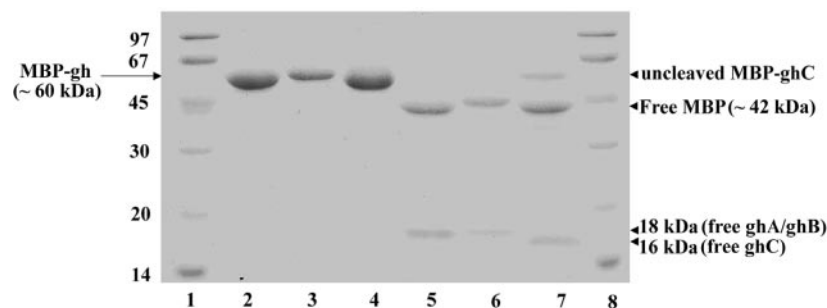
#### Labeling of apoptotic cells with MBP-ghA, -ghB, and -ghC

Apoptosis was induced by irradiation of PBMCs with a calibrated UV lamp at a dose of 2500 mJ/cm<sup>2</sup> at a density of  $1 \times 10^6$ /ml in 6-well plates in PBS. Irradiated cells were cultured for a further 6 h in PBS and stained with either FITC-annexin V and propidium iodide (PI) to monitor cell death, or with C1q. Different amounts of biotinylated C1q, MBP-ghA, -ghB, or -ghC in PBS were incubated with  $1 \times 10^6$  irradiated PBMC for 30 min at 4°C. Cells were washed twice in PBS containing 1% w/v BSA (PBS + BSA) and bound proteins detected with FITC-conjugated anti-biotin mAb (Sigma-Aldrich, St. Louis, MO). Cells were analyzed by flow cytometry (FACS; BD Biosciences, Mountain View, CA) in the presence of PI. Apoptotic cells were detected on the basis of scatter profile and PI staining. For competition assays, irradiated PBMCs were incubated with either buffer alone, or 5  $\mu$ g of MBP, MBP-ghA, -ghB, or -ghC in PBS + BSA for 30 min at 4°C. After washing two times in PBS/BSA, cells were incubated with biotinylated C1q (1  $\mu$ g) and analyzed by FACS.

## Results

### The ghA, ghB, and ghC modules of human C1q can be expressed as soluble fusion proteins

The globular head regions of C1q A, B, and C chains were expressed as MBP fusion proteins in *E. coli* BL21 cells containing pKBM-A, pKBM-B, and pKBM-C, respectively. Following induction with 0.4 mM isopropyl  $\beta$ -D-thiogalactoside for 3 h, each fusion protein appeared as overexpressed protein band of ~60 kDa, as judged by SDS-PAGE under reducing conditions. The majority of the MBP fusion proteins bound to the affinity column and eluted as >95% pure soluble fractions (~18 mg of MBP-ghA, ~22 mg of MBP-ghB, and ~30 mg of MBP-ghC per liter of bacterial culture) (Fig. 1, lanes 2, 3, and 4). Further purification was achieved using a Q-Sepharose anion-exchange column (fusion proteins bound at 0.1 M NaCl and eluted as a sharp peak at ~0.6 M NaCl)



**FIGURE 1.** SDS-PAGE (12% w/v, under reducing conditions) analysis of the purified fusion proteins, MBP-ghA, -ghB, and -ghC. Lanes 1 and 8, Standard protein molecular mass markers; lanes 2, 3, and 4, MBP-ghA, -ghB, and -ghC fusion proteins, respectively, following affinity purification on an amylose resin column. The ghA, ghB, and ghC modules were released from MBP following factor Xa cleavage of the fusion proteins. Lane 5, Factor Xa-cleaved MBP-ghA; lane 6, factor Xa-cleaved MBP-ghB; lane 7, factor Xa-cleaved MBP-ghC. The ghA (lane 5) and ghB (lane 6) migrated to ~18 kDa whereas ghC migrated to ~16 kDa (lane 7). A small proportion of MBP-ghC fusion protein, not cleaved by factor Xa and migrating to ~60 kDa, can be seen in lane 7.

before factor Xa protease digestion to release the globular head fragments free from MBP (Fig. 1). The ghA (lane 5) and ghB (lane 6) migrated as bands of  $\sim 18$  kDa whereas the ghC band migrated as  $\sim 16$  kDa (lane 7). However, the free globular regions (ghA, ghB, and ghC) showed tendencies to aggregate and, therefore, the MBP fusions of each globular region were used for functional assays.

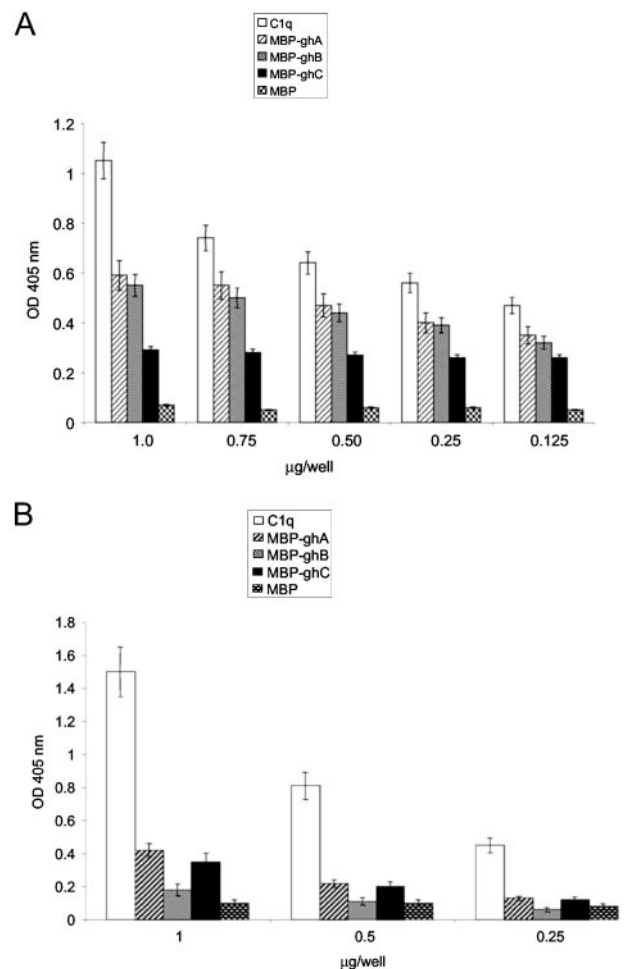
The recombinant globular head modules ghA, ghB, and ghC (free from MBP) were recognized by rabbit anti-human C1q antisera, as judged by Western blot. Polyclonal Abs, raised in rabbit against each module, also recognized the whole C1q molecule in the Western blot and ELISA. The N-terminal amino acid analysis revealed the sequences: GSIEGRPKDQ for ghA, TSGFPGDYK-ATQKIAFSAT for ghB, and GSIEGRPKQK for ghC (with the sequences of each globular sequence given in italics) that confirmed the identity of each recombinant protein. The fusion proteins, when loaded onto a Superose 12 gel filtration column (Pharmacia, Piscataway, NJ) in the buffer containing 20 mM Tris-HCl, 100 mM NaCl, and 1 mM EDTA, pH 7.4, eluted between  $\sim 120$  and  $\sim 68$  kDa molecular size markers, suggesting that the fusion proteins were either dimers or monomers which may be retarded during gel permeation. MBP alone, as expected, eluted as a monomer of  $\sim 42$  kDa. A small proportion of MBP-ghC also eluted on a gel filtration column as a large aggregate that was separated from the more homogeneously eluting fraction for use in various assays. When run on a 12% SDS-PAGE under nonreducing conditions in the presence of iodoacetamide, the majority of the MBP fusion proteins migrated at  $\sim 60$  kDa, suggesting that the MBP-containing C1q globular head fragments were not cross-linked via aberrant disulfide bonding.

#### MBP-ghA, -ghB, or -ghC bind heat-aggregated IgG and IgM differentially

Different concentrations of C1q, MBP-ghA, -ghB, or -ghC, coated to the microtiter wells, were allowed to bind heat-aggregated IgG or IgM, and then probed with goat anti-human IgG or IgM conjugated to AP. C1q, MBP-ghA, and MBP-ghB bound heat-aggregated IgG in a dose-dependent manner, whereas MBP-ghC showed comparatively weaker binding ( $A_{405} < 0.3$ ) that did not change with an increase in its concentration (Fig. 2A). C1q, MBP-ghA, and MBP-ghC bound IgM well, compared with MBP-ghB and MBP alone (Fig. 2B). The binding of the MBP-ghA and MBP-ghC to heat-aggregated IgM was comparable, although 4-fold lower than C1q. The MBP-ghB showed relatively weak background binding, which was not dose-dependent (26). It is worthwhile to note that the amount of the recombinant MBP fusion proteins in molar terms is higher than C1q for the same concentration of proteins by weight. For example as in Fig. 2A, a comparison between C1q ( $\sim 21$  nM) and MBP-gh ( $\sim 167$  nM) binding to aggregated IgG at  $1 \mu\text{g}/100 \mu\text{l}$  reveals nearly an 8-fold excess of recombinant proteins. Because the native C1q has 18 globular heads (which are clustered) and the recombinant ghA, ghB, and ghC are single polypeptide chains, it is difficult to compare such interactions with respect to multiple heads of C1q vs single heads of recombinant modules.

#### MBP-ghA, -ghB, and -ghC differentially inhibit C1q-dependent hemolysis

To examine the inhibitory effects of MBP-ghA, -ghB, and -ghC on C1q-dependent hemolysis, SRBC (E) were sensitized with hemolysin (A; anti-sheep erythrocyte IgG or IgM fraction) to yield EA<sub>IgG</sub> or EA<sub>IgM</sub> cells. Reconstitution of C1q-deficient serum with  $1 \mu\text{g}$  of C1q was found to completely lyse ( $>95\%$ ) the sensitized EA cells. EA<sub>IgG</sub> and EA<sub>IgM</sub> cells were pretreated with various

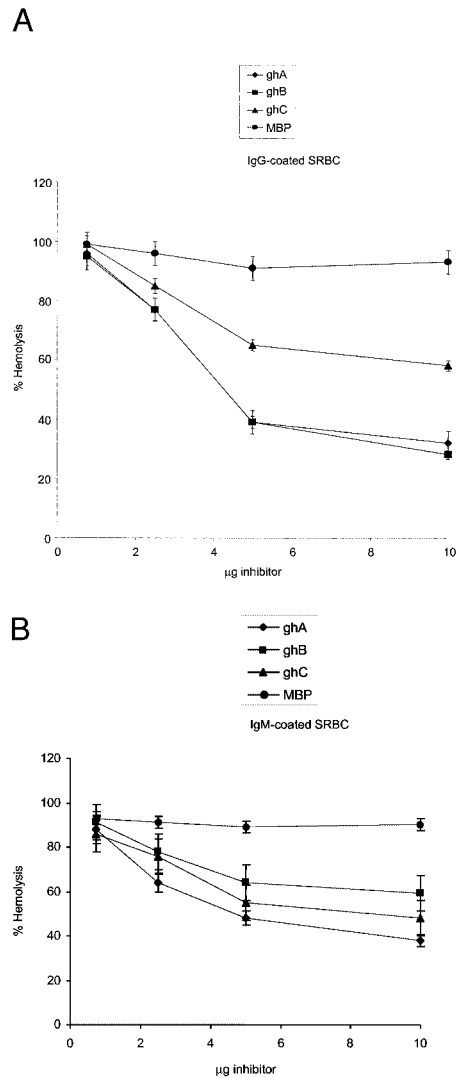


**FIGURE 2.** Binding of heat aggregated IgG and IgM to solid phase MBP-ghA, -ghB, and -ghC. Various concentrations of C1q, MBP-ghA, -ghB, or -ghC were coated to the microtiter wells and a fixed concentration of either (A) heat-aggregated human IgG ( $10 \mu\text{g}/\text{well}$ ), or (B) heat-aggregated human IgM ( $20 \mu\text{g}/\text{well}$ ), were added and probed with (A) anti-human IgG, or (B) anti-human IgM, respectively, conjugated to AP. The color was developed using *p*-nitrophenyl phosphate and  $A_{405}$  values were measured. All experiments were performed in duplicate and MBP was used as a negative control.

concentrations of MBP-ghA, MBP-ghB, MBP-ghC, or MBP alone ( $0.625$ – $10 \mu\text{g}$ ) before reconstituting C1q-deficient serum with exogenous human C1q. As shown in Fig. 3A,  $\sim 4 \mu\text{g}$  of MBP-ghA or MBP-ghB were required to reduce C1q-mediated hemolysis to 50%, whereas  $10 \mu\text{g}$  of MBP-ghC failed to achieve 50% inhibition for EA<sub>IgG</sub>. To achieve  $>50\%$  inhibition of EA<sub>IgM</sub>,  $\sim 4 \mu\text{g}$  of MBP-ghA and  $\sim 10 \mu\text{g}$  of MBP-ghC were required, whereas  $10 \mu\text{g}$  of MBP-ghB failed to achieve 50% inhibition (Fig. 3B). Therefore, the potencies of the three recombinant proteins as inhibitors of C1q, based on C1q-dependent hemolysis of sensitized sheep erythrocytes, are in the order ghA  $\approx$  ghB  $>$  ghC for EA<sub>IgG</sub>, and ghA  $>$  ghC  $>$  ghB for EA<sub>IgM</sub>. Normal serum (1/20 dilution) and C1q-deficient serum (1/40 dilution) were used as control for complete and background lysis, respectively. MBP did not interfere with C1q-dependent hemolysis.

#### The ghA module preferentially binds HIV-1 gp41 peptide 601-613

C1q (Fig. 4A) and MBP-ghA (Fig. 4B) bound HIV-1 gp41 peptide 601-613 specifically, whereas background level binding was

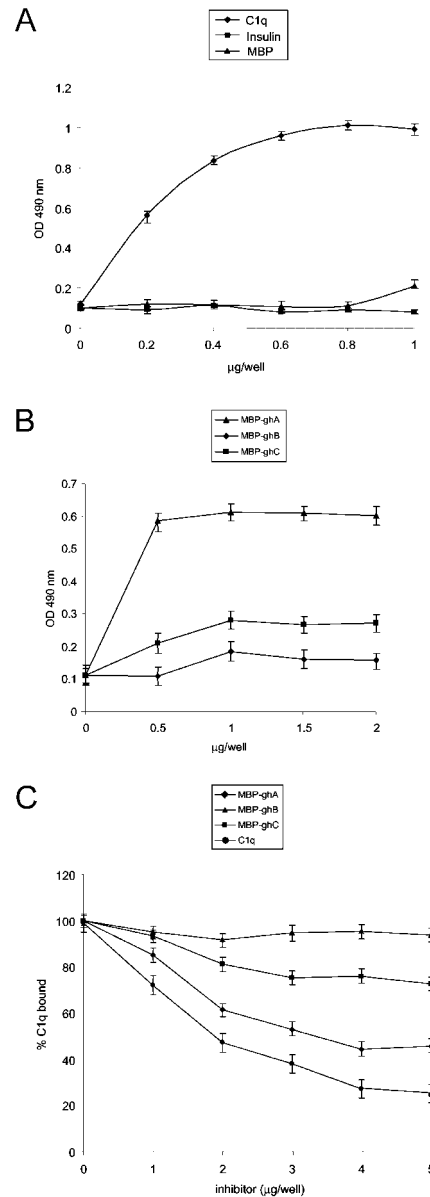


**FIGURE 3.** Inhibition by MBP-ghA, -ghB, or -ghC of C1q-dependent hemolysis of SRBC sensitized with (A) IgG and (B) IgM. EA cells ( $1 \times 10^7$ ), sensitized with IgG (EA<sub>IgG</sub>) or IgM (EA<sub>IgM</sub>), were pretreated with various concentrations of MBP-ghA, -ghB, or -ghC for 1 h at 37°C. C1q (1 µg) together with 1/40 C1q-deficient serum was then added to the pretreated EA cells to bring about hemolysis. The nonlysed cells were centrifuged and A<sub>412</sub> values of the supernatants were measured. The percentage lysis was determined relative to complete hemolysis using water. The means of three experiments are presented for each set of experiments.

shown by MBP-ghB and MBP-ghC (Fig. 4B). When recombinant globular head fragments were used to compete with C1q for binding to peptide 601-613, the MBP-ghA appeared to compete with C1q preferentially and dose-dependently (Fig. 4C).

#### The ghC module shows a preference for HTLV-I gp21 peptide 400-429

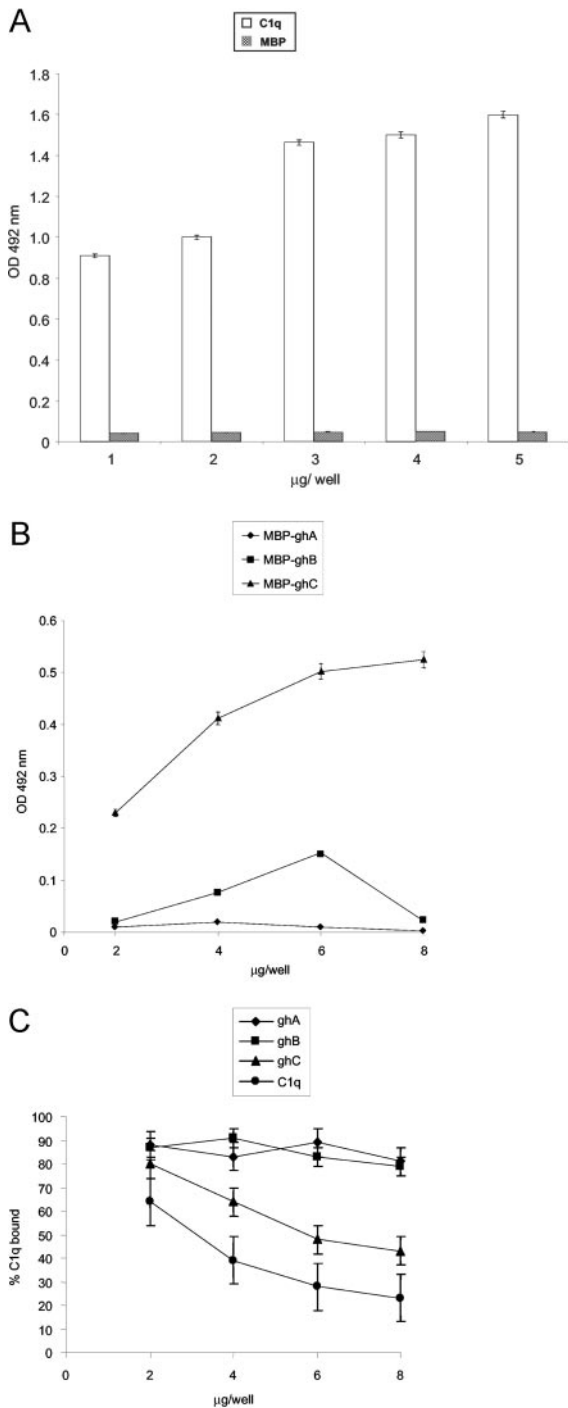
When the HTLV-I gp21 peptide 400-429 (1 µg/well) was coated on microtiter wells and probed with biotinylated C1q, MBP-ghA, -ghB, or -ghC, the C1q and MBP-ghC bound specifically with background binding of MBP-ghA and MBP-ghB similar to MBP alone (Fig. 5, A and B). In a competitive ELISA, where nonbiotinylated C1q, MBP-ghA, -ghB, and -ghC were allowed to inhibit binding of biotinylated C1q to the peptide, MBP-ghC inhibited ~40% binding at a concentration of 50 µg/ml (5 µg/well; Fig. 5C).



**FIGURE 4.** Binding of HIV-1 gp41 peptide 601-613 to human C1q (A) and MBP-ghA, -ghB, or -ghC (B). ELISAs were conducted for the detection of interaction between solid-phase HIV-1 gp41 peptide 601-613 and human C1q (A) and recombinant MBP-ghA, -ghB, and -ghC modules (B). MBP and insulin were used as control proteins (SD < 0.06). C, Inhibition of C1q binding to HIV-1 gp41 peptide 601-613 by MBP-ghA, -ghB, or -ghC. An ELISA for detection of inhibitory activity of MBP-ghA, -ghB, and -ghC fragments on the interaction between native C1q and peptide 601-613 was conducted by adding biotinylated C1q (1 µg) to the peptide-coated wells in the presence of various concentrations of the MBP-ghA, -ghB, or -ghC.

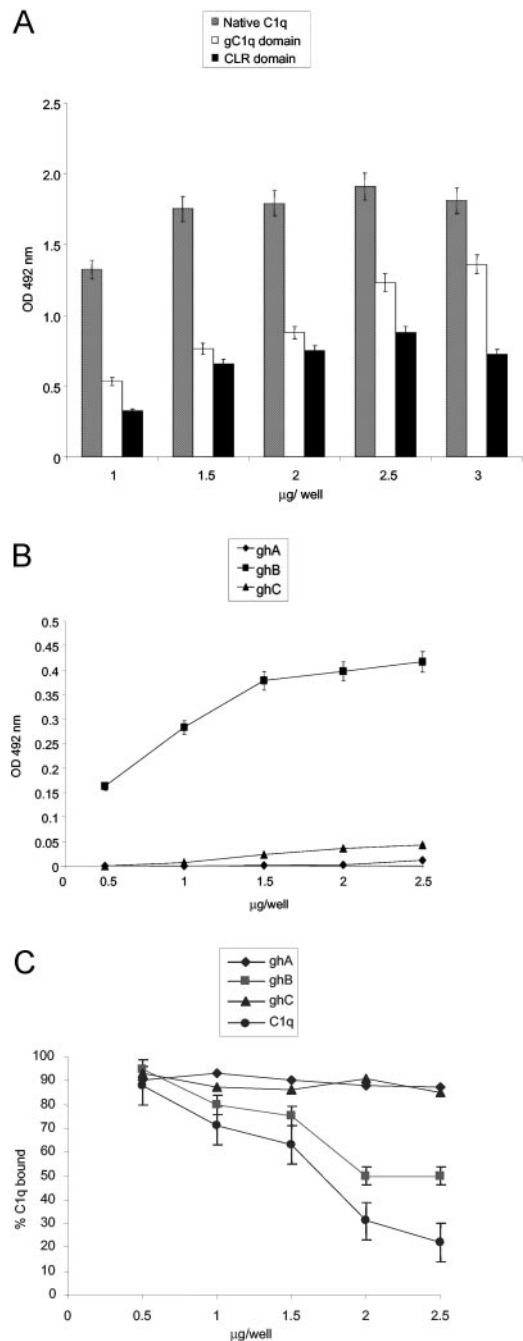
#### The β-amyloid peptide specifically interacts with the ghB module

The Aβ<sub>1-42</sub> bound native C1q, gC1q, and CLR in a dose-dependent manner in the order C1q > gC1q > CLR (Fig. 6A). The MBP-ghB showed highly specific, preferential and dose-dependent binding to solid-phase Aβ<sub>1-42</sub> (Fig. 6B). In a competitive ELISA, where varying concentrations of nonbiotinylated C1q, MBP-ghA, -ghB, and -ghC were allowed to inhibit binding of biotinylated C1q (1 µg) to Aβ<sub>1-42</sub> (1 µg), the MBP-ghB inhibited ~50% binding at 20 µg/ml (2 µg/well; Fig. 6C). These results



**FIGURE 5.** Binding of HTLV-I gp21 peptide 400-429 to human C1q and MBP-ghA, -ghB, or -ghC. The microtiter wells were coated with HTLV-I gp21 peptide 400-429 in carbonate buffer and probed with biotinylated C1q (A) or MBP-ghA, -ghB, and -ghC (B). After blocking and washing the wells, the binding was detected using streptavidin-HRP conjugate and OPD as substrate. C, Inhibition of C1q binding to HTLV-I gp21 peptide 400-429 by MBP-ghA, -ghB, or -ghC. Microtiter wells were coated with HTLV-I gp21 peptide 400-429 and blocked. Different concentrations of nonbiotinylated C1q, MBP-ghA, -ghB, and -ghC were used as inhibitors of biotinylated C1q before being added to the wells and incubated at 37°C for 2 h. Bound C1q was subsequently probed with streptavidin-HRP conjugate.

suggest that like the CLR domain, the gC1q might have additional binding sites for A $\beta$ 1-42, which appears to be located within the ghB module.



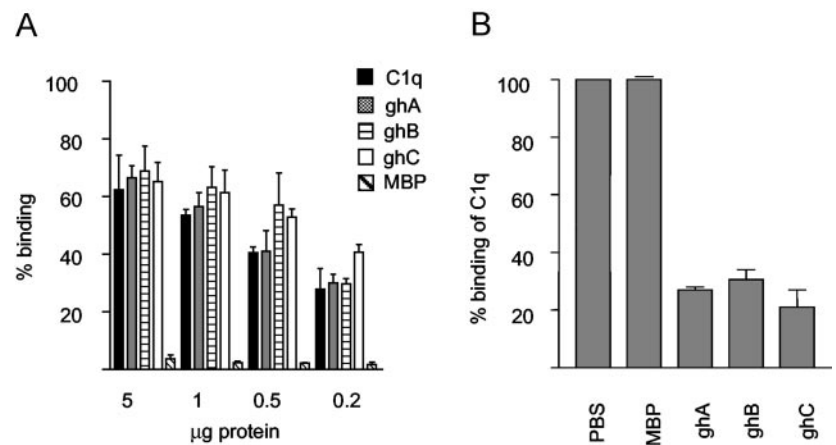
**FIGURE 6.** A, Binding of  $\beta$ -amyloid peptide (A $\beta$ 1-42) to human C1q, gC1q, and CLR. The peptide (1  $\mu$ g/well) was coated onto the microtiter wells, which were then probed with biotinylated human C1q, gC1q, or CLR. The color was developed using streptavidin-HRP conjugate and OPD as substrate. B, Binding of A $\beta$ 1-42 to MBP-ghA, -ghB, or -ghC. The solid phase A $\beta$ 1-42 (1  $\mu$ g) was probed with a range of concentrations of biotinylated MBP-ghA, -ghB, and -ghC (37°C, 2 h). The color was developed using streptavidin-HRP conjugate. C, Inhibition of C1q binding to A $\beta$ 1-42 by MBP-ghA, -ghB, or -ghC. The recombinant fusion proteins, C1q, MBP-ghA, -ghB, and -ghC were allowed to inhibit binding of biotinylated human C1q (1  $\mu$ g/well) to A $\beta$ 1-42 (1  $\mu$ g/well). The ghB module inhibited >50% C1q binding to A $\beta$ 1-42 at a concentration of 20  $\mu$ g/ml (2  $\mu$ g/well).

*MBP-ghA, -ghB, or -ghC bind apoptotic cells specifically*

C1q bound apoptotic PBMCs in a dose-dependent manner, consistent with previous reports (28, 29). Biotinylated C1q bound to 62% ( $\pm$ 0.12) of the apoptotic cells at a 5  $\mu$ g/ml concentration and



**FIGURE 7.** A, Binding of C1q and MBP-ghA, -ghB, or -ghC to apoptotic cells. Apoptotic PBMCs were incubated with various concentrations of biotinylated human C1q, MBP-ghA, -ghB, or -ghC, detected by anti-biotin Ab, and analyzed by FACS. Data of at least three independent experiments are presented as the percentage of apoptotic cells bound by C1q, MBP-ghA, -ghB, or -ghC. B, Inhibition of C1q binding to apoptotic cells by MBP-ghA, -ghB, or -ghC. Apoptotic cells were incubated with buffer, or MBP, MBP-ghA, -ghB, or -ghC (5  $\mu$ g each), washed and then exposed to 1  $\mu$ g of biotinylated C1q. Binding of C1q was detected by FACS. The percentage binding described is from at least three independent experiments. The inhibition of binding of C1q to apoptotic cells in the presence of MBP-ghA, -ghB, or -ghC was significant for all three chains with  $p < 0.05$  (paired Student's *t* test).



to 28% ( $\pm 0.071$ ) of the apoptotic cells at 0.2  $\mu$ g/ml (Fig. 7A). When apoptotic cells were labeled with various concentrations of biotinylated MBP-ghA, -ghB, or -ghC, all three recombinant globular head fragments bound to apoptotic cells, in a similar manner to that of C1q (Fig. 7A). Binding of recombinant proteins to apoptotic cells was specific, because nonbiotinylated MBP-ghA, -ghB, or -ghC (5  $\mu$ g each) inhibited binding of biotinylated C1q (1  $\mu$ g) by 73, 70, and 79%, respectively (Fig. 7B). MBP alone had no effect on binding of C1q to apoptotic cells.

## Discussion

A distinctive, heterotrimeric gC1q domain, situated at the C-terminal end of the triple-helical CLR, characterizes the structural organization of C1q. As a charge pattern recognition protein, C1q is known to interact with a range of ligands via gC1q. However, the role and contributions of ghA, ghB, and ghC in binding to IgG and IgM have not been precisely defined. We generated the recombinant forms of ghA, ghB, and ghC to understand whether the C-terminal globular regions of human C1q A, B, and C chains are functionally autonomous modules (with ghA, ghB, and ghC having distinct binding properties), or the ability of C1q to bind its ligands is dependent upon a combined, globular structure. In addition to aggregated IgG and IgM, we also included in our study some of the known non-Ig targets of C1q, such as peptide 601-613 derived from the transmembrane envelope glycoprotein gp41 of HIV-1, the gp21 peptide 400-429 from HTLV-I,  $\beta$ -amyloid peptide, and apoptotic PBMC.

Based on the Acrp-30 numbering, four residues are conserved throughout the members of C1q and TNF families: Tyr<sup>161</sup>, Gly<sup>159</sup>, Phe<sup>237</sup>, and Leu<sup>242</sup> (14). Each residue seems important for the correct packing of the hydrophobic core of the protomers. Experiments involving chemical modification of distinct amino acid residues and subsequent cross-linking to heterologous IgG have implicated two regions of the gC1q domain (of C1q) in IgG binding (30, 31): "site 1" is thought to be located in a section ranging from 114 to 129 of the B chain with Arg<sup>114</sup> and Arg<sup>129</sup> being the important residues. "Site 2" is considered to involve all three C1q chains, in particular Arg<sup>162</sup> in the A chain, Arg<sup>163</sup> in the B chain, and Arg<sup>156</sup> in the C chain. Interestingly, each of these sites maps to the exterior of the Acrp-30 trimer. These two C1q sites can also be mapped to two separate loops in the Acrp-30 crystal structure. Based on the ELISAs, our results appear to suggest that the ghA is the most effective region for interacting with heat-aggregated IgG and IgM. Because the ghB and ghC showed preference for aggregated IgG and IgM, respectively, it seems likely that a stable interaction between C1q and IgM would require multivalent involve-

ment of all three chains. Alternatively, it may also reflect on the requirement of multiple contact sites between gC1q and IgM. These observations are consistent with the results of C1q-dependent hemolytic assays where both ghA and ghB could also inhibit C1q-dependent hemolysis of IgG- and IgM-sensitized sheep erythrocytes. However, for IgM-coated erythrocytes, the ghC was a better inhibitor of C1q than the ghB.

C1q has been shown to interact with residues 601-613 (loop region) of the envelope protein gp41 of HIV-1, which facilitates infection of complement-receptor bearing cells, instead of viral lysis (32-34). HIV-1-infected B cells, which may serve as an alternative reservoir for HIV-1, also activate CCP in a gp41-dependent manner (35). Structurally, the gp41 loop region exhibits an absence of charged residues and an abundance of exposed hydrophobic side chains (characteristics of protein-protein interaction sites). When modeled on the gp41 structure of simian immunodeficiency virus, the HIV-1 gp41 loop region forms a cleft, which is likely to be complementary to the binding of gC1q (36). We found a specific interaction between ghA and peptide 601-613 compared with ghB and ghC (37). In the competitive ELISA, there was a dose-dependent decrease in binding of C1q to peptide 601-613 when MBP-ghA was used as an inhibitor of this interaction. The inhibitory effect of MBP-ghA was maximally 50% implicating a prominent role of the gC1q domain, especially ghA, in the interaction with gp41. Because HIV-1-induced CCP activation is stronger upon shedding of gp120, the gp120 and gC1q are likely to have overlapping binding sites within the gp41 loop region. Previously, Lys<sup>608</sup>, Leu<sup>609</sup>, and Ile<sup>610</sup> within peptide 601-613 have been identified as central to interaction between gp41 and C1q (38). The Ala substitution of all hydrophobic residues (including the 4-carbon aliphatic moiety of the Lys<sup>608</sup> side chain) within the loop abolishes C1q-gp41 interaction. Because this interaction involves hydrophobic contacts, it is curious that only ghA binds to peptide 601-613 preferentially. It will be interesting to examine whether ghA can interfere with the gp41-C1q interaction and thus inhibit complement-dependent enhancement of viral infection.

C1q has been shown to bind and inhibit the infectivity of the cell-free HTLV-I virion via its interaction with the extramembrane region of gp21, a transmembrane protein considered responsible for fusion between the virus and target cell membrane (39, 40). Although the C1q binding site has been mapped to residues 400-429 and 426-460 of gp21, only peptide 400-429 has been shown to inhibit the syncytium formation induced by cocultivation of HTLV-I-bearing C91/PL cells with indicator MOLT-4 cells (41). The N-terminal region of gp21 bears a 29-residue long hydrophobic stretch (a feature of a fusion peptide) and an anchorage domain,



corresponding to a hydrophobic sequence (residues 446-465) located near the C terminus of the gp21. Thus, gp21 peptide 400-429 corresponds to the site between the anchorage domain and the fusion domain of gp21. Because syncytium formation induced by virus-bearing cells involves binding to the virus receptor and folding of the envelope leading to the exposure of the fusion domain and membrane fusions, the interaction between peptide 400-429 and C1q may directly affect the fusion process required for syncytium formation. Our result suggests that the C1q-HTLV-I gp21 interaction is likely to take place via the gC1q domain and the ghC module is likely to have a central role.

C1q has the ability to bind the fibrils of  $\beta$ -amyloid (A $\beta$ 1-42), which accumulate around cerebral blood vessels. It is the major protein component of the senile or neuritic plaques in the brains of patients with Alzheimer's disease and is known to activate the CCP (42, 43). Recently, it has been shown that C1q interaction with fibrils of the A $\beta$ 1-42 is likely to take place through the gC1q region (44), contrary to an earlier report suggesting an involvement of residues 14-26 within the C1q A chain CLR in C1q-A $\beta$ 1-42 interaction (45). Our results provide evidence for a highly specific and preferential interaction of the ghB module with  $\beta$ -amyloid fibrils. However, we also noticed a dose-dependent binding of CLR to A $\beta$ 1-42 (Fig. 6A), compared to the other two peptides examined, i.e., HIV-1 gp41 peptide 601-613 and HTLV-I gp21 peptide 400-429 (data not included). We think that both gC1q and CLR are capable of engaging  $\beta$ -amyloid fibrils and such interactions should be viewed in the context of fibril aggregation and the activation of the CCP.

C1q is considered to protect against autoimmunity by serving as "opsonin" in the efficient recognition and physiological clearance of cells undergoing programmed death by apoptosis. The apoptotic cells generate discrete subcellular structures, called "apoptotic blebs", that contain either nuclear or cytoplasmic constituents, many of which are targeted by autoantibodies in systemic lupus erythematosus (SLE) patients. The finding that C1q can bind specifically to the surface blebs of apoptotic cells (28), and that the common autoantigens targeted in SLE can be found in high concentrations on the surface of the apoptotic cells (46), have led to the view that C1q deficiency may cause SLE as a result of an impaired clearance of apoptotic cells (47-49). C1q has been shown to bind directly to apoptotic blebs of vascular endothelial cells and PBMCs via the gC1q domain (29), which causes CCP activation and deposition of C4 and C3 on the surface of these cells and on cell-derived blebs (50). Thus, appropriate recognition of apoptotic cells by gC1q and targeted clearance of the molecular contents of surface blebs to complement receptors via exposed CLR (for example, CR1 on the surface of erythrocytes) may be critical for the maintenance of immune tolerance. The recombinant ghA, ghB, and ghC showed specific, dose-dependent binding to the apoptotic PBMC and competed with C1q in binding to the apoptotic cells. Our results are consistent with a recent study where globular head-specific mAbs as well as native gC1q (prepared following collagenase digestion of native human C1q) were able to inhibit binding of C1q to apoptotic cells (29). The recombinant globular head modules may prove to be useful reagents in identifying potential autoantigens, which are likely to be clustered within apoptotic blebs.

In summary, the expression and functional characterization of the individual globular head regions has allowed examination of the modular organization of the C-terminal regions of human C1q. The specificity as well as selectivity of individual globular heads potentially offers enormous flexibility and versatility to the intact C1q molecule, which is considered to perform broad spectrum immune functions by engaging a range of ligands, cell types, and

putative cell surface receptor molecules. The recognition of a C1q/TNF superfamily, whose members have a wide range of functions in host defense, inflammation, apoptosis, autoimmunity, cell differentiation, and homeostasis, emphasizes the possibility that the globular regions of C1q may fulfill more binding functions than previously envisaged. The recombinant ghA, ghB, and ghC therefore could be useful reagents in identifying TNF-related functions (15, 16). It should also be possible to re-examine the interaction of gC1q with a range of ligands, either via direct binding studies, or as inhibitors of whole C1q molecule. Such studies have consistently been influenced by difficulties of generating reasonable amounts of relatively soluble gC1q domains, prepared by collagenase digestion of native C1q.

However, there are significant quantitative differences in molar terms between C1q and recombinant globular heads wherever interaction of native and recombinant proteins with ligands have been examined. A direct comparison between C1q and recombinant fragments of globular heads is further compounded by the fact that intact C1q is likely to have up to 18 binding sites (or at least 6 interacting sites: 6  $\times$  ghA, 6  $\times$  ghB, and 6  $\times$  ghC) compared with a single site within the recombinant ghA, ghB, and ghC. It is also likely that avidity and kinetics of C1q binding to its ligands will be different because of multivalency of globular regions in the intact molecule. Therefore, it is important to consider these issues while interpreting the results of direct binding and competitive inhibition described in the present study.

Future work would involve reconstitution of ghA, ghB, and ghC, following denaturation and renaturation procedure, to generate a soluble and stable heterotrimer, as is considered to be present in the native molecule. If successful, such a "recombinant" heterotrimeric gC1q molecule should provide opportunities to engineer point mutations within ghA, ghB, or ghC modules and study the effects of single residue mutations within a heterotrimeric organization. At a functional level, these recombinant gC1q fragments could also be used as "C1q inhibitors" to block the CCP at the first step of its activation (15, 16, 27, 51, 52).

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## References

- Sellar, G. C., D. J. Blake, and K. B. M. Reid. 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.
- Reid, K. B. M., and R. R. Porter. 1976. Subunit composition and structure of subcomponent C1q of the first component of human complement. *Biochem. J.* 155:19.
- Kishore, U., and K. B. M. Reid. 1999. Modular organization of proteins containing C1q-like globular domains. *Immunopharmacology* 4:15.
- Yamaguchi, N., P. D. Benya, M. van der Rest, and Y. Nimomiya. 1989. The cloning and sequencing of  $\alpha_1$  VIII collagen cDNAs demonstrate that the type VIII collagen is a short chain collagen and contains triple-helical and carboxy-terminal non-triple helical domains similar to those of type X collagen. *J. Biol. Chem.* 270:16022.
- Ninomiya, Y., M. Gordon, M. van der Rest, T. Schmid, T. Linsenmayer, and B. R. Olsen. 1986. The developmentally regulated type X collagen gene contains a long open reading frame without introns. *J. Biol. Chem.* 261:5041.
- Urade, Y., J. Oberdick, R. Molinar-Rode, and J. I. Morgan. 1991. Precerebellin is a cerebellum-specific protein with similarity to the globular domains of complement protein C1q B chain. *Proc. Natl. Acad. Sci.* 88:1069.
- Takamatsu, N., K. I. Ohba, J. Kondo, and T. Shiba. 1993. Hibernation associated gene regulation of plasma proteins with a collagen-like domain in mammalian hibernators. *Mol. Cell. Biol.* 13:1516.

8. Hayward, C. P. M., J. A. Hassell, G. A. Denomme, R. A. Rachubinski, C. Brown, and J. G. Kelton. 1995. The cDNA sequence of human endothelial cell, multimarin: a unique protein with RGDs, coiled-coil and epidermal growth factor-like domains and a carboxy-terminus similar to the globular domain of component C1q and collagen type VIII and X. *J. Biol. Chem.* 270:18246.
9. Scherer, P. E., S. Williams, M. Fogliano, G. Baldini, and H. F. Lodish. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J. Biol. Chem.* 270:26746.
10. Davis, J. G., J. C. Oberholtzer, F. R. Burns, and M. I. Greene. 1995. Molecular cloning and characterization of an inner ear specific structural protein. *Science* 263:1031.
11. Doliana, R., M. Mongiat, F. Bucciotti, E. Giacomello, R. Deutzmann, D. Volpin, G. M. Bressan, and A. Colombatti. 1999. EMILIN, a component of the elastic fiber and a new member of the C1q/tumor necrosis factor superfamily of proteins. *J. Biol. Chem.* 274:16773.
12. Zhang, Y., and Q. Chen. 1999. The non-collagenous domain 1 of type X collagen: a novel motif for trimer and higher order multiple formation without a triple helix. *J. Biol. Chem.* 274:22409.
13. Dublet, B., T. Vernet, and M. van der Rest. 1999. Schmid's metaphyseal chondrodysplasia mutations interfere with folding of the C-terminal domain of human collagen X expressed in *Escherichia coli*. *J. Biol. Chem.* 274:18909.
14. Shapiro, L., and P. E. Scherer. 1998. The crystal structure of a C1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr. Biol.* 8:335.
15. Kishore, U., and K. B. M. Reid. 2000. C1q: structure, function and receptors. *Immunopharmacology* 42:159.
16. Kishore, U., M. S. Kojouharova, and K. B. M. Reid. 2002. Recent progress in the understanding of the structure-function relationships of the globular head regions of C1q. *Immunobiology* 205:355.
17. Jones, E. Y., D. I. Stuart, and N. P. Walker. 1989. Structure of tumor necrosis factor. *Nature* 338:225.
18. Smith, K. F., P. I. Harris, D. Chapman, K. B. M. Reid, and S. J. Perkins. 1994. The  $\beta$ -sheet secondary structure of the trimeric globular domain of C1q of complement and collagen types VIII and X by Fourier-transform infrared spectroscopy and averaged structure predictions. *Biochem. J.* 301:249.
19. Sledge, C. R., and D. H. Bing. 1973. Binding properties of the human complement protein, C1q. *J. Biol. Chem.* 248:2818.
20. Schumaker, V. N., M. A. Calcott, H. L. Spiegelberg, and H. J. Muller-Eberhard. 1976. Ultracentrifuge studies of the binding of IgG of different subclasses to the C1q subunit of the first component of complement. *Biochemistry* 15:5175.
21. Hughes-Jones, N. C., and B. Gardner. 1978. The reaction between the complement subcomponent C1q, IgG complexes and polyionic molecules. *Immunology* 34:459.
22. Hughes-Jones, N. C. 1977. Functional affinity constants of the reaction between <sup>125</sup>I-labelled C1q and C1q binders and their use in the measurement of plasma C1q concentrations. *Immunology* 32:191.
23. Duncan, A. R., and G. Winter. 1988. The binding site for C1q on IgG. *Nature* 332:738.
24. Perkins, S. J., A. S. Nealis, B. J. Sutton, and A. Feinstein. 1991. Solution structure of human and mouse immunoglobulin M by synchrotron x-ray scattering and molecular modelling: a possible mechanism for complement activation. *J. Mol. Biol.* 221:1345.
25. Kishore, U., R. D. Sontheimer, K. N. Sastry, K. S. Zaner, E. G. Zappi, G. R. V. Hughes, M. A. Khamashta, P. Strong, K. B. M. Reid, and P. Eggleton. 1997. Release of calreticulin from neutrophils may alter C1q-mediated immune functions. *Biochem. J.* 322:543.
26. Kishore, U., L. E. A. Leigh, P. Eggleton, P. Strong, M. V. Perdikoulis, A. C. Willis, and K. B. M. Reid. 1998. Functional characterization of a recombinant form of the carboxyl terminal, globular head region of the B chain of human serum protein, C1q. *Biochem. J.* 333:27.
27. Kishore, U., P. Strong, M. V. Perdikoulis, and K. B. M. Reid. 2001. A recombinant homotrimer, composed of the  $\alpha$ -helical neck region of human surfactant protein D and C1q B chain globular domain, is an inhibitor of the classical complement pathway. *J. Immunol.* 166:559.
28. Korb, L. C., and J. M. Ahearn. 1997. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. *J. Immunol.* 158:4525.
29. Navratil, J. S., S. C. Watkins, J. J. Wisniewski, and J. M. Ahearn. 2001. The globular heads of C1q specifically recognize surface blebs of apoptotic vascular endothelial cells. *J. Immunol.* 166:3231.
30. Marques, G., L. C. Anton, E. Barrio, A. Sanchez, S. Ruiz, F. Gavilanes, and F. Vivanco. 1993. Arginine residues of the globular regions of human C1q involved in the interaction with immunoglobulin G. *J. Biol. Chem.* 268:10393.
31. Kaul, M., and M. Loos. 1997. Dissection of C1q capability of interacting with IgG: time-dependent formation of a tight and only partly reversible association. *J. Biol. Chem.* 272:33234.
32. Theilens, N. M., I. M. Bally, C. F. Ebenbichler, M. P. Dierich, and G. J. Arlaud. 1993. Further characterization of the interaction between the C1q subcomponent of human C1 and the transmembrane envelope glycoproteins gp41 of HIV-1. *J. Immunol.* 151:6583.
33. Tacnet-Delorme, P., V. Boyer, N. M. Thielen, J. F. Hernandez, I. Bally, R. B. Sim, C. Desgranges, and G. J. Arlaud. 1999. In vitro analysis of complement-dependent HIV-1 cell infection using a model system. *J. Immunol.* 162:4088.
34. Marschang, P., U. Kruger, C. Ochsenbauer, L. Gurtler, A. Hittmair, V. Bosch, J. R. Patsch, and M. P. Dierich. 1997. Complement activation by HIV-1-infected cells: the role of transmembrane glycoprotein gp41. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* 14:102.
35. Moir, S., A. Malaspina, Y. Li, T. W. Chun, T. Lowe, J. Adelsberger, M. Baseler, L. A. Ehler, S. Liu, R. T. Davey Jr., et al. 2000. B cells of HIV-1-infected patients bind virions through CD21-complement interactions and transmit infectious virus to activated T cells. *J. Exp. Med.* 192:637.
36. Caffrey, M. 2001. Model for the structure of the HIV gp41 ectodomain: insight into the intermolecular interactions of the gp41 loop. *Biochim. Biophys. Acta* 1536:116.
37. Kojouharova, M. S., I. D. Panchev, M. I. Tchobadjieva, K. B. M. Reid, and H. J. Hoppe. 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.
38. Quinkal, I., J. F. Hernandez, S. Chevallier, G. J. Arlaud, and T. Vernet. 1999. Mapping of the interaction between the immunodominant loop of the ectodomain of HIV-1 gp41 and human complement protein C1q. *Eur. J. Biochem.* 265:656.
39. Ikeda, F., Y. Haraguchi, A. Jinno, Y. Iino, Y. Morishita, H. Shiraki, and H. Hoshino. 1998. Human complement component C1q inhibits the infectivity of cell-free HTLV-I. *J. Immunol.* 161:5712.
40. Nagy, K., P. Clapham, R. Chelngons-Popov, and R. Weiss. 1983. Human T-cell leukemia virus type I: induction of syncytium and inhibition by patients' sera. *Int. J. Cancer* 32:321.
41. Sagara, Y., Y. Inoue, H. Shiraki, A. Jinno, H. Hoshino, and Y. Maeda. 1996. Identification and mapping of functional domains on human T-cell lymphotropic virus type 1 envelope proteins by using synthetic peptides. *J. Virol.* 170:1564.
42. Gasque, P., Y. D. Dean, E. P. McGreal, J. Van Beek, and B. P. Morgan. 2000. Complement components of the innate immune system in health and disease in the central nervous system. *Immunopharmacology* 49:171.
43. Webster, S., C. Glabe, and J. Rogers. 1995. Multivalent binding of complement protein C1q to the amyloid  $\beta$ -peptide (A $\beta$ ) promotes the nucleation phase of A $\beta$  aggregation. *Biochem. Biophys. Res. Commun.* 217:869.
44. Tacnet-Delorme, P., S. Chevallier, and G. J. Arlaud. 2001.  $\beta$ -amyloid fibrils activate the C1 complex of complement under physiological conditions: evidence for a binding site for A $\beta$  on the C1q globular regions. *J. Immunol.* 167:6374.
45. Jiang, H., D. Burdick, C. G. Glabe, C. W. Cotman, and A. J. Tenner. 1994.  $\beta$ -amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain. *J. Immunol.* 152:5050.
46. Casciola-Rosen, L. A., G. Anhalt, and A. Rosen. 1994. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* 179:1317.
47. Botto, M., C. Dell'Agnola, A. E. Bygrave, E. M. Thompson, H. T. Cook, F. Petry, M. Loos, P. P. Pandolfi, and M. J. Walport. 1998. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* 19:56.
48. Mevorach, D., J. L. Zhou, X. Song, and K. B. Elkou. 1998. Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J. Exp. Med.* 188:387.
49. Mitchell, D. A., P. R. Taylor, H. T. Cook, J. Moss, A. E. Bygrave, M. J. Walport, and M. Botto. 1999. C1q protects against the development of glomerulonephritis independently of C3 activation. *J. Immunol.* 162:5676.
50. Nauta A. J., L. A. Trouw, M. R. Daha, O. Tijms, R. Nieuwland, W. J. Schwaeble, A. R. Gingras, A. Mantovani, E. C. Hack, and A. Roos. 2002. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *Eur. J. Immunol.* 32:1726.
51. Roos, A., A. J. Nauta, D. Broers, M. C. Faber-Krol, L. A. Trouw, J. W. Drijfhout, and M. R. Daha. 2001. Specific inhibition of the classical complement pathway by C1q-binding peptides. *J. Immunol.* 167:7052.
52. Lambiris, J. D., K. B. M. Reid, and J. E. Volanakis. 1999. The evolution, structure, biology and pathophysiology of complement. *Immunol. Today* 20:207.