Interaction of the globular domain of human C1q with Salmonella typhimurium lipopolysaccharide

ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · JUNE 2008

Impact Factor: 4.66 · DOI: 10.1016/j.bbapap.2008.04.029 · Source: PubMed

Biochimica et Biophysica Acta 1784 (2008) 1271–1276

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Interaction of the globular domain of human C1q with *Salmonella typhimurium* lipopolysaccharide

Lubka T. Roumenina [a,b], Krustyo T. Popov [a], Svetlana V. Bureeva [c], Mihaela Kojouharova [a], Mihaela Gadjeva [d], Shweta Rabheru [e], Roshni Thakrar [e], Alexander Kaplun [c], Uday Kishore [e,*]

[a] *Department of Biochemistry, Sofia University, St. Kliment Ohridski, 8 Dragan Tsankov St., Sofia 1164, Bulgaria*
[b] *Centre de Recherche des Cordeliers, INSERM UMRS 872, 15 rue de l'Ecole de Médecine 75270. PARIS Cédex 06, France*
[c] *Department of Biotechnology, M.V. Lomonosov State Academy of Fine Chemical Technology, 86 Vernadskogo St., Moscow, 119571, Russia*
[d] *Brigham and Women's Hospital, Department of Pathology, Harvard Medical School, Boston, MA 02115, USA*
[e] *Laboratory of Human Immunology, Division of Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge UB8 3PH, West London, United Kingdom*

ARTICLE INFO

Article history:
Received 19 December 2007
Received in revised form 25 April 2008
Accepted 29 April 2008
Available online 10 May 2008

Keywords:
C1q
Salmonella lipopolysaccharide
IgG
Ca^{2+}
Recognition

ABSTRACT

Gram-negative bacteria can bind complement protein C1q in an antibody-independent manner and activate classical pathway via their lipopolysaccharides (LPS). Earlier studies have implicated the collagen-like region of human C1q in binding LPS. In recent years, a number of C1q target molecules, previously considered to interact with collagen-like region of C1q, have been shown to bind via the globular domain (gC1q). Here we report, using recombinant forms of the globular head regions of C1q A, B and C chains, that LPS derived from *Salmonella typhimurium* interact specifically with the B-chain of the gC1q domain in a calcium-dependent manner. LPS and IgG-binding sites on the gC1q domain appear to be overlapping and this interaction can be inhibited by a synthetic C1q inhibitor, suggesting common interacting mechanisms.

1. Introduction

C1q is the first subcomponent of the classical complement pathway and a major link between innate and acquired immunity. The human C1q molecule is made up of six copies of three polypeptide chains (A, B and C). Each of these chains has an N-terminal triple-helical collagen-like region (CLR) and a C-terminal globular (gC1q) domain [1]. The gC1q domain is composed of the C-terminal regions of A, B and C chains held together by CLR, thus making it a heterotrimeric structure. C1q has been shown to be capable of recognizing over 50 structurally diverse ligands. Some of these ligands are bound by the gC1q domain (for example aggregated IgG and IgM), while ligands such as DNA and C-reactive protein (CRP) have been shown to bind via CLR. A number of C1q ligands, including CRP and β-amyloid peptide, previously shown to interact with C1q CLR, have recently been demonstrated to bind the gC1q domain [1], thus shedding new lights on the modulation of the classical complement pathway by C1q binders.

The versatility of the gC1q domain to engage a wide array of target molecules is helped by its modular organization of the individual globular head modules contributed by A (ghA), B (ghB), and C (ghC) chains. The recombinant expression and characterization of ghA, ghB and ghC modules of human C1q has suggested that each module can have structural and functional autonomy based on its differential ligand-binding properties [2]. The modular organization has been confirmed by the crystal structure of the native heterotrimeric gC1q domain solved at 1.9 Å resolution [3] that has revealed a compact, spherical heterotrimer with a pseudo-3-fold symmetry. It also reflects that individual modules within the heterotrimeric gC1q domain are capable of interacting with diverse ligands owing to differential charge and orientation.

The gC1q domain has an exposed Ca^{2+} ion located near its apex, which influences the ligand recognition properties of C1q towards immunoglobulins and pentraxins [4]. The calcium ion governs the direction of molecular electric moment vector of gC1q — a parameter that has recently been proposed to be important for electrostatic recognition between macromolecules. A loss of Ca^{2+} leads to changes in the direction of the electric moment vector from co-axial (in HOLO,

Abbreviations: B2S, betulin disulfate; gC1q, the globular domain of human C1q; CLR, collagen-like region of C1q; CRP, C-reactive protein; ghA, ghB, ghC, the recombinant forms of the globular regions of C1q A, B and C chains, respectively; F2S, 9,9-bis(4′ hydroxyphenyl)fluorene disulfate; KDO, 2-keto-3-deoxyoctonoic acid; LPS, lipopolysaccharides; MBP, maltose-binding protein; pIgG, pooled human IgG; PBS, phosphate-buffered saline; PTX3, long prototypic pentraxin 3; PBST, phosphate-buffered saline containing 0.05% Tween-20; HRP, horseradish peroxidase

* Corresponding author. Tel.: +44 1895 266362; fax: +44 1895 269873.
E-mail addresses: ukishore@hotmail.com, uday.kishore@brunel.ac.uk (U. Kishore).

1570-9639/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2008.04.029

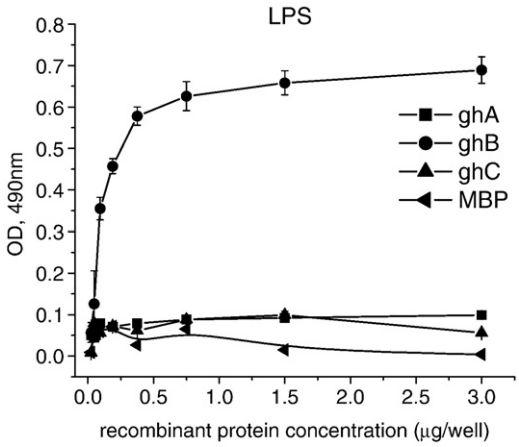

Fig. 1. ELISA analysis of the interaction between the recombinant globular modules of C1q (ghA, ghB and ghC) and LPS or pIgG. LPS was coated to the microtitre wells and probed with recombinant ghA, ghB or ghC modules expressed as MBP fusions (3, 1.5, 0.75, 0.375, 0.188, 0.094 and 0.045, 0.023 μg/well). The bound recombinant proteins (being expressed as fusion proteins with MBP) were detected by mouse anti-MBP monoclonal antibodies, followed by anti-mouse IgG–HRP conjugate. MBP was used as a negative control protein. The data shown are means ± SD of triplicate measurements.

Ca^{2+} saturated form) to perpendicular (in APO, Ca^{2+} depleted form) to the molecular axis. Two planes, perpendicular to these vectors define APO (the equatorial surface of the B-chain) and HOLO (gC1q apex) planes. Electrostatic modeling [4] as well as mutational analysis [5–8] of the recombinant ghA, ghB and ghC modules have led to the suggestion that the binding sites on gC1q domain for IgG1, IgM, CRP and pentraxin 3 (PTX3) are overlapping, and in most cases, located within HOLO and APO surfaces. Thus, a novel model of target recognition and complement activation by C1q has been proposed [4]. C1q in serum is present in Ca^{2+}-bound form and its molecular electrical moment is directed to the apex of the gC1q domain. The initial recognition of the target/ligand is achieved by the gC1q apex, followed by subsequent loss of calcium and reorientation of molecular moment vectors, thus leading to rotation of the gC1q domains. The apparent increase in the angle between the stalks in the hinge region of the CLR leads to transmission of the activation signal to C1r [5,6].

Lipopolysaccharides (LPS) derived from a number of Gram-negative bacteria have been shown to interact with C1q, in an immunoglobulin-independent manner [9,10]. C1q interacts with the lipid A portion that is highly exposed in Re-mutant. C1q–LPS interaction is influenced by the core and the O-specific sugar portion (O-antigens) of LPS [10], especially the anionic charges of 2-keto-3-deoxyoctonoic acid (KDO) [11]. It has been shown that C1q binding to bacteria occurs with higher affinity for shorter LPS molecules [12]. *Escherichia coli* LPS can fix complement and this C1q–LPS binding involves interaction with the CLR via histidine (but not arginine) residues [11]. This binding site is likely to reside within the hinge region of C1q, the CLR site that is occupied by $C1r_2$–$C1s_2$ tetramer.

The aim of the current work was to examine interaction of the gC1q domain with *Salmonella* LPS using recombinant forms of ghA, ghB and ghC modules. Our data suggest the existence of a binding site for LPS on the gC1q domain. The ghB module appears centrally involved in this interaction. The binding sites for IgG and LPS are likely to be overlapping. These interactions are calcium-sensitive, and thus, may share a common complement activation mechanism.

2. Materials and methods

2.1. Purified proteins and antibody conjugates

C1q was purchased from Quidel (San Diego, CA, USA) and LPS derived from *Salmonella typhimurium* from Sigma-Aldrich (St. Louise, MO, USA). Pooled human therapeutic IgG preparation (Imunovenin Intact, BulBio, Sofia, Bulgaria) was used as a source of IgG (pIgG) that was heat-aggregated prior to assay. Goat anti-rabbit IgG-horseradish peroxidase (HRP) conjugate, mouse anti-maltose-binding protein (MBP)-monoclonal antibodies and *o*-phenylenediamine dihydrochloride (OPD) were purchased from Sigma-Aldrich. Rabbit anti-human C1q polyclonal antibodies and rabbit anti-human IgG-HRP conjugate were purchased from DAKO.

2.2. Intracellular expression and purification of ghA, ghB, and ghC and their mutant forms

The recombinant globular head regions of the A-chain (ghA, residues 88–223), the B-chain (ghB, 90–226), and the C-chain (ghC, 87–217) and the three mutant form of ghB (Arg^{B129}Ala, Arg^{B163}Glu and Tyr^{B175}Leu) were expressed as fusion proteins linked to MBP in *E. coli* BL21 and purified, as described previously [13].

2.3. ELISA for detecting interaction of recombinant ghA, ghB and ghC modules with LPS and pIgG

Microtitre wells were coated for 1 h at 37 °C with either 1 μg/well of LPS or 1 μg/well pIgG in carbonate buffer pH 9.6. Any non-specific binding sites were blocked with 200 μl/well 1% w/v BSA for 1 h at 37 °C. The wells were washed with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBST), and then incubated with different amounts of recombinant ghA, ghB and ghC (3, 1.5, 0.75, 0.375, 0.188, 0.094 and 0.045, 0.023 μg/well) 1 h at 37 °C. After three rounds of washing with PBST, the wells were incubated with mouse anti-MBP antibodies (1:4000 dilution) for 1 h at 37 °C. Bound recombinant modules were detected by rabbit anti-mouse IgG–HRP conjugate (1:1000 dilution). The color was developed using OPD as substrate. Data are given as an average of three repetitions ± standard deviation.

2.4. ELISA for detecting interaction between C1q and LPS or pIGG with or without calcium

Microtitre wells were coated for 1 h at 37 °C with either 1 μg/well of LPS or 1 μg/well pIgG in carbonate buffer pH 9.6. Any non-specific binding sites were blocked with

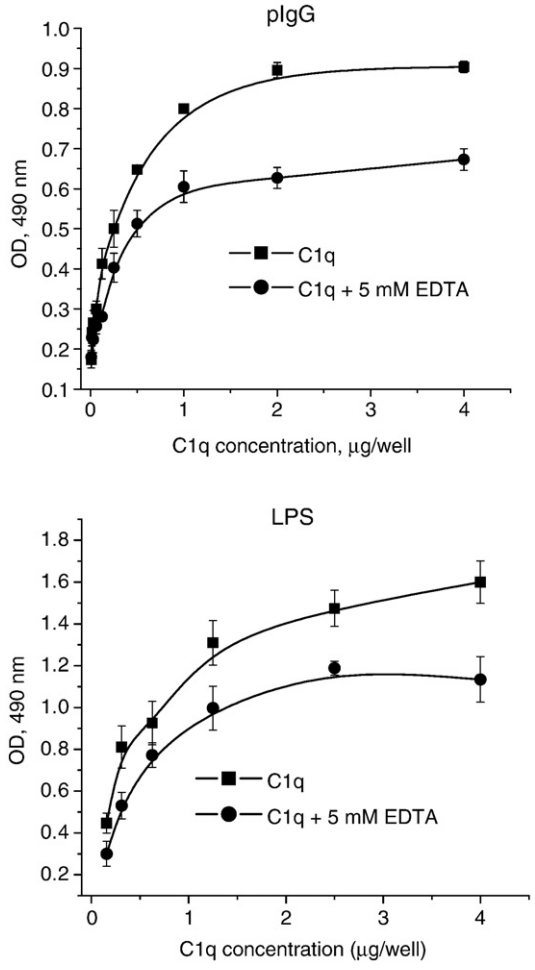

Fig. 2. ELISA to assess the Ca^{2+} dependence of C1q interaction with: (a) LPS and (b) pIgG. One μg/well LPS or pIgG was coated on the microtitre wells for 1 h at 37 °C. After blocking in PBS containing 1% (w/v) BSA and subsequent washing, the wells were incubated with C1q at different concentrations (0.0313, 0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 μg/well) with or without 5 mM EDTA overnight at 4 °C. The bound C1q was detected by rabbit anti-human C1q polyclonal antibodies and goat anti-rabbit IgG, conjugated with HRP. The data shown are means ± SD of triplicate measurements.

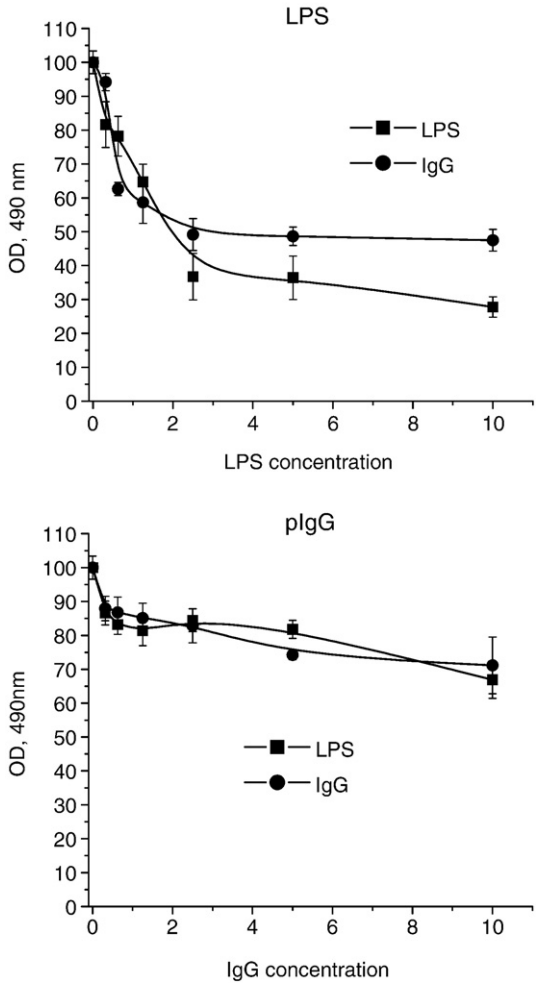

Fig. 3. ELISA for detecting inhibitory effects of (a) LPS and (b) pIgG on the interaction between C1q and LPS. The microtitre wells were coated with LPS or pIgG (1 μg/well), blocked and washed. C1q (1 μg/well) was pretreated with different amounts of LPS or pIgG (0, 0.313, 0.625, 1.25, 2.5, 5 and 10 μg/well), incubated for 1 h, and then added to the microtitre wells. The amount of bound C1q to LPS or pIgG, coated to the wells, was detected by using rabbit anti-C1q polyclonal antibodies and HRP-conjugated goat anti-rabbit IgG. Data are given in percentage as an average of three repetitions ± standard deviation. The results presented are representative of three independent experiments. The % reduction in the binding after the addition of a competitor molecule was calculated for all studied interactions. The degree of C1q binding to the corresponding target molecule in the absence of a competitor was taken as 100%.

200 μl/well 1% w/v BSA for 1 h at 37 °C. Then, the wells were washed with PBST, followed by incubation with different amounts of human C1q (4, 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.0313 μg/well). The incubation was carried out in PBST alone, or PBST with 5 mM EDTA (without Ca^{2+}) overnight at 4 °C. After washing, the wells were incubated with rabbit anti-human C1q polyclonal antibodies (1:1000 dilution) for 1 h at 37 °C. Bound C1q was detected using goat anti-rabbit IgG–HRP conjugate (1:5000 dilutions). The color was developed using OPD. Data are given as an average of three repetitions ± standard deviation. The % reduction of the binding after Ca^{2+} removal was calculated for the studied interactions. The plateau values of binding in PBST were taken as 100%. The plateau value in the presence of EDTA was taken to calculate % reduction.

2.5. ELISA for detecting the interaction of recombinant globular module of C1q B-chain (ghB) and its substitution mutants with LPS

Microtitre wells were coated for 1 h at 37 °C with 1 μg/well LPS in carbonate buffer pH 9.6. Any non-specific binding sites were blocked using 200 μl/well 1% w/v BSA for 1 h at 37 °C. The wells were then washed with PBST, and incubated with a serial dilution of ghB and its substitution point mutants: $Arg^{B129}Ala$, $Arg^{B163}Glu$ and $Tyr^{B175}Leu$ [5,7] (concentration ranging between 0.015 μg/well and 2 μg/well) in PBST for 1 h at 37 °C. The amount of bound protein was detected with mouse anti-MBP monoclonal antibodies as described above. The data are given as an average of three experiments ±standard deviation. The plateau values for the wild-type recombinant modules were considered as 100% and those of the mutant form were used to calculate the percent reduction in LPS binding.

2.6. Competitive ELISA

The inhibitory effects of fluid phase LPS or pIgG on the interaction between C1q and solid phase pIgG or LPS were examined. The microtitre wells were coated with LPS or pIgG (1 μg/well), blocked and washed. Purified human C1q (at 1 μg/well) was coincubated with different amounts of LPS or pIgG (0, 0.313, 0.625, 1.25, 2.5, 5 and 10 μg/well) for 1 h prior to being added to the wells. The amount of C1q bound to solid phase LPS or IgG was detected using rabbit anti-C1q polyclonal antibodies (1:1000 dilution) and goat anti-rabbit IgG–HRP conjugate (1:5000 dilution). The color was developed using OPD. Data are given in percentage as an average of three repetitions ± standard deviation. The % reduction in binding was calculated. The degree of binding of C1q to the corresponding target molecule at the plateau in the absence of a competitor was taken as 100%.

2.7. ELISA to detect inhibition of the interaction of C1q with LPS and pIgG by betulin disulfate (B2S) and 9,9-bis(4′hydroxyphenyl)fluorene disulfate (F2S)

Microtitre wells were coated with LPS or pIgG, 1 μg/well in carbonate buffer and any residual binding sites were blocked with 2% w/v BSA in PBS for 1 h at 37 °C. After washing, wells were incubated (1.5 h at 37 °C) with C1q or ghB, previously incubated with B2S or F2S in different concentrations (96, 48, 24, 12, 6, 3, 1.5 and 0 μM for B2S and 225, 112, 56, 28, 14, 7, 3.5 and 0 μM for F2S) diluted in PBST. After washing, the microtitre wells were incubated with rabbit anti-human C1q antibodies (1:1000 dilution) or mouse anti-MBP antibodies (1:4000 dilution) for 1 h at 37 °C. Bound proteins were detected using HRP-conjugated goat anti-rabbit IgG (1:1000 dilution) or rabbit anti-mouse IgG (1:1000 dilution) conjugated to HRP. The color was developed using OPD. Data are expressed as percentage using C1q or ghB binding to LPS or pIgG in the absence of betulin disulfate. The results presented are an average of three repetitions ± standard deviation.

3. Results

3.1. Localization of LPS-binding site on C1q

C1q has previously been shown to interact, via its CLR, with lipid A and LPS derived from different bacterial sources. Ligands such as CRP [14], serum amyloid protein (SAP) [15] and β-amyloid [16], previously known to bind CLR, have recently been shown to interact with the gC1q domain using improved techniques and reagents [4,17–19]. Thus, we readdressed the issue of C1q–LPS interaction using the recombinant modules. We show here that C1q can interact with LPS via its gC1q domain. The ghB module appeared central to this interaction and constitute the main LPS-binding site (Fig. 1). The recombinant globular modules ghA and ghC, as well as the fusion partner MBP did not interact with LPS.

3.2. Effect of Ca^{2+} on the interaction of C1q with LPS and pIgG

Previously, calcium associated with the gC1q domain as well as ghB has been shown to be important for C1q interaction with IgG1, IgM and CRP binding and subsequent complement activation [4]. Thus, we examined

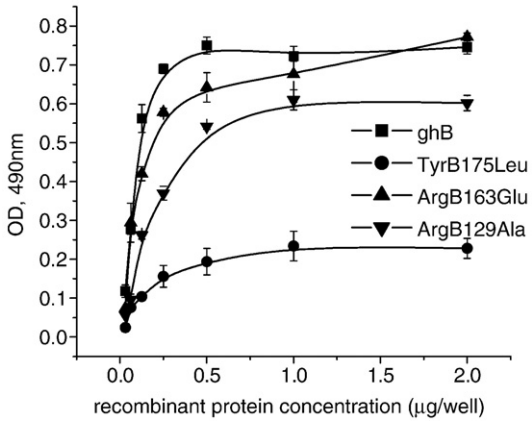

Fig. 4. Interaction of wild-type ghB and its mutant forms $Arg^{B129}Ala$, $Arg^{B163}Glu$ and $Tyr^{B175}Leu$ with LPS. Microtitre wells were coated with 1 μg/well LPS. After blocking and washing, different quantities (0.063, 0.125, 0.25, 0.5, 1.0, and 2 μg/well) of ghB, $Arg^{B129}Ala$, $Arg^{B163}Glu$ or $Tyr^{B175}Leu$ were added to the wells and incubated for 1 h. Mouse anti-MBP antibodies, followed by rabbit anti-mouse IgG–HRP conjugate, detected the amount of bound wild-type ghB module or its substitution mutants. The data shown are the mean ± SD of triplicate measurements.

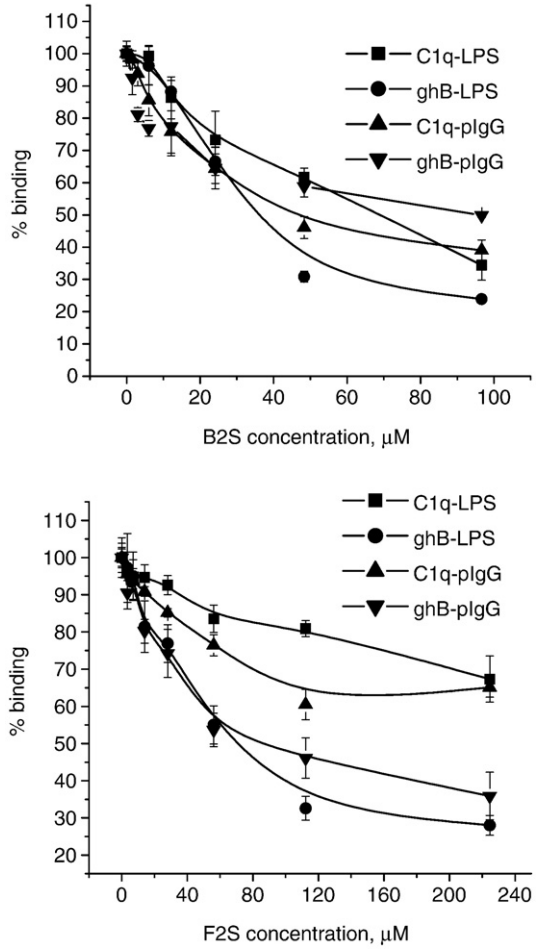

Fig. 5. ELISA analysis to examine the inhibitory effect of (a) B2S and (b) F2S on the interaction of C1q and ghB modules with LPS and pIgG, LPS and IgG were coated to the microtitre wells, followed by addition of betulin disulfate pretreated with C1q or ghB (96, 48, 24, 12, 6, 3, 1.5 and 0 μM B2S). Alternatively F2S was added to C1q and ghB preparations (225, 112, 56, 28, 14, 7, 3.5 and 0 μM for F2S). The bound C1q or ghB was detected using rabbit anti-human C1q antibodies and goat anti-rabbit IgG, conjugated with HRP or by mouse anti-MBP antibodies, recognized by anti-mouse IgG–HRP conjugate respectively. The data shown are means ± SD of triplicate measurements. The results presented are % binding in the presence of the inhibitor, where the C1q binding to the target in the absence of betulin disulfate was taken as 100%.

whether C1q interaction with LPS, a classical pathway activator, was also calcium-sensitive. The interaction between C1q and target molecules was performed in the presence or absence of Ca^{2+} in the incubation buffer. The depletion of Ca^{2+} was achieved by adding EDTA. In the presence of 5 mM EDTA the apparent target-binding ability of C1q was reduced by ~30% for LPS and by ~25% for pIgG (Fig. 2). These results are consistent with the data obtained previously for IgG1 (30%), IgM (28%) and CRP (15%) [4].

3.3. LPS and pIgG have overlapping binding sites on gC1q domain

Given that B-chain was found to be central to the gC1q–LPS interaction (as is the case with C1q–IgG interaction), we set out to examine if the binding sites on the gC1q domain for LPS and IgG were overlapping and shared. In a competitive ELISA, pre-incubation of C1q with LPS inhibited binding of C1q to immobilized LPS and to IgG, in a dose-dependent manner (Fig. 3a), and the resulting curve trends are comparable. Similarly, pIgG in a fluid phase also inhibited the binding of C1q to immobilized LPS and to pIgG in a dose-dependent manner (Fig. 3b). The inhibition by pIgG is slight, because aggregation is required for stable C1q binding to be achieved in solution.

In order to identify residues within the ghB module that may be contributing to the C1q–LPS interaction, three substitution mutants that have previously been shown to be important for ghB–IgG interaction were used [5,7]. As shown in Fig. 4, it was demonstrated that mutation of Tyr^{B175}Leu mutant reduced the ghB binding down to about 70% and Arg^{B129}Ala to ~15%. The Arg^{B163}Glu substitution mutant did not have a significant effect on the ghB–LPS interaction.

3.4. Inhibition of C1q–LPS interaction by charged low molecular weight compounds

To further confirm that IgG and LPS shared common binding sites on the gC1q domain, we examined whether low molecular weight compounds, B2S and F2S, which were inhibitors of C1q–IgG interaction [20,21], could also inhibit binding of C1q to LPS. Since we observed that the LPS-binding site lied within the ghB module, the abilities of these C1q inhibitors to alter the interaction of ghB with LPS as well as pIgG were also examined. In most assays, comparable inhibition was achieved at similar concentrations for various interactions. The data obtained for B2S is shown in Fig. 5a. B2S is also an inhibitor of the interaction between C1q (and ghB) and CRP, PTX3, or IgG [28]. Similar results were obtained using F2S, another small molecule inhibitor (Fig. 5b).

4. Discussion

A growing list of C1q binders and activators of the classical complement pathway, previously shown to bind to the C1q CLR region, are being reported to interact via the gC1q domain using improved assay techniques and reagents [1]. With the availability of the recombinant forms of wild-type and mutant forms of the globular head regions (ghA, ghB and ghC), together with the crystal structure of the native heterotrimeric gC1q domain, a number of C1q ligands are being reported to bind to the gC1q domain of C1q. The recent obseravations have put complement activation mechanisms in perspective, with a real possibility of developing therapeutic inhibitors of the classical pathway [2,3,5,17,19,23]. In the current study, the interactions between the ghB module and LPS derived from *S. typhimurium* appear to highlight this issue. This interaction appears to be calcium-sensitive and inhibitable by low molecular weight compounds of C1q–IgG binding. LPS bound to recombinant ghB module specifically in a dose-dependent manner, indicating the presence of a LPS-binding site, located in this module, which also contains IgG-binding site [7]. The binding sites for a number of C1q target molecules have been shown to be identical or closely overlapping. These include: IgG, CRP, PTX3 [5]; CRP and IgG [19]; PTX3, CRP, IgG, SAP and gC1qR [23]; and IgG, OmpK36 (outer membrane protein) and non-immunoglobulin agglutinin (NIA) [24]. In another case, the IgG and IgM-binding sites within the gC1q domain are partially overlapping [2,6,24]. The viral glycoproteins gp41 from HIV-1, gp21 from HTLV-1 [2,25] and tumor metalloproteinase [26] bind to different sites, all non-identical with that for IgG. Our mutational analysis (Fig. 4) suggest that the same binding surface within the gC1q domain is engaged with the binding to immunoglobulins, pentraxins as well as to LPS. The results indicate that Tyr^{B175} has a leading role in the interaction (about 70% reduction in the binding). Presumably the binding is achieved due to hydrogen bond with the hydroxyl group of Tyr^{B175}Leu, since the substitution involves a long hydrophobic residue, which could participate in hydrophobic interactions, typical for the aromatic ring of Tyr. The same residue, although to a less extent, is involved in IgG interaction [5,7]. It has a leading role in the C1q interaction with PTX3 and IgM, which share overlapping binding sites with IgG [5,6]. The results are consistent with those obtained for IgG1 [5]. Bacterial porins, including OmpK36, also bind to the gC1q domain in the same manner as IgG [24,27].

Existence of LPS-binding site within the gC1q domain was further confirmed by the observations that binding of LPS to either C1q or

ghB was inhibited by B2S and F2S (Fig. 5) and heme [22], a low molecular weight inhibitors of C1q–IgG binding [21]. Both of these low molecular weight compounds are known inhibitors of C1q–IgG interaction [28]. B2S and F2S are also inhibitors of the interaction between IgG1, CRP and PTX3 with C1q: targets that have overlapping binding sites on gC1q domain. It is likely that the inhibition of C1q–LPS interaction by B2S and F2S shares similar mechanisms.

Our results suggest existence of additional LPS-binding site(s) within the gC1q domain, following earlier report of localization of binding site within the CLR [11]. Such an involvement of both CLR and gC1q domain in C1q ligand binding has recently been reported for fucoidan, an algal polysaccharide [29,30]. Fucoidan binds to CLR hinge region but also to the B-chain of the gC1q domain and Arg^{B109} is important for this interaction [30]. The same residue (Arg^{B109}) has also been shown to be important for the interaction of gC1q domain with IgG, IgM and most likely to CRP, using recombinant ghA, ghB and ghC modules and their point mutants [4,6]. A special role for Arg^{B108} and Arg^{B109}, which reside between the gC1q apex and the side surface of the B-chain, has been highlighted by biophysical studies through electrostatic calculations [4]. It appears that there are two distinct modes of LPS binding to C1q. The site within the gC1q domain can bind to Lipid A (Tan, Kishore, Sim, unpublished data) and thus leading to the activation of the complement. The LPS interacting site within the CLR probably binds to the negatively charged carbohydrate moiety of LPS. The outcome of CLR–LPS interaction in terms of complement activation remains unclear. The structure and the composition of the complex C1q-binding surface on the bacterial cell wall would probably determine the possibility of involvement of either gC1q domain or CLR [11,12]. This dual mode of interaction between C1q and LPS may have impact upon the pathophysiological outcomes leading to either complement susceptibility (activation of classical complement cascade) or resistance (downregulation of the complement activation) with respect to the invading pathogen.

A number of diseases such as Alzheimer's disease, dementia, ischemia/reperfusion injury, and stroke are associated with uncontrolled activation of the classical pathway [31]. Currently there are no well-tested inhibitors of C1q that can inhibit the classical pathway at its earliest step. Ideally, the C1q inhibitor should suppress only the pathological C1q–ligand interaction, leaving intact the ability of the classical pathway to protect the organism from pathogen invasion. The evidence that the gC1q domain appears to recognize immunoglobulins, pentraxins and LPS in a similar manner, makes the design of ligand-specific inhibitors a difficult proposition.

Acknowledgements

We are thankful to Prof. Boris Atanasov (Sofia, Bulgaria) for the helpful and stimulating discussion. L.R. is supported by an EMBO Long Term Fellowship and K.P. is a recepient of a FEBS Summer Fellowship. U.K. is supported by Brunel University's BRIEF funding. S.R. and R.T. carried out their B.Sc. final year projects (2007–08) in the Brunel laboratory of Human Immunology and Infection Biology.

References

[1] U. Kishore, R. Ghai, T.J. Greenhough, A.K. Shrive, D.M. Bonifati, M.G. Gadjeva, P. Waters, M.S. Kojouharova, T. Chakraborty, A. Agrawal, Structural and functional anatomy of the globular domain of complement protein C1q, Immunol. Lett. 95 (2004) 113–128.
[2] U. Kishore, S.K. Gupta, M.V. Perdikoulis, M.S. Kojouharova, B.C. Urban, K.B.M. Reid, Modular organization of the carboxyl-terminal, globular head region of human C1q A, B, and C chains, J. Immunol. 171 (2003) 812–820.
[3] C. Gaboriaud, J. Juanhuix, A. Gruez, M. Lacroix, C. Darnault, D. Pignol, D. Verger, J.C. Fontecilla-Camps, G.J. Arlaud, The crystal structure of the gobular head of complement protein C1q provides a basis for its versatile recognition properties, J. Biol. Chem. 278 (2003) 46974–46982.
[4] L.T. Roumenina, A.A. Kantardjiev, B.P. Atanasov, P. Waters, M. Gadjeva, K.B. Reid, A. Mantovani, U. Kishore, M.S. Kojouharova, Role of Ca^{2+} in the electrostatic stability and the functional activity of the globular domain of human C1q, Biochemistry 44 (2005) 14097–14109.
[5] L.T. Roumenina, M. Rouseva, A. Zlatarova, M. Kolev, R. Ghai, N. Olova, M. Gadjeva, A. Agrawal, A. Mantovani, B. Bottazzi, K.B.M. Reid, U. Kishore, M.S. Kojouharova, 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 (2006) 4093–4104.
[6] A. Zlatarova, M. Rouseva, L.T. Roumenina, M. Gadjeva, M. Kolev, I. Dobrev, N. Olova, R. Ghai, J. Jensenius, K.B. Reid, U. Kishore, M. Kojouharova, Existence of different, but overlapping, IgG- and IgM-binding sites on the globular domain of human C1q, Biochemistry 45 (2006) 9979–9988.
[7] M.S. Kojouharova, M.G. Gadjeva, I.G. Tsacheva, A. Zlatarova, L.T. Roumenina, M.I. Tchorbadjieva, B.P. Atanasov, P. Waters, B.C. Urban, R.B. Sim, K.B.M. Reid, U. Kishore, 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 (2004) 4351–4358.
[8] A. Zlatarova, L.T. Roumenina, M. Rouseva, M. Gadjeva, U. Kishore, M.S. Kojouharova, Glu162 of the B-chain of human complement component C1q possesses a key role in IgG recognition, Compt. Rendus ABS 58 (2005) 215–220.
[9] M. Loos, B. Euteneuer, F. Clas, Interaction of bacterial endotoxin (LPS) with fluid phase and macrophage membrane associated C1q. The Fc-recognizing component of the complement system, Adv. Exp. Med. Biol. 256 (1990) 301–317.
[10] C. Dumestre-Perard, E. Doerr, M.G. Colomb, M. Loos, Involvement of complement pathways in patients with bacterial septicemia, Mol. Immunol. 44 (2007) 1642–1649.
[11] A. Zohair, S. Chesne, R.H. Wade, M.G. Colomb, Interaction between complement subcomponent C1q and bacterial lipopolysaccharides, Biochem. J. 257 (1989) 865–873.
[12] M. Loos, F. Clas, Antibody-independent killing of Gram-negative bacteria via the classical pathway of complement, Immunol. Lett. 14 (1987) 203–208.
[13] U. Kishore, L.E. Leigh, P. Eggleton, P. Strong, M.V. Perdikoulis, A.C. Willis, K.B. Reid, Functional characterization of a recombinant form of the C-terminal, globular head region of the B-chain of human serum complement protein, C1q. Biochem. J. 333 (Pt 1) (1998) 27–32.
[14] H. Jiang, F.A. Robey, H. Gewurz, Localization of sites through which C-reactive protein binds and activates complement to residues 14–26 and 76–92 of the human C1q A chain, J. Exp. Med. 175 (1992) 1373–1379.
[15] S.C. Ying, A.T. Gewurz, H. Jiang, H. Gewurz, Human serum amyloid P component oligomers bind and activate the classical complement pathway via residues 14–26 and 76–92 of the A chain collagen-like region of C1q, J. Immunol. 150 (1993) 169–176.
[16] H. Jiang, D. Burdick, C.G. Glabe, C.W. Cotman, A.J. Tenner, Beta-Amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain, J. Immunol. 152 (1994) 5050–5059.
[17] P. Tacnet-Delorme, S. Chevallier, G.J. Arlaud, Beta-Amyloid fibrils activate the C1 complex of complement under physiological conditions: evidence for a binding site for Ab on the C1q globular regions, J. Immunol. 167 (2001) 6374–6381.
[18] A. Agrawal, A.K. Shrive, T.J. Greenhough, J.E. Volanakis, Topology and structure of the C1q-binding site on C-reactive protein, J. Immunol. 166 (2001) 3998–4004.
[19] F.D. McGrath, M.C. Brouwer, G.J. Arlaud, M.R. Daha, C.E. Hack, A. Roos, Evidence that complement protein C1q interacts with C-reactive protein through its globular head region, J. Immunol. 176 (2006) 2950–2957.
[20] S. Bureeva, J. Andia-Pravdivy, G. Petrov, M. Igumnov, S. Romanov, E. Kolesnikova, A. Kaplun, L. Kozlov, Inhibition of classical pathway of complement activation with negative charged derivatives of bisphenol A and bisphenol disulphates, Bioorg. Med. Chem. 13 (2005) 1045–1052.
[21] S. Bureeva, J. Andia-Pravdivy, A. Symon, A. Bichucher, V. Moskaleva, V. Popenko, A. Shpak, V. Shvets, L. Kozlov, A. Kaplun, Selective inhibition of the interaction of C1q with immunoglobulins and the classical pathway of complement activation by steroids and triterpenoids sulfates. Bioorg. Med. Chem. 15 (10) (2007) 3489–3498.
[22] J.D. Dimitrov, L.T. Roumenina, V.R. Doltchinkova, T.L. Vassilev, Iron ions and heme modulate the binding properties of complement subcomponent C1q and of immunoglobulins. Scand. J. Immunol, 65 (2007) 230–239.
[23] A.J. Nauta, B. Bottazzi, A. Mantovani, G. Salvatori, U. Kishore, W.J. Schwaeble, A.R. Gingras, S. Tzima, F. Vivanco, J. Egido, O. Tijsma, E.C. Hack, M.R. Daha, A. Roos, Biochemical and functional characterization of the interaction between pentraxin 3 and C1q, Eur. J. Immunol. 33 (2003) 465–473.
[24] M.S. Kojouharova, I. Tsacheva, M. Tchorbadjieva, K.B. Reid, U. Kishore, 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 (2003) 64–74.
[25] M.S. Kojouharova, I.D. Panchev, M.I. Tchorbadjieva, K.B.M. Reid, H.-J. Hoppe, 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 (1998) 4325–4331.
[26] D.V. Rozanov, S. Sikora, A. Godzik, T.I. Postnova, V. Golubkov, A. Savinov, S. Tomlinson, A. Strongin, Non-proteolytic, receptor-ligand interactions associate cellular membrane type-1 matrix metalloproteinase with the complement component C1q, J. Biol. Chem. 279 (2004) 50321–50328.
[27] S. Alberti, G. Marques, S. Hernandez-Alles, X. Rubires, J.M. Tomas, F. Vivanco, V.J. Benedi, Interaction between complement subcomponent C1q and the *Klebsiella pneumoniae* porin OmpK36, Infect. Immun. 64 (1996) 4719–4725.
[28] L.T. Roumenina, S. Bureeva, A.A. Kantardjiev, D. Karlinski, J. Andia-Pravdivy, R.B. Sim, A. Kaplun, M. Popov, U. Kishore, B.P. Atanasov, Inhibition of complement gC1q target recognition by electric moment disturbance, J. Mol. Recogn. 20 (5) (2007) 405–415.

[29] B. Tissot, R. Daniel, C. Place, Interaction of the C1 complex of complement with sulfated polysaccharide and DNA probed by single molecule fluorescence microscopy, Eur. J. Biochem. 270 (2003) 4714–4720.
[30] B. Tissot, F. Gonnet, A. Iborra, C. Berthou, N. Thielens, G.J. Arlaud, R. Daniel, Mass spectrometry analysis of the oligomeric C1q protein reveals the B chain as the target of trypsin cleavage and interaction with fucoidan, Biochemistry 44 (2005) 2602–2609.
[31] A. Roos, T.H. Ramwadhdoebe, A.J. Nauta, C.E. Hack, M.R. Daha, Therapeutic inhibition of the early phase of complement activation, Immunobiology 205 (2002) 595–609.