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*J Immunol* 2010; 185:808-812; Prepublished online 14 June 2010;
doi: 10.4049/jimmunol.1000184

http://www.jimmunol.org/content/185/2/808

Supplementary Material  http://www.jimmunol.org/content/suppl/2010/06/14/jimmunol.1000184.DC1

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Cutting Edge: C1q Binds Deoxyribose and Heparan Sulfate through Neighboring Sites of Its Recognition Domain

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C1q, the recognition subunit of the C1 complex of complement, is an archetypal pattern recognition molecule with the striking ability to sense a wide variety of targets, including a number of altered self-motifs. The recognition properties of its globular domain were further deciphered by means of x-ray crystallography using deoxy-D-ribose and heparan sulfate as ligands. Highly specific recognition of deoxy-D-ribose, involving interactions with Arg C98, Arg C111, and Asn C113, was observed at 1.2 Å resolution. Heparin-derived tetrasaccharide interacted more loosely through Lys C129, Tyr C155, and Trp C190. These data together with previous findings define a unique binding area exhibiting both polyanion and deoxy-D-ribose recognition properties, located on the inner face of C1q. DNA and heparin compete for C1q binding but are poor C1 activators compared with immune complexes. How the location of this binding area in C1q may regulate the level of C1 activation is discussed. The Journal of Immunology, 2010, 185: 808–812.

Well characterized as the innate immune recognition protein responsible for triggering the classical C pathway (1), C1q is a 460-kDa hexameric protein with the overall shape of a bouquet of flowers. Its three chains, A, B, and C, associate to form six heterotrimetric collagen-like fibers prolonged by C-terminal globular regions (GRs). Whereas the collagen domains hold the hexameric architecture and interact with effector molecules, the GRs support the recognition properties of C1q (2–4).

C1q binds IgM, IgG, or C-reactive protein deposited on pathogen surfaces (5, 6), but also recognizes various repeating molecular patterns on pathogens (bacterial porins, LPSs) (7, 8). C1q-triggered C activation on these non–self-surfaces leads to their phagocytosis and lysis and to inflammatory signals (1). Moreover, C1q also senses various altered self-structures, such as abnormal proteins [prion (9) and β-amyloid fibrils (10)] and apoptotic cells (11). In this latter case, C1q binding elicits clearance, but inflammation and cytolysis are inhibited (12, 13), which is essential for immune tolerance (14). It is not known whether, in addition to the control exerted by C regulators (15, 16), this differential effect may also result from a regulation at the level of C1 activation.

Two molecules known to provide eat-me signals on apoptotic cells, phosphatidylserine and DNA, were shown to be recognized by C1q (17, 18). The C1q–DNA interaction at the apoptotic cell surface involves recognition of the deoxy-D-ribose moiety of DNA (18). To decipher the structural determinants for the specific C1q binding to deoxy-D-ribose compared with ribose, x-ray analyses of C1q–GR crystals were performed in the presence of these two molecules. In contrast, sulfated molecules, such as triterpenoid sulfates, heparin (Hp) sulfates, or chondroitin sulfates, have been shown to interact with C1q and inhibit activation of the classical C pathway (19–21). This prompted us to investigate C1q binding to heparan sulfates through the combined use of surface plasmon resonance (SPR) and x-ray analyses. Interestingly, these studies reveal that deoxy-D-ribose and hp-derived tetrasaccharide (HS–4) bind to an area of the C1q-GR close to a site previously identified for phosphoserine (PS) (17). The recognition specificity of this region and its possible implication in the regulation of C1 activation will be discussed.

Materials and Methods
C1q was purified from human serum (22), and its GRs were produced by collagenase treatment as described previously (4, 10).

SPR analysis
Six-kiloDalton heparin (Hp6) was biotinylated and immobilized on a Biacor CM4 sensorchip (Biacore, GE Healthcare, Uppsala, Sweden), as described previously (23). Briefly, two flow cells were prepared by sequential injections of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/N-hydroxysuccinimide, streptavidin, and ethanolamine. One cell was used as a negative control surface, whereas biotinylated Hp6 was injected on the other cell to reach an immobilization level of 40–50 response units. All analyses were performed using 10 mM HEPES, 150 mM NaCl, and 0.005% surfactant P20 (pH 7.4) at a flow rate of 20 µl/min. A range of C1q-GR concentrations (0–80 µg/ml) was injected for 8 min over both surfaces, and the complexes formed

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Coordinates and structure factors have been submitted to Protein Data Bank (www.pdb.org) under accession numbers 2wnu and 2wnv.

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The online version of this article contains supplemental material.

Abbreviations used in this paper: gC1q, C1q globular domain; GR, globular region; Hp, heparin; HS–4, heparin-derived tetrasaccharide; IC, IgG–OVA complex; PS, phosphoserine; SPR, surface plasmon resonance.

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were allowed to dissociate by washing the surfaces with the buffer for 6 min. Regeneration of the Hp6-bound surface was achieved by injection of 10 mM HEPEs, 2 mM NaCl, and 0.005% surfactant P20 (pH 7.4). The sensorgrams shown correspond to the online subtraction of the negative control to the Hp6-bound surface signal. Data analysis was performed using the BIAeval 3.1 software (Biacore, GE Healthcare).

**C1 activation assay**

C1 was reconstituted from purified C1q and the proenzyme C1s-C1r-C1r-C1s tetramer (10). The complex (0.25 µM) was incubated for 90 min at 37°C in 50 mM triethanolamine-HCl, 145 mM NaCl, and 1 mM CaCl2 (pH 7.4) in the presence of 1 µM C1 inhibitor and varying amounts of IgG-OVA immune complexes, Hp(15) (Sigma-Aldrich, Saint Quentin, France), or calf thymus DNA (Invitrogen, Cergy-Pontoise, France). The C1 activation extent was measured by SDS-PAGE followed by Western blot analysis using an anti-thymus DNA (Invitrogen, Cergy-Pontoise, France). The C1 activation extent was measured by SDS-PAGE followed by Western blot analysis using an anti-C1s Ab (10).

**X-ray analysis**

C1q-GR crystals suitable for diffraction were obtained as described previously (4). Microseeding was used to obtain more reproducible native crystals. Crystals were soaked in highly concentrated ligand solutions (200 mM ribose or deoxy-d-ribose for 5 h or 10 mM HS-4 for 8 d). HS-4 is an Hp-derived tetrasaccharide prepared as previously described (24). X-ray diffraction data were collected at the European Synchrotron Radiation Facility (Grenoble, France) beamlines ID14-eh2 and ID14-eh1 and processed using XDS (Max Planck Institute for Medical Research, Heidelberg, Germany) (25). The structure was solved by molecular replacement with PHASER (University of Cambridge, Cambridge, U.K.) (26) using native C1q-GR as a search model. Refinement was performed using Refmac5 (27), and model corrections using CCP4 Matthews and Contact procedures, respectively (29).

**Results and Discussion**

**SPR analysis reveals binding of C1q-GR to Hp**

The binding of C1q and its GR to an Hp6-functionalized sensor chip was monitored by SPR. C1q was found to bind avidly to Hp6, but no model could fit the data, likely because of the presence of different kinds of binding sites (19). The isolated C1q-GR also bound to Hp6 (Fig. 1), with a $K_d$ of 154 ± 6 nM, comparable to the value of 500 nM previously determined for the interaction of this domain with PS (17). This represents high binding affinity, considering that this value is expected to increase through avidity at the level of the hexameric C1q molecule (17).

**Deoxyribose and HS-4 bind neighboring sites on C1q-GR**

X-ray structural analyses were carried out to determine precise interactions between C1q-GR and its ligands deoxy-d-ribose and HS-4. Despite our efforts, no crystal of a C1q-GR/ligand complex could be generated by cocrystallization experiments. We therefore used the soaking technique to introduce ligands into native C1q-GR crystals, as this approach was used successfully to investigate interaction with PS (17). C1q-GR crystals were found to collapse in highly concentrated HS-4-containing solutions; therefore, the ligand concentration was reduced to 10 mM, and the soaking time was increased. X-ray diffraction data were collected, and ligands could be observed in the electron density maps after soaking the crystals into deoxy-d-ribose- and HS-4-containing solutions. In contrast, the density maps remained unmodified when using ribose, in agreement with previous data showing that deoxy-d-ribose, but not ribose, prevents C1q binding to DNA, galactose, or N-acetyl glucosamine (18).

The complete data collection and refinement statistics corresponding to the crystallographic analyses performed with deoxy-d-ribose and HS-4 are provided in Supplemental Table I. The C1q-GR/HS crystals diffracted only to 2.3 Å resolution compared with native data sets previously obtained at 1.9 Å resolution (4). The final $R_{work}$ and $R_{free}$ factors were 0.198 and 0.256, respectively. The additional electron density was much smaller and less elongated than the ligand itself, indicating that only part of the HS-4 molecule was stabilized through identical binding interactions in the crystal. The observed binding site is located in the outer part of the C chain, between the surface loops 98–113 and 125–130 (Fig. 2A). The ligand moiety corresponding to the extra electron density was interpreted as a sulfate group and part of its associated carbohydrate ring, including the link to the next carbohydrate. As depicted in Fig. 2B, this part of the molecule is accommodated into a site lined by Tyr155, Trp190, and Lys129. This is reminiscent of the binding of Hp to trombospondin-1, where a funnel lined by several arginines, a lysine, and a tryptophan was described (30). In this latter case, discrete electron density patches were observed and interpreted as a signature pattern corresponding to the binding of sulfate groups (30). In our case, the main electron density patch also likely corresponds to a sulfate group, stabilized by a polar interaction with the side-chain nitrogen of Trp190. The hydroxyl group of Tyr155 interacts with the carbohydrate ring. In addition, an electrostatic interaction with Lys129 likely stabilizes the sulfate group of the neighboring carbohydrate unit (Fig. 2B).

Soaking C1q-GR crystals into deoxy-d-ribose-containing solutions significantly improved the diffraction resolution to 1.25 Å. The final $R_{work}$ and $R_{free}$ factors were 0.175 and 0.202, respectively. A clear density patch corresponding to deoxy-d-ribose was observed, also located in the vicinity of the 98–113 surface loop of the C1q globular domain (gC1q) C subunit (Fig. 2A). As depicted in Fig. 2C, the deoxy-d-ribose molecule is inserted between Arg98 and Arg111. Polar interactions with Asn113 and Arg111 stabilize its 3-OH group. Additional water-mediated interactions with Arg98 and Arg111 stabilize its 5-OH group. This ligand orientation provides a structural basis for the C1q-binding specificity toward deoxy-d-ribose (18), because grafting the additional OH group of ribose onto the bound deoxy-d-ribose would induce steric hindrance with Asn113.

As illustrated in Fig. 2D, these analyses thus provide evidence that both HS and deoxy-d-ribose are attracted to the same area within subunit C, close to the PS-binding site previously identified, involving Arg111, Ser126, and Thr127 (17). However,
binding is significantly improved for the smaller deoxy-D-ribose ligand compared with larger ones. This observation prompted us to investigate whether crystal packing could result in restricted access to large ligands. Two solvent channels, C1 and C2 (Fig. 2D), represent ∼41% of the crystal content. The binding area identified in this study (shown in magenta in Fig. 2A) is located on the edge of the smaller C1 channel. More precisely, Gln99, Thr100, Gln102, Arg111, Asn113, and Ser126 of gC1qC mediate polar and Van der Waals crystal packing interactions with the 162–165 loop of gC1qA. Thus, whereas the relatively small deoxy-D-ribose molecule has clearly reached its specific binding pocket, larger physiological ligands have reduced access to their binding site in this crystal context. In particular, access to arginines C98 and C111, which can attract anionic molecules, is clearly hampered by crystal packing, preventing interaction with larger sulfate or phosphate groups, as discussed previously in the case of PS (17).

C1q-GR binds the polyanionic Hp and DNA molecules through neighboring sites

Because deoxy-D-ribose is likely a molecular determinant recognized by C1q in DNA (18), we have checked whether its positioning in the x-ray structure is actually compatible with its recognition in the context of a nucleotide. The model depicted in Fig. 2E suggests that a DNA 3’ end can indeed be accommodated in this binding site, the 5’ phosphate group being stabilized by Arg C98, and the nitrogenous base being stacked between Asn C113 and Thr C125. We observe binding of a sulfate group to Tyr C155 and Trp C190 and previously showed binding of a phosphate group to Arg C111 and Ser C126 (17). In addition, it is likely that another HS sulfate group could bind to Lys C129 and that a

FIGURE 2. Structural characterization of the gC1qC binding area. A, Overall structure of C1q-GR, highlighting the location of the binding area observed in gC1qC (magenta). Labels indicate the color code of the C1q subunits. The yellow sphere represents the Ca²⁺ ion. A deoxy-D-ribose molecule is shown in sticks in its binding orientation. B, Zoom on the HS binding site. Sticks correspond to the HS moiety fitted into the additional electron density map observed after soaking, whereas thin lines represent the adjacent mobile parts (black, yellow, and red). The C1q binding site residues Y155, W190, and K129 are shown in sticks. Polar interactions and their corresponding distance are shown with dotted red lines. The position of the nearby mobile Arg111 is also shown. C, Zoom on the deoxy-D-ribose binding site, using the same color code as in B. The 2Fo-Fc electron density map corresponding to the deoxy-D-ribose molecule is shown in blue. D, Crystal packing environment in the C1q–HS complex. Solvent channels are labeled C1 and C2. Stars indicate the approximate position of the HS, PS, and deoxyribose binding sites. The color code for gC1q is the same as in A, except for the A162–A165 loop (orange) and the B160–B169 loop (dark blue). E, A thymidine nucleotide was modeled from the bound deoxy-D-ribose, and its additional interactions are shown. F, Model of the whole C1q molecule highlighting the position of the known C1q-GR binding sites.

FIGURE 3. C1 activation by IC, Hp, and DNA. C1 (0.25 μM) was incubated for 90 min at 37°C in the presence of C1 inhibitor (1 μM) and increasing concentrations of IC, Hp, or DNA. C1 activation was monitored as described under Materials and Methods. DNA, calf thymus DNA; Hp, Hp15; and IC, IgG–OVA complexes.
phosphate group could bind to Arg C98. This latter interaction cannot be observed by x-ray analysis because of the crystal packing restraints. Altogether, these observations strongly suggest that this particular region of gC1qC could be dedicated to the specific recognition of polyanionic molecules. To further investigate this question and confirm that Hp and DNA bind to neighboring sites of C1q-GR, competition experiments were performed by SPR. Indeed, as shown in Supplemental Fig. 1, both soluble DNA and Hp efficiently and dose dependently inhibited binding of C1q-GR to immobilized Hp6, with IC_{50} values of ~50 and 5 nM, respectively, in full agreement with x-ray crystallography analyses.

Location of ligand binding sites on C1q-GR regulates the level of subsequent C1 activation

According to the current version of our three-dimensional C1q model (4), the binding sites for deoxy-d-ribose, HS, and PS would be oriented toward the target surface on the inner face of the C1q cone, in contrast to the IgG binding site thought to lie on the outer equatorial part of the C1q-GRs (Fig. 2F). Resolution of the x-ray structure of the zymogen C1r catalytic domain has revealed the relative locations of the catalytic and cleavage sites, leading to the conclusion that an outward movement of the C1q stems is required to dissociate the resting C1r head-to-tail dimeric structure and thereby trigger C1 activation (3, 4, 31, 32). In this hypothesis, any ligand interacting with the outer part of the C1q-GRs, as postulated in the case of IgG, is expected to trigger efficient C1 activation. In contrast, the inner location of the binding area described in the current study appears unlikely to generate such an outward mechanical stress, hence preventing or at least limiting C1 activation upon interaction with the corresponding ligands. If this hypothesis is correct, then DNA and Hp are expected to be poor C1 activators compared with IgG-containing immune complexes. In agreement with this hypothesis, it was shown early by kinetic analyses that, under nearly physiological conditions, C1 inhibitor has no effect on C1 activation by immune complexes, but strongly inhibits activation by DNA and Hp (33). Using a different C1 activation assay in the presence of excess C1 inhibitor, we have performed the same type of experiment and also show that increasing concentrations of IgG–OVA complexes efficiently activate C1, in contrast to DNA and Hp (3, Fig. 3).

Uncontrolled activation of the classical C pathway is involved in many inflammatory pathologies, such as ischemia/reperfusion injury, and selective inhibitors for this pathway are needed (1, 21). In contrast, C1q deficiency is directly correlated with autoimmune diseases, such as systemic lupus erythematosus and glomerulonephritis, associated with accumulation of apoptotic cells (34, 35). Thus, the beneficial role of C1q in efficient removal of altered self must be preserved. Phosphatidylinerine and DNA are two eat-me signals recognized by C1q on the surface of apoptotic cells (16–18), whereas Hp belongs to a group of polyanionic molecules known to inhibit C activation. We propose that, due to the location of their binding sites within the same area of the C1q-GR, recognition of these ligands by C1q results in limited C1 activation, hence contributing to the control of inflammatory reactions. The identification in C1q-GR of different binding sites associated with contrasted outcomes in terms of C activation and inflammation should be helpful in the development of inhibitors specifically targeting the noxious effects of C1 activation.

Acknowledgments

We thank the scientists of the ID14 ESRF beamlines, Claudine Darnault for obtaining the first C1q-GR crystals, and Monique Lacroix for performing the C1 activation assays.

Disclosures

The authors have no financial conflicts of interest.

References


