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Evidence That Complement Protein C1q Interacts with C-Reactive Protein through Its Globular Head Region

Fabian D. G. McGrath,* Mieke C. Brouwer,* Gérard J. Arlaud,† Mohamed R. Daha,‡ C. Erik Hack,2* and Anja Roos3‡

C1q acts as the recognition unit of the first complement component, C1, and binds to immunoglobulins IgG and IgM, as well as to non-Ig ligands, such as C-reactive protein (CRP). IgG and IgM are recognized via the globular head regions of C1q (C1qGR), whereas CRP has been postulated to interact with the collagen-like region (C1qCLR). In the present study, we used a series of nine mAbs to C1q, five directed against C1qGR and four against C1qCLR, to inhibit the interaction of C1q with CRP. The F(ab°)2 of each of the five mAbs directed against C1qGR inhibited binding of C1q to polymerized IgG. These five mAbs also successfully inhibited the interaction of C1q with CRP. Moreover, these five mAbs inhibited C1 activation by CRP as well as by polymerized IgG in vitro. In contrast, none of the four mAbs against C1qCLR inhibited C1q interaction with CRP or IgG, or could reduce activation of complement by CRP or polymerized IgG. These results provide the first evidence that the interaction of C1q with CRP or IgG involves sites located in the C1qGR, whereas sites in the CLR do not seem to be involved in the physiological interaction of C1q with CRP. The Journal of Immunology, 2006, 176: 2950–2957.

The classical pathway of complement becomes activated under inflammatory conditions such as immune complex diseases or ischemia/reperfusion injury (1, 2). The mechanism of activation in the latter condition involves natural Abs (3, 4) as well as the acute phase protein C-reactive protein (CRP) (5–7). These molecules bind to newly exposed epitopes or ligands present on ischemic and/or apoptotic cells (8–10). Excessive activation of the classical pathway induces additional tissue injury in ischemia/reperfusion injury (11). Hence, inhibitors that attenuate classical pathway activation by IgGs or CRP provide novel therapeutic options in ischemia/reperfusion injury and possibly other conditions.

Activation of the classical pathway is triggered by binding of C1, the first component of the pathway, to an activator. C1 consists of a complex of one C1q, two C1r, and two C1s molecules in which C1q acts as the recognition subunit while C1r and C1s associate to form a calcium-dependent tetramer that functions as the catalytic subunit (1, 12). C1q molecule has a molecular mass of 460 kDa, and is composed of three different polypeptide chains, A, B, and C, containing 223-, 226-, and 217-aa residues, respectively. Six copies of each chain are found per C1q molecule (13, 14). Overall, the molecule looks like a bouquet of six tulips, each tulip consisting of an A, B, and C chain. The N-terminal region of each chain consists of a sequence that is involved in the formation of A-B and C-C interchain disulphide bonds. This sequence is followed by a collagen-like sequence of ~81 aa (15), in which the A, B, and C chains form a heterotrimeric collagenous triple helix. This part is referred to as the C1q collagen-like region (C1qCLR), and makes up the stalk of each tulip. At their C terminus each C1q chain contains a globular head region consisting of ~135 residues. These domains of A-, B-, and C-chain together form the globular head regions (C1qGR) of the tulip, six of these being present per C1q molecule.

C1q can bind various activators such as immunoglobulins IgG (16) and IgM (17), as well as non-Ig proteins like CRP (7), SAP (18), PTX3 (19), and β-amyloid fibrils (Aβ) (20). A common binding site on C1q for all these activators may serve as target for potential inhibitors. However, binding sites on C1q for the Fc domain of aggregated IgG or IgM have been mapped to the C1qCLR (21, 22), whereas those for some other ligands were shown to be located within its CLR, as was first reported for the interaction with CRP (23). In particular, the sequences involving the amino acid residues 14–26 and 76–92 within the CLR of the A chain are thought to be important for the interaction with CRP (23). Whether, in addition to these, common binding sites for ligands such as CRP and IgM or IgG exist on C1q, is not known. In a search for such common binding sites, we evaluated the effect of several mAbs against human C1q on the binding of C1q to IgG and CRP, and on the activation of complement by these activators. Our results indicate that both binding sites, involved in the interaction of C1q with either activator, are located on the globular domain.

Materials and Methods

Proteins

C1q was purified from recalcified frozen plasma according to Tenner et al. (24). The final preparation was dialyzed against PBS (pH 7.4). C1q concentration in the preparation was derived from its absorbance at 280 nm using \( \epsilon_{280} = 6.82 \). Purity of the preparation was assessed from 12% SDS-PAGE analysis (Invitrogen Life Technologies) using silver staining.
(Fig. 1). C1q was radiolabeled as described (125I-labeled C1q (125I-C1q)) (25).

C1qCLR were prepared by pepsin digestion of purified C1q (15). Briefly, C1q was incubated with pepsin (Sigma-Aldrich) at 30:1, w/w, for 3.5 h at 37°C in 0.2 M sodium acetate, 100 mM NaCl (pH 4.4), after which the reaction was stopped by addition of 1 M Tris (pH 9.0). Pepsin and residual intact C1q were removed by sucrose gradient ultracentrifugation. The 6.3S-peak fractions were pooled and dialyzed against PBS. Purity of C1qCLR was confirmed using 12% SDS-PAGE and Coomassie staining (Fig. 1). The concentration of C1qCLR was determined by analysis on 12% SDS-PAGE using (respectively at 19, 17, and 15 kDa).

For both preparations, the preparation was determined by analysis on 12% SDS-PAGE using silver staining. C1q shows a doublet running between IgG and albumin (expected molecular mass is 125 kDa).

FIGURE 1. SDS-PAGE analysis of C1q and C1q fragments. Human C1q, C1qCLR, and C1qGR (2 µg/lane) were analyzed by SDS-PAGE (12%, reducing conditions), followed by silver staining. C1q shows a double band containing the A and B chains (31 and 30 kDa, respectively), and the C chain at 26 kDa. C1qCLR runs in a single band of ~16 kDa, and C1qGR shows three bands corresponding to the A, B, and C moieties (respectively at 19, 17, and 15 kDa).
BSA) and 100 μl of these dilutions were added to the appropriate wells. The plates were incubated again for 1 h, followed by incubations with biotinylated rabbit polyclonal anti-C1q Ab (Sanquin), and HRP-conjugated streptavidin (Amersham Biosciences), both diluted in hypertonic assay buffer. Finally, 100 μl of 3.3' 5.5'-tetra-methyl-benzidine, 100 μg/ml in 0.11 M sodium acetate (pH 5.5) containing 0.003%, v/v, H2O2, was added to each well and color development was observed. The reaction was stopped by addition of 100 μl of 2 M H2SO4 to each well. The OD of each well was then read at 450/540 nm and results were recorded.

Radioimmunoassays (RIA) to study the interaction of C1q with IgG or CRP

Human IgG was coupled to cyanogen bromide-activated-Sepharose (Amersham) according to manufacturer’s instructions. The IgG-Sepharose beads were then washed twice and resuspended in RIA buffer (VBS containing 5 mM CaCl2, 0.3% BSA, and 0.1% Tween 20) to a concentration of 10 μg/ml. Fifty microliters of anti-C1q F(ab’)2 (25 μg/ml in PBS) was preincubated with 50 μl of 125I-C1q (10,000 cpm) for 1 h at room temperature. Then, 0.5 ml of IgG-Sepharose suspension was added and the tubes were incubated overnight by head-over-head rotation. Following incubation the Sepharose beads were washed four times in PBS, the supernatant was removed, and the radioactivity of the spun down Sepharose was read on a LKN Wallac Multigamma II (Wallac Oy). Results were expressed as the percent of input. All tests were done in duplicate.

The effect of anti-C1q mAbs on the interaction of C1q with CRP was tested in a similar way. Immobilized p-aminophenyl-phosphorylcholine coupled to Sepharose (PC-Sepharose) was purchased from Pierce. The PC-Sepharose was read on a LKN Wallac Multigamma II (Wallac Oy). Results were expressed as the percent of input. All tests were done in duplicate.

RIA to assess mutual effect of CRP and IgG on binding of C1q

Similar procedures as described above to investigate the ability of anti-C1q mAbs to inhibit binding of C1q to IgG or CRP were used to assess the inhibition of C1q binding to immobilized CRP by IgG, and vice versa. rCRP directly coupled to Sepharose was used in these experiments, as the use of PC-Sepharose incubated with rCRP precluded the use of rCRP as a control competitor, because free PC sites on the PC-Sepharose bound the antibody. In initial experiments, the rCRP- and IgG coupled Sepharoses were titrated (in 50 μl of RIA buffer) in the presence of 0.5 μl of each competitor or control, at varying dilutions, was added rCRP. In initial experiments, the rCRP- and IgG-coupled Sepharoses were titrated (in 50 μl of RIA buffer) to yield 25–50% binding of 125I-C1q input, to enhance the sensitivity of the competition assays. Dilutions of competitors were prepared in RIA buffer to give stock concentrations of 17.4 × 10^{-6} M rCRP, 4 × 10^{-7} M IgG, 3.3 × 10^{-5} M AHG, and 3 × 10^{-4} M human serum albumin (HSA).

In the RIA, 50 μl of each competitor or control, at varying dilutions, was preincubated with 50 μl of [125I-C1q (10,000 cpm) for 1 h at room temperature. After this, 50 μl of the appropriate Sepharose was added and the tubes were incubated on a shaker for 4 h. The beads were washed four times in PBS, the supernatant removed and the radioactivity of the spun down Sepharose was read. All tests were done in duplicate. Results were assessed as percentage of maximum 125I-C1q bound, in the absence of competing ligands.

Effects of anti-C1q mAbs on complement activation by AHG or CRP

Microtiter plates (Nunc Maxisorp) were coated with CRP (5 μg/ml) or IgG (5 μg/ml) overnight at room temperature, in 0.1 M carbonate buffer (pH 9.6), 100 μl/well. The plates were blocked with PBS containing 2% BSA, followed by addition of normal human serum diluted in PBS containing 0.5 mM MgCl2, 2 mM CaCl2, 0.05% Tween 20, and 0.1% gelatin (pH 7.5). For inhibition experiments, diluted normal human serum (1/1000) was preincubated with F(ab’2)2 anti-C1q (10 μg/ml) for 30 min at room temperature, followed by addition of the mixture to the plate and incubation for 1 h at 37°C. Complement activation was detected using digoxigenin-conjugated polyclonal rabbit anti-C1q (Fab from Boehringer Mannheim), both diluted in PBS containing 1% BSA and 0.05% Tween 20. Enzyme activity of HRP was detected using ABTS, followed by measurement of the OD at 415 nm.

Results

Characterization of anti-C1q mAbs

To localize the epitopes of different anti-C1q mAbs on C1q, each of the nine anti-C1q mAbs was immobilized and binding of intact C1q and C1q fragments was studied by ELISA. These binding experiments were conducted at 0.5 M NaCl to prevent nonspecific binding of C1q to the Fc domains of IgG. All nine anti-C1q mAbs strongly recognized the intact C1q molecule (Fig. 4A). Anti-C1q-85, -89, -124, -171, and -195 showed a strong binding to C1qGR, in contrast to anti-C1q-2, -42, -52, and -139 (Fig. 4B). Conversely, mAbs anti-C1q-2, -42, -52, and -139 showed strong reactivity with C1qCLR, whereas anti-C1q-85, -89, -124, -171, and -195 showed hardly any binding to C1qCLR (Fig. 4C). These results indicated that mAbs anti-C1q-2, -42, -52, and -139 are directed against epitopes on the CLR region of C1q, while mAbs anti-C1q-85, -89, -124, -171, and -195 recognize epitopes in the C1qGR region of C1q. Non-specific mouse Ab yielded negative results with C1q.
CLR, and GR (Fig. 4), demonstrating the specificity of these assays.

In experiments not shown here, competition between anti-C1q mAbs for binding to C1q was observed to varying degrees indicating closely related epitopes. With respect to mAbs directed against C1qGR and CLR, respectively, there were at least two noncompeting mAbs in each group: anti-C1q-2 and -42 did not compete for binding to C1qCLR and anti-C1q 85 and 124 did not compete for binding to C1qGR.

Effects of anti-C1q mAbs on the interaction of C1q with IgG-Sepharose

To exclude interference by Fc fragments of the mAbs in the interaction between C1q and IgG or CRP, and to allow the use of buffers at physiologic ionic strength, F(ab′)_2 of each of the nine anti-C1q mAbs were used in subsequent experiments. These F(ab′)_2 were studied for their ability to inhibit the binding of radiolabeled C1q to IgG-Sepharose. F(ab′)_2 of anti-C1q-2, -42, -52, and -139 did not inhibit binding of 125I-C1q to immobilized IgG relative to the control F(ab′)_2 (Fig. 5A). In contrast, F(ab′)_2 of mAbs anti-C1q-85, -89, -124, -171, and -195 showed >80% inhibition of this binding (Fig. 5A). These results indicate that the five mAbs directed against C1qGR inhibit the interaction between C1q and IgG.

Effects of anti-C1q mAbs on interaction of C1q with CRP-PC-Sepharose

rCRP was incubated with PC-Sepharose to allow its immobilization in a physiologic conformation. Using this immobilized CRP, binding of radiolabeled C1q was studied in the presence of F(ab′)_2 against C1q. The results showed that mAbs anti-C1q-2, -42, -52, and -139 did not inhibit the binding of 125I-C1q to CRP relative to the control F(ab′)_2, whereas anti-C1q-85, -89, -124, -171, and -195 showed >90% inhibition (Fig. 5B). Thus, the F(ab′)_2 of the anti-C1q mAbs with C1qGR specificity were able to prevent binding of C1q not only to solid-phase IgG but also to ligand-bound CRP. In contrast, F(ab′)_2 from anti-C1q mAbs with C1qCLR specificity were unable to block these interactions.

**FIGURE 5.** Anti-C1q F(ab′)_2 against C1qGR inhibit interaction of C1q with IgG and CRP. A. Inhibition of 125I-C1q interaction with IgG-Sepharose by anti-C1q mAb F(ab′)_2. B. Inhibition of 125I-C1q interaction with CRP-PC-Sepharose by anti-C1q mAb F(ab′)_2. C1q was preincubated with anti-C1q F(ab′)_2, after which appropriate Sepharose beads were added. After incubation, beads were washed, and radioactivity bound to Sepharose was counted. Results are given as the percent of input of radiolabeled C1q. Anti-FcRII mAb 8G3 was used as a control.

Competition between CRP and IgG for binding to C1q

The above results suggested that CRP bound to C1qGR, similar to IgG, and not to C1qCLR as has been reported in the literature (23, 28). To further confirm that both CRP and IgG bind to C1qGR, we performed cross-competition experiments, using the binding of fluid-phase radiolabeled C1q to immobilized IgG and to immobilized CRP, respectively, as readouts. Preincubation of 125I-C1q with increasing concentrations of fluid-phase IgG strongly inhibited binding of 125I-C1q to rCRP-PC-Sepharose, whereas 125I-C1q binding was not inhibited by HSA, used as a negative control (Fig. 6A). Furthermore, in the reverse experiment, fluid-phase rCRP was able to inhibit the binding of 125I-C1q to IgG-Sepharose in a dose-dependent manner (Fig. 6B). Binding of 125I-C1q to IgG-Sepharose was also dose-dependently inhibited by fluid-phase IgG, but not by HSA (Fig. 6C). Together, these results show that interaction between C1q and CRP in the fluid phase inhibits C1q binding to immobilized IgG, whereas fluid-phase interaction between C1q and IgG inhibits the binding of C1q to immobilized CRP.

To directly compare competition between CRP and IgG in a single experiment, competition experiments were performed using CRP directly immobilized on Sepharose. In experiments not shown here, we observed that this rCRP-Sepharose bound labeled C1q in a similar way as rCRP-PC-Sepharose, and that this binding was inhibited by the same anti-C1q mAbs as those inhibiting binding of C1q to rCRP-PC-Sepharose. Competition experiments showed that both IgG and aggregated IgG were able to inhibit the binding to 125I-C1q to CRP-Sepharose in a dose-dependent manner, although aggregated IgG was ~10 times more efficient than monomeric IgG (Fig. 7). Furthermore, fluid-phase CRP inhibited binding of 125I-C1q to immobilized CRP with an efficacy that was better than that of IgG but less than that of aggregated IgG (Fig. 7). The specificity of the competition experiments was again shown using albumin, which did not modify 125I-C1q binding.

**Ability of anti-C1q mAbs to inhibit C1 activation**

CRP is known to activate the classical pathway of complement via its interaction with C1q. rCRP as well as CRP purified from human plasma were equally able to activate the classical complement pathway when immobilized on ELISA plates, as demonstrated by the activation of C4 following incubation with increasing concentrations of fresh human serum (Fig. 8A). Control-coated wells did not show C4 deposition (Fig. 8A). To investigate the involvement of the globular region of C1q in complement activation by CRP, we assessed the effects of F(ab′)_2 of anti-C1q mAbs on C4 activation. The F(ab′)_2 of anti-C1q mAbs-2, -42, -52, and -139 showed little or no inhibition of C4 activation, whereas those of anti-C1q mAbs -85, -89, -124, -171, and -195 showed complete
inhibition of C4 activation, both for rCRP (Fig. 8B) and for CRP purified from human plasma (Fig. 8C). Similar results were obtained when complement activation was induced by immobilized IgG (Fig. 8D). The requirement for complement activation in this assay was clearly shown by experiments with heat-inactivated serum or in the presence of EDTA, resulting into complete inhibition of C4 deposition, as indicated by dashed lines in Fig. 8, B–D. The results thus show that only F(ab')2 from mAbs with C1qGR specificity inhibited C1 activation by both recombinant and native CRP or IgG whereas those with C1qCLR specificity were unable to inhibit C1 activation by CRP or IgG.

Discussion
In the present study, we show that five mAbs against C1qGR inhibited the binding of C1q to CRP, whereas none of the four mAbs against C1qCLR shared this feature. Moreover, binding of C1q to CRP could be inhibited by IgG and conversely, indicating that the binding sites on C1q for IgG and CRP are both on the GR rather than on the CLR, which has earlier been suggested to harbor the binding sites for CRP (28, 29).

The mAbs used for this study were characterized regarding binding to C1qGR and C1qCLR. Five mAbs turned out to be directed to the C1qGR, and these mAbs inhibited the binding of C1q to solid-phase IgG, in agreement with the notion that the binding sites on C1q for IgG are located in the GR (16, 17). Moreover, competition experiments not shown here revealed that these five mAbs were directed against at least two different epitopes on the globular region of C1q. Thus, these mAbs provided unique molecular probes to study the interaction between C1q and CRP.

It should be noted that earlier studies used trimerized CRP (30) as a ligand for C1q interaction studies. The extrapolation of these results to native CRP is unclear and recent information about the interaction of CRP with C1q (31) emphasizes that this chemical cross-linking may influence the characteristics of this interaction. Studies on the topology of the C1q binding site on CRP (31) showed an extended deep cleft that contains the binding site for C1q, which is located on the face of the pentamer opposite that of the PC-binding site. Critical residues for C1q binding in this cleft include Asp112 and Tyr175 whereas the interaction is hindered by a lysine at position 114. Because trimerization of CRP involves chemical cross-linking via lysine residues (30), this modification might have altered its interaction with C1q, possibly contributing to the previous conclusion that CRP binds to C1qCLR. Furthermore, it is interesting to note that in earlier studies CRP trimers were observed to interact with C1qGR although competition studies were not conducted (30).

It is also important to note that many of the interactions via the CLR portion of C1q are ascribed to two regions of the A chain, 14-26 and 76-92. These are highly cationic sequences (20) with, for example, 5 basic residues in the 13 residues of C1qA 14-26. The interactions ascribed to this segment of C1q could be mainly based on low affinity charge interactions, a hypothesis strengthened by the experimental detail that half-physiologic ionic strength buffers were used to detect the binding between C1qGR and CRP (28).
Next to these possible explanations for the discrepancies in the localization of the C1q binding site for CRP, it is important to note that earlier interaction studies used C1q and C1q fragments coated to microtiter plates, whereas in this situation, interaction with the C1qGR may not be easily accessible, because C1q binding to the plate is primarily via these regions (20). In our experiments, fluid-phase C1q and its fragments were allowed to bind to immobilized CRP.

Finally, strong support that C1q-CRP interaction is via the GR comes from the recent elucidation of the crystal structure of C1qGR, refined to 1.9 Å resolution (26). Using molecular modeling, this study revealed that the tip of C1qGR, which is predominantly basic, would fit into the central pore of the CRP pentamer. Moreover, the residues Asp112 and Tyr175 of CRP, which were experimentally shown to be critical for C1q binding, would be the contact points in this model, which strongly supports this theoretical model. Taken together, much of our present understanding of C1q and its ligands (32) now points to its interaction with CRP via C1qGR. However, we believe that the present study provides the first experimental evidence to demonstrate that this is the case under physiological conditions.

Not only CRP but also PTX3, another member of the pentraxin family, was recently shown to interact with the globular head of C1q (19). Furthermore, competition studies strongly suggested that PTX3, CRP and the pentraxin family member SAP recognize the same domain of C1q (19). Next to the pentraxin molecules, recent evidence was provided that the β-amyloid peptide, a molecule involved in Alzheimer’s disease, also binds to C1qGR (20). Therefore, taken together, these results indicate that complement-activating C1q ligands generally bind to the GR region, leaving the CLR region available for interaction with the complement-activating enzymes C1r and C1s, and for receptor interactions with cells (Fig. 9).

Our results show that recombinant and native CRP are both equally able to activate the classical pathway of complement, as assessed at the level of C4 deposition. This complement activation was completely inhibited by Abs against the C1qGR, but not by Abs against C1qCLR, strongly suggesting that the mechanisms of C1 activation by CRP and by IgG are similar.

In the present study, we use fluid-phase competition studies to demonstrate that binding sites for CRP and IgG on C1q are overlapping or in close proximity. In addition, these studies clearly indicate that CRP is able to bind to C1q in the fluid phase. Although in general, complement-activating ligands for C1q were proposed to need presentation in a multimeric fashion to allow C1q binding and subsequent complement activation, also for the classical interaction of C1q with Abs, it has become increasingly clear

![FIGURE 8](http://www.jimmunol.org/)

**FIGURE 8.** Complement activation by recombinant and native CRP requires its interaction with C1qGR. A, Wells were coated with recombinant CRP, native CRP purified from human plasma, or coating buffer only, followed by incubation with increasing concentrations of normal human serum as a complement source and assessment of C4 activation. B–D, Normal human serum was preincubated in the presence of anti-C1q mAb F(ab')₂ control F(ab')₂ mAb, as indicated, followed by incubation in wells coated with rCRP (B), plasma CRP (C), or human IgG (D). Activation of C4 was assessed by ELISA.

![FIGURE 9](http://www.jimmunol.org/)

**FIGURE 9.** The heads of C1q are required for recognition of complement-activating ligands. A schematic representation of the C1q molecule is provided, containing six trimeric heads and tails. The position of globular heads and collagenous fragments, their recognition by mAbs used in the present study, and the interaction with serine proteases, receptors, and complement-activating ligands including CRP is indicated.
that this is largely a matter of affinity. In this respect, it has been previously shown that C1q can bind to IgG and IgM in the fluid phase, and that this interaction can compete for the binding of C1q to immobilized IgG (33), as is confirmed in the present study. Furthermore, it has been previously shown that the interaction of C1q with PTX3 also occurs in the fluid phase (19). Therefore, we propose that C1q has a low affinity interaction with CRP in the fluid phase, whereas multimeric immobilized CRP binds to C1q with high affinity. The latter interaction results in complement activation. Our experiments with CRP and C1q in the fluid phase, as well as experiments using CRP directly conjugated to Sepharose or immobilized in ELISA wells, indicate that binding of C1q and activation of the classical complement pathway by CRP does not require its interaction with the natural CRP-ligand PC. A direct interaction of C1q with CRP (28), PTX3 (19), and SAP (34), without a need for ligand binding, has also been shown previously. Fluid-phase interaction between CRP and C1q, as we now present, excludes the alternative explanation that immobilization of CRP on plastic exposes a previously hidden C1q-binding site on CRP. It has been proposed by Agrawal et al. (31) that attachment of CRP to PC leads to a conformational change in CRP, exposing the affinity of CRP for C1q. The latter interaction results in complement activation.

CRP, once ligand-bound and aggregated, is capable of activating complement with the resultant proinflammatory effects. Many studies now show the importance of CRP levels as a predictive factor in cardiac disease both for the occurrence of a coronary event (35, 36) and postmyocardial infarct survival (37, 38). CRP has been shown to colocalize with complement in acute myocardial infarction (39) and together they have been demonstrated to be important mediators of the resulting tissue damage (2, 36). Inhibition of the early phase of complement activation has been demonstrated in an animal model to have cardioprotective effects following ischemia/reperfusion injury (40), and using complement inhibition as a therapy is the subject of many studies (reviewed in Refs. 41 and 42). Given reports of the importance of the C1q-CRP inhibition as a therapy is the subject of many studies (reviewed in Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent, ed. Springer, Berlin, pp. 39–50.)

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**Disclosures**

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