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CRP after 2004

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Abstract

C-reactive protein (CRP) that has been conserved throughout evolution is a host-defense molecule. Its attraction towards phosphocholine-ligands, such as modified low-density lipoprotein, and apoptotic cells leads to the "masking" of these substances that have the capabilities to otherwise engage in deleterious activities. Complement activation by CRP complexes and the modulation by CRP of complement activation by its ligands add up to its beneficial effects. In the presence of CRP, production of membrane-damaging last product of the complement pathway is arrested. CRP is currently serving as an indicator of cardiovascular diseases, but to pinpoint the role of CRP in atherosclerosis, a drug that can lower cholesterol levels, but not the CRP levels, is needed for experimentation.

Keywords

C-reactive protein; Complement; Phosphocholine; Atherosclerosis

1. Introduction

C-reactive protein (CRP), categorized as an acute phase protein in humans, has gained much attention because its blood concentration increases even under chronic inflammatory conditions, such as the development of atherosclerosis. CRP binds to many biological materials, and subsequently, activates complement although only through half way. What purpose do these two properties of CRP serve? Why does complement activation by CRP not proceed to completion? We are curious to learn the answer and extend that to understand the contribution of CRP to atherosclerosis. The truth remains to be discovered, however, most recent data project that it is advantageous to have CRP around.

2. "Masking" of phosphocholine-rich surfaces by CRP

CRP has five identical subunits arranged symmetrically as a pentamer with a naturally formed pore in the center (Agrawal et al., 1993; Shrive et al., 1996). CRP readily gets complexed in a Ca^{2+}-dependent manner to substances with exposed phosphocholine (PCh) groups, such as modified low-density lipoproteins (LDL) (Bhakdi et al., 2004; Chang et al., 2002; Taskinen et al., 2002), apoptotic cells (Chang et al., 2002; Gershov et al., 2000) and C-polysaccharide of the cell wall of *Streptococcus pneumoniae* (Agrawal et al., 1997). A PCh-binding site is present on each CRP subunit. Since, the subunits have same orientation in the assembled pentamer, all five PCh-binding sites fall on the same face, commonly known as the 'recognition face' of CRP (Thompson et al., 1999; Volanakis, 2001). Thus, pentameric CRP can be pictured lying flat through its recognition face on a PCh-bearing

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surface. If the ligand surface is rich in PCh moieties, that is, densely populated with PCh, then CRP will apparently "mask" the original characteristics of its ligand. Only the surface opposite to the recognition face of CRP popularly designated as the 'effector face' would then be available for the consequences.

3. Initiation of complement activation by CRP

CRP–Ca^{2+}–PCh-mediated activation of the classical pathway of complement is ignited by C1q recognition (Agrawal and Volanakis, 1994; Volanakis, 2001). Complement has been indicated to participate in several proposed anti-inflammatory actions of CRP, such as phagocytosis of apoptotic cells (Gershov et al., 2000; Nauta et al., 2003a,b), protection from bacterial infections (Mold et al., 2002; Szalai et al., 1996), and maintenance of LDL cholesterol homeostasis (Bhakdi et al., 2004). Mechanistic details of the actions of CRP following CRP–C1q interaction, however, are not clear because CRP-mediated activation of complement proceeds only through half way (Berman et al., 1986). The process generates C3 convertase but does not result in the formation of an effective C5 convertase. Therefore, the assembly of the membrane-attack complex (MAC; a multi-protein complex composed of C5b–C9) is not observed in the complement pathway triggered by CRP, and pro-inflammatory molecules like C5a are not produced (Mold et al., 1999; Volanakis, 2001). Presumably, the ligands bound to CRP, and opsonized by C3 fragments alone under the actions of C3 convertase, are targeted for opsonophagocytosis. Thus, CRP, assisted by C1q, plays the role of an opsonin and acts as an anti-inflammatory acute phase protein of the innate immune system.

Binding of Ca^{2+} to CRP subunits is necessary for PCh-binding but not for C1q-binding. For example, CRP-polycation complexes recognize C1q although they are devoid of Ca^{2+} (Claus et al., 1977). CRP forms complexes with polycations at its recognition face and does not require either Ca^{2+} or the critical residues from the PCh-binding site (Agrawal et al., 2002; Black et al., 2003). Remarkably, for C1q to be able to bind to CRP and activate complement, just one CRP pentamer is not enough. Supporting evidences are: First, C1q can recognize chemically cross-linked trimers, but not dimers, of CRP pentamers (Jiang et al., 1991). Second, C1q is much larger than CRP, therefore, a requirement for more than one CRP pentamer to form stable interactions with C1q is justified (Agrawal et al., 2001). Lastly, CRP complexed only to PCh-containing substances and not to PCh salt interacts with C1q. So, to initiate complement activation, the ligand must possess several CRP pentamers.

4. Regions in activity on CRP and C1q

The C1q is accommodated in an area formed by the five clefts present one on each CRP subunit and located on the effector face of CRP. The cleft is long, starts at about the center of each subunit, and extends to the central pore of the pentamer. It is deep and narrow at its origin but is wider and shallow towards the center (Shrive et al., 1996). Site-directed mutagenesis of amino acids located in the CRP cleft indicated that the wider and shallow end of the cleft close to the pore was the C1q-binding site. Asp^{112} and Tyr^{175} in CRP appear to be the contact residues and participate directly in CRP–C1q interactions (Agrawal and Volanakis, 1994; Agrawal et al., 2001). The conformation of the CRP cleft is not changed by the presence or absence of Ca^{2+} at the recognition face consistent with the findings that both Ca^{2+}-independent CRP complexes and artificial trimers of CRP-pentamers are capable of binding C1q. Based on the proposals (Agrawal et al., 2001; Nauta et al., 2003a,b) that CRP binds to the globular head (gC1q) and not the collagenous tail of C1q, a molecular model of PCh-CRP with gC1q head was built to define the CRP-binding site on C1q (Gaboriaud et al., 2003). The basic top of the gC1q could fit in the acidic central pore of CRP and all three polypeptide chains of C1q were shown to form the CRP-binding site.

The modeling studies highlight steric restraint between gC1q and the single CRP pentamer bound to free PCh, and it was suggested that a slight conformational change in the CRP structure would relieve it (Gaboriaud et al., 2003). However, no major structural difference has been reported between the C1q-binding site of unliganded and PCh-liganded CRP. The binding of PCh to CRP just seems to facilitate bringing CRP pentamers close together. If a structural change or tilting of the CRP subunits to fully open the C1q recognition area to the surface is necessary as proposed, it may occur simultaneously with the binding of gC1q domains to many CRP molecules arranged on the PCh-rich ligand.

5. Failure of MAC assembly

The cause for the failure of PCh–Ca^{2+}–CRP–C1q complex formed on any PCh-containing macromolecule/particle to generate MAC is not determined yet. It has been shown that, in vitro, CRP binds to factor H, the complement protein that binds C3b and regulates alternative complement pathway (Jarva et al., 1999) and that this provides a possible mechanism for prevention of MAC in the classical pathway (Gershov et al., 2000; Mold et al., 1999). Models of CRP–gC1q and IgG–gC1q complexes indicate that gC1q sits on its two different partners in two different ways (Gaboriaud et al., 2003). IgG is recognized by the equatorial region of a single chain of gC1q, while CRP is recognized by the top of the head involving all three chains of gC1q. Itwas not evident though that the unique orientation of gC1q on ligand-bound CRP would offer steric hindrances in the pathway leading to the formation of MAC.

MAC is huge. It is unlikely that a PCh-containing target, such as an appropriate bacterium or a damaged cell or an LDL molecule, once totally covered with CRP, will still be able to present enough space on its surface for the assembly of the multi-protein complex MAC. CRP indeed is known to protect assembly of MAC on the CRP-bound targets, such as apoptotic cells (Gershov et al., 2000) and modified LDL (E-LDL) (Bhakdi et al., 2004). The extent of complement activation by CRP complexed with a substance with fewer and irregular distribution of PCh moiety on it is yet to be elucidated. However, experiments employing similar strategy have been recently published (Bhakdi et al., 2004). CRP-dependent complement activation was found to be dependent on the amount of E-LDL (Bhakdi et al., 2004). In the absence of CRP, E-LDL activated complement and MAC was formed. In the presence of CRP, MAC was not detected with the limited concentration of E-LDL; MAC could be detected when unlimited concentration of E-LDL was used. The relative abundance of CRP and the CRP-ligand dictated the production of MAC. It would be interesting to see if the availability of more and more CRP would prevent MAC formation even by unlimited concentration of E-LDL. The expectation is that the MAC will not arrive if CRP concentration exceeds ligand concentration.

6. Concluding remarks

The appearance of CRP and the functions of CRP both have benefits. The serum CRP concentration serves as an alarm/indicator of either chronic or acute inflammation occurring in the body: somewhere (Danesh et al., 2004; Kushner and Sehgal, 2002; Ridker et al., 2001). Functioning in a variety of ways, CRP acts as an entire host-defense system although some pro-inflammatory qualities have also been assigned to it (Devraj et al., 2003; Paul et al., 2004; Torzewski et al., 2000). The capture of modified LDL by CRP and the manner in which CRP limits the extent of complement activation by modified LDL are of most relevant to atherosclerosis. A drug that can lower cholesterol levels, but not the CRP levels, should be of choice over statins (which lowers both) (Ridker et al., 2001) in the experiments aimed at determining any role of CRP in the development of atherosclerosis. Recently (Binder et al., 2003), the occurrence of “molecular mimicry” has been shown between *S.*

pneumoniae and oxidized LDL, and the antibodies to PCh have been shown to decrease atherosclerotic lesion formation. I believe that the phenomenon of CRP action may well be identical to that of anti-PCh antibodies action in atherosclerosis.

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