Minireview

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C-reactive Protein*

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C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein, with homologs in vertebrates and many invertebrates, that participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states, a characteristic that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. Recently, an association between minor CRP elevation and future major cardiovascular events has been recognized, leading to the recommendation by the Centers for Disease Control and the American Heart Association that patients at intermediate risk of coronary heart disease might benefit from measurement of CRP. This review will largely focus on our current understanding of the structure of CRP, its ligands, the effector molecules with which it interacts, and its apparent functions.

CRP[1] was discovered in Oswald Avery's laboratory during the course of studies of patients with *Streptococcus pneumoniae* infection (1). Sera obtained from these patients during the early, acute phase of the illness were found to contain a protein that could precipitate the "C" polysaccharide derived from the pneumococcal cell wall. Forty years later, Volanakis and Kaplan identified the specific ligand for CRP in the pneumococcal C polysaccharide as phosphocholine, part of the techoic acid of the pneumococcal cell wall (2). Although phosphocholine was the first defined ligand for CRP, a number of other ligands have since been identified. In addition to interacting with various ligands, CRP can activate the classical complement pathway, stimulate phagocytosis, and bind to immunoglobulin receptors (FcγR).

In humans, plasma levels of CRP may rise rapidly and markedly, as much as 1000-fold or more, after an acute inflammatory stimulus, largely reflecting increased synthesis by hepatocytes. CRP induction is part of a larger picture of reorchestration of liver gene expression during inflammatory states, the *acute phase response*, in which synthesis of many plasma proteins is increased, whereas that of a smaller number, notably albumin, is decreased. At least 40 plasma proteins are defined as acute phase proteins, based on changes in circulating concentration of at least 25% after an inflammatory stimulus. This group includes clotting proteins, complement factors, anti-proteases, and transport proteins (reviewed in Ref. 3). These changes presumably contribute to defensive or adaptive capabilities.

Regulation of CRP Expression

The CRP gene, located on the short arm of chromosome 1, contains only one intron, which separates the region encoding the signal peptide from that encoding the mature protein. Induction of CRP in hepatocytes is principally regulated at the transcriptional level by the cytokine interleukin-6 (IL-6), an effect which can be enhanced by interleukin-1β (IL-1β) (4). Both IL-6 and IL-1β control expression of many acute phase protein genes through activation of the transcription factors STAT3, C/EBP family members, and Rel proteins (NF-κB). The unique regulation of each acute phase gene is due to cytokine-induced specific interactions of these and other transcription factors on their promoters. Thus, for the fibrinogen genes, STAT3 is the major factor, for the serum amyloid A genes, NF-κB is essential, and for CRP, the C/EBP family members C/EBPβ and C/EBPδ are critical for induction. In addition to C/EBP binding sites, the proximal promoter region of the CRP gene contains binding sites for STAT3 and Rel proteins. Interactions among these factors that result in enhanced stable DNA binding of C/EBP family members result in maximum induction of the gene (5). Extrahepatic synthesis of CRP has also been reported in neurons, atherosclerotic plaques, monocytes, and lymphocytes (6, 7). The mechanisms regulating synthesis at these sites are unknown, and it is unlikely that they substantially influence plasma levels of CRP.

Protein Structure

CRP consists of five identical, noncovalently associated ~23-kDa protomers arranged symmetrically around a central pore. The term "pentraxins" has been used to describe the family of related proteins with this structure. Each protomer has been found by x-ray crystallography to be folded into two antiparallel β sheets with a flattened jellyroll topology similar to that of lectins such as concanavalin A (8, 9). Each protomer has a recognition face with a phosphocholine binding site consisting of two coordinated calcium ions adjacent to a hydrophobic pocket. The co-crystal structure of CRP with phosphocholine (Fig. 1) suggests that Phe-66 and Glu-81 are the two key residues mediating the binding of phosphocholine to CRP (9). Phe-66 provides hydrophobic interactions with the methyl groups of phosphocholine whereas Glu-81 is found on the opposite end of the pocket where it interacts with the positively charged choline nitrogen. The importance of both residues has been confirmed by mutagenesis studies (10, 11).

The opposite face of the pentamer is the effector face, where complement C1q binds and Fcγ receptors are presumed to bind. A cleft extends from the center of the protomer to the central

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[1] The abbreviations used are: CRP, C-reactive protein; FcγR, immunoglobulin Fcγ receptor; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; SLE, systemic lupus erythematosus; STAT, signal transducers and activators of transcription; C/EBP, CCAAT enhancer-binding protein.

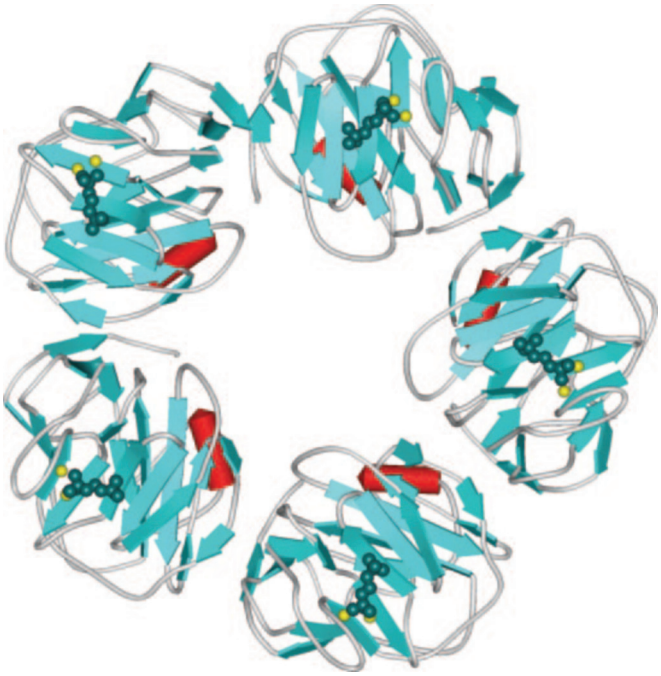

FIG. 1. **Crystal structure of C-reactive protein complexed with phosphocholine from Thompson *et al.* (9).** ViewerPro 4.2 software (Accelrys, San Diego, CA) was used to generate the ribbon diagram of the x-ray crystal structure of CRP-phosphocholine complex obtained from Brookhaven Protein Data Bank (PDB entry 1B09). The calcium ions are *yellow*, and phosphocholine is *green*.

pore of the pentamer, and several residues along the boundaries of this cleft have been shown to be critical for the binding of CRP to C1q, including Asp-112 and Tyr-175 (12, 13). The crystal structure of the globular head domain of C1q was recently solved (14), and a model for C1q binding to CRP was proposed in which the top of the predominantly positively charged C1q head interacts with the predominantly negatively charged central pore of the CRP pentamer. In this model, which displays shape complementarity, the globular head of C1q spans the central pore of CRP and interacts with two of the five protomers of the pentamer (Fig. 2). The strict steric requirements for CRP interaction with C1q in this model imply that optimal C1q binding is accompanied by slight conformational changes in the CRP structure (14). These conformational changes appear to differ depending on the ligand to which CRP is bound (11).

In Vitro Effects

Further insight into the biologic function or functions of CRP is provided by the ligands and effector molecules with which it interacts. Phosphocholine is found in a number of bacterial species and is a constituent of sphingomyelin and phosphatidylcholine in eukaryotic membranes. However, the head groups of these phospholipids are inaccessible to CRP in normal cells, so that CRP can bind to these molecules only in damaged and apoptotic cells (15–18). In addition to phosphocholine, CRP can bind to a wide variety of other ligands, including phosphoethanolamine, chromatin, histones, fibronectin, small nuclear ribonucleoproteins, laminin, and polycations (11, 19). Ligand-bound or aggregated CRP efficiently activates the classical complement pathway through direct interaction with C1q. There is evidence that CRP can interact with the immunoglobulin receptors FcγRI and FcγRII as well, eliciting a response from phagocytic cells. The ability to recognize pathogens with subsequent recruitment and activation of complement, as well as effects on phagocytic cells, constitute important components of the first line of host defense.

Like many mediators of inflammatory processes, CRP has pleiotropic effects. Both "pro-inflammatory" and "anti-inflammatory" activities have been described. In addition to the *in vivo* anti-inflammatory effects described below, CRP has been shown to induce the expression of interleukin-1 receptor antagonist (20) and increase release of the anti-inflammatory cytokine interleukin-10 (21, 22) while repressing synthesis of interferon-γ (22). However, many other functions that can be regarded as pro-inflammatory are recognized. For example, CRP activates complement and enhances phagocytosis. CRP up-regulates the expression of adhesion molecules in endothelial cells, inhibits endothelial nitric-oxide synthase expression in aortic endothelial cells (23), stimulates IL-8 release from several cell types, increases plasminogen activator inhibitor-1 expression and activity, and increases the release of IL-1, IL-6, IL-18, and tumor necrosis factor-α (24). Although some of these *in vitro* properties are consistent with the net *in vivo* effects of CRP observed in mice and described below, it is likely that the function of CRP is context-dependent and that it can either enhance or dampen inflammatory responses depending on the circumstance.

Complement—The complement system, consisting of about 30 proteins, plays an important role in host defense mechanisms against infectious agents and in the inflammatory response. Three pathways through which complement can be activated are currently recognized: the classical, alternative, and mannose-binding lectin pathways (reviewed in Ref. 25). C1–C9 are the major components of the classical activation cascade, most commonly initiated by binding of immune complexes to C1q. The initial stage of activation generates cleavage products of C3 and C4, which act as opsonins. The later stage of classical complement activation involves C5–C9, which are highly inflammatory, generating powerful chemotactic peptides and forming the membrane attack complex, which can result in lysis of the bacteria or cells to which it binds.

Complexing of ligand-bound CRP to C1q leads to formation of C3 convertase (26), which assembles in a fashion similar to that initiated by antibody-antigen complexes. However, examination of individual complement components suggests that CRP-mediated complement activation is limited to the initial stage of complement activation involving C1–C4, with little activation of the late complement proteins C5–C9 (26). This is in contrast to the complement cascade initiated by antigen-antibody complexes, in which late phase components are activated. The difference between complement activation by CRP and that resulting from immune complexes is presumably due to the ability of CRP to interact with factor H, leading to inhibition of the pathways that result in formation of C5 convertases. As a result, the strong inflammatory responses typically associated with C5a and the C5–C9 membrane attack complex are limited. An additional mechanism through which CRP may limit the amount of complement activation has recently been described, in which CRP up-regulates endothelial cell expression of three complement inhibitory factors: decay-accelerating factor, membrane cofactor protein, and CD59 (27). The net effect is that CRP can participate in host defense systems while limiting the potentially damaging inflammatory effects of the late stage complement components.

CRP Receptor—Functional effects of CRP on phagocytic cells, as well as binding of CRP to such cells, have been recognized for many years. Only recently have the receptors for CRP been identified as the already known receptors for IgG, FcγRI and FcγRII. Two general classes of FcγRs are now recognized, the stimulatory receptors, characterized by an associated cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) sequence, and an inhibitory receptor, characterized by the

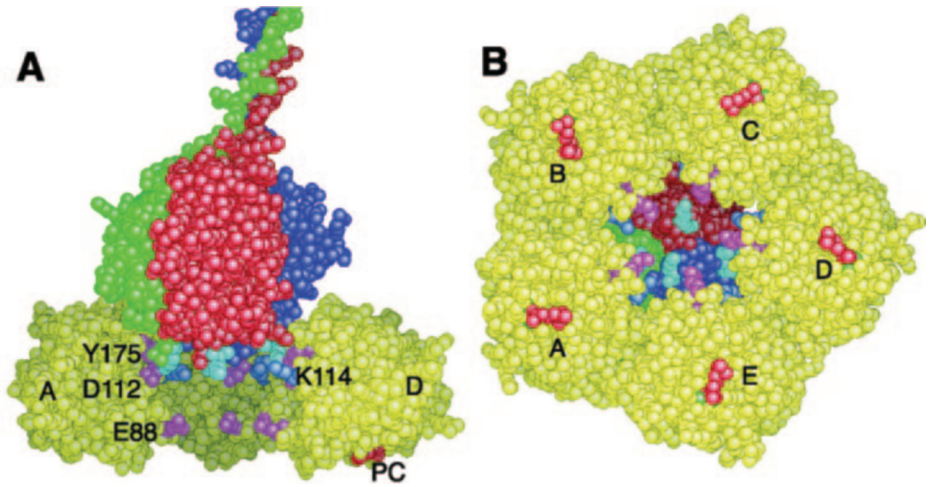

FIG. 2. **Model of the interaction of CRP with C1q from Gaboriaud *et al.* (14).** *A*, side view. Subunits B and C of CRP have been omitted for clarity. *B*, perpendicular bottom view. Modules A, B, and C of the C1q subunit are shown in *blue*, *green*, and *red*, respectively. The lysines at the top of the C1q head (Ala-173, Ala-200, Ala-201, Cys-170) and Tyr^{B175} are in *light blue*. *A–E* designate the CRP protomers as described by Shrive *et al.* (8). The phosphocholine (*PC*) ligand is in *red*, and the nearby Ca^{2+} ion is in *green*. Color coding for CRP mutations is as follows. Mutations impairing complement activation (Glu-88, Asp-112, Tyr-175) are *magenta*, and mutations enhancing complement activation (Lys-114) are *blue*.

presence of an immunoreceptor tyrosine-based inhibition motif (ITIM) sequence. Biological responses triggered by ITAM-containing FcγRs include phagocytosis, respiratory bursts, and secretion of cytokines. ITIM-containing FcγRs, when found co-aggregated with ITAM-containing FcγRs, negatively regulate ITAM-mediated activity (reviewed in Ref. 28). In both humans and mice, CRP binds to ITAM- and ITIM-containing receptors, which include FcγRI and FcγRII. Although some investigators have voiced doubts about CRP binding to FcγRs (29), several groups have demonstrated the importance of these receptors.

Phagocytosis of CRP-opsonized particles and apoptotic cells has been shown to proceed through FcγRI in the mouse (30, 31). CRP has also been shown to induce signaling through human FcγRIIa, an ITAM-containing receptor, in granulocytes (32). As discussed previously, activation of the classical complement pathway can lead to an enhancement of leukocyte phagocytosis, but even in the absence of complement, CRP has been reported to enhance *in vitro* leukocyte phagocytosis of several pathogenic species, including *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella aerogenes* (33). The enhancement of phagocytosis by CRP is likely due to its interactions with FcγRs.

In Vivo Effects

In contrast to humans, plasma levels of mouse CRP rarely exceed 2 μg/ml following inflammatory stimuli. Ablation of the murine CRP gene by homologous recombination has not been reported. Rather, the murine CRP response represents an evolutionary oddity, a natural knockdown, that has been exploited in a variety of studies utilizing exogenous or transgenic CRP to study the effects of CRP *in vivo*.

The ability of CRP to protect mice against bacterial infection by various species has been well established. These species include *S. pneumoniae* (34, 35) and *Haemophilus influenza* (36, 37), which have phosphocholine-rich surfaces, and *Salmonella enterica* serovar Typhimurium, which has no known surface phosphocholine, although its cell membrane is known to be rich in another CRP ligand, phosphoethanolamine (38). Protection is presumably mediated through CRP binding to phosphocholine or phosphoethanolamine, followed by activation of the classical complement pathway. CRP protection of mice infected with *S. pneumoniae* has been shown to require an intact complement system (39) but does not require interaction with FcγRs (39, 40).

CRP protective effects are not limited to bacteria. CRP has been shown to play a protective role in a variety of inflammatory conditions, including protecting mice from lethal challenge with bacterial lipopolysaccharide and various mediators of inflammation (41). The former has been shown to require Fcγ receptors (21). In addition, CRP has been found to delay the onset and development of experimental allergic encephalomyelitis, an aseptic animal model of multiple sclerosis (22). In a murine model of chemotactic factor-induced alveolitis, CRP has also been shown to inhibit the influx of neutrophils and protein into the lungs (42, 43). Taken together, these experiments suggest that the net effect of CRP in mice is anti-inflammatory.

It is of interest that CRP may exert an ameliorative effect upon murine models of systemic lupus erythematosus (SLE). SLE is an autoimmune condition, which is characteristically accompanied by antibodies against cellular, particularly nuclear, components, many of which are CRP ligands, and in which CRP levels are often unexpectedly low (44). Two reports have shown that injection or transgenic expression of CRP in a murine strain prone to development of a disease resembling human SLE resulted in a slight delay in mortality (45, 46). In addition to these mouse models, a polymorphism in the human CRP gene resulting in a lower basal level of CRP has been associated with an increased risk of developing systemic lupus erythematosus (47). These findings raise the possibility that decreased amounts of CRP may contribute to the pathogenesis of SLE. It has long been held that an important function of CRP is to target for clearance the cellular debris of necrotic and apoptotic cells by binding to damaged cell membranes and nuclear material. Decreased clearance of such material might well enhance development of autoantibodies to them.

"Modified" CRP

Denatured and aggregated forms of CRP (neo-CRP or modified CRP) have been reported to be powerfully pro-inflammatory in a number of experimental systems, although the existence of this material *in vivo* has not been unequivocally established (reviewed in Ref. 48). It is conceivable that at local sites of deposition, small amounts of modified CRP may be generated with a set of properties distinct from those of the native protein. It has recently been reported that modified CRP increased the release of the inflammatory mediators monocyte chemoattractant protein-1 and IL-8 and up-regulated the expression of ICAM-1 in endothelial cells. In this model, modified CRP was shown to be a much more potent inducer than native CRP (49).

Minor CRP Elevation

Although about two-thirds of the American population has plasma CRP levels under 3 μg/ml, circulating CRP levels under 10 μg/ml have historically been regarded as clinically insignificant. In recent years, a plethora of studies have demonstrated an association between slightly elevated CRP plasma levels, between 3 and 10 μg/ml, and the risk of developing cardiovas-

cular disease (reviewed in Ref. 50), metabolic syndrome, and colon cancer. It is felt that many of these conditions involve a low level of underlying chronic inflammation that could be reflected by these minor increases. Minor increases in CRP levels have also been reported to be associated with a number of medical conditions that do not appear to be inflammation-associated, as well as with several genetic polymorphisms of the CRP and other genes, ethnicity, various dietary patterns, and obesity.

CRP and Atherosclerosis

Evidence in support of the possibility that CRP itself plays a role in the pathogenesis of atherosclerosis has been summarized in a recent review (6). Examples include the finding that CRP binds the phosphocholine of oxidized low density lipoprotein (18), up-regulates the expression of adhesion molecules in endothelial cells, increases low density lipoprotein uptake into macrophages (51), inhibits endothelial nitric-oxide synthase expression in aortic endothelial cells (23), and increases plasminogen activator inhibitor-1 expression and activity. A recent study utilizing a mouse strain expressing transgenic CRP and deficient in apolipoprotein E reported a modest acceleration in aortic atherosclerosis in male animals expressing high levels of CRP (52). A second report demonstrated increased arterial occlusion in transgenic mice expressing CRP in a model of vascular injury (53). Despite these suggestive findings, a role for CRP in the pathogenesis of atherosclerosis is far from established.

Summary

CRP is an ancient protein whose initial role as a pattern recognition molecule may have been to defend against bacterial infections, but whose present biological role appears quite complex. It is protective against a variety of bacterial infections and inflammatory stimuli in mice. It is likely that the activity of CRP in humans, either pro- or anti-inflammatory is dependent on the context in which it is acting. Recent data have raised the possibility that it may participate in the pathogenesis of disease.

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