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Structure-Function Relationships of C-Reactive Protein in Bacterial Infection

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Structure-Function Relationships of C-Reactive Protein in Bacterial Infection

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One host defense function of C-reactive protein (CRP) is to protect against *Streptococcus pneumoniae* infection as shown by experiments employing murine models of pneumococcal infection. The protective effect of CRP is due to reduction in bacteremia. There is a distinct relationship between the structure of CRP and its anti-pneumococcal function. CRP is functional in both native and non-native pentameric structural conformations. In the native conformation, CRP binds to pneumococci through the phosphocholine molecules present on the C-polysaccharide of the pneumococcus and the anti-pneumococcal function probably involves the known ability of ligand-complexed CRP to activate the complement system. In the native structure-function relationship, CRP is protective only when given to mice within a few hours of the administration of pneumococci. The non-native pentameric conformation of CRP is created when CRP is exposed to conditions mimicking inflammatory microenvironments, such as acidic pH and redox conditions. In the non-native conformation, CRP binds to immobilized complement inhibitor factor H in addition to being able to bind to phosphocholine. Recent data using CRP mutants suggest that the factor H-binding function of non-native CRP is beneficial: in the non-native structure-function relationship, CRP can be given to mice any time after the administration of pneumococci irrespective of whether the pneumococci became complement-resistant or not. In conclusion, while native CRP is protective only against early stage infection, non-native CRP is protective against both early stage and late stage infections. Because non-native CRP displays phosphocholine-independent anti-pneumococcal activity, it is quite possible that CRP functions as a general anti-bacterial molecule.

Keywords: C-reactive protein, factor H, phosphocholine, pneumococcal C-polysaccharide, *Streptococcus pneumoniae*

INTRODUCTION

C-reactive protein (CRP) is a multifunctional molecule of the innate immune system in humans [1–4]. CRP is a cyclic pentameric protein comprised of five identical non-covalently attached subunits. Each subunit has an intra-disulfide bond and the molecular weight of each subunit is ~23 kDa [5, 6]. A phosphocholine (PCh)-binding site is located on the same face of each subunit in the homopentamer. The amino acids Phe^{66}, Thr^{76}, and Glu^{81} in CRP are critical for the formation of

the PCh-binding site (7–9). Once CRP is complexed with a substance with exposed PCh group, the complex activates the complement system through the classical pathway (10–12).

Streptococcus pneumoniae are gram positive bacteria that asymptomatically colonize the upper respiratory tract (1, 13–15). It is the most common bacterium that causes community-acquired pneumonia and is also a significant cause of septicemia and meningitis (1, 13–15). Systemic pneumococcal infection raises the level of CRP in serum by up to several hundred-fold in humans as a part of the acute phase response (16–18). CRP binds to pneumococci through Ca^{2+}-dependent interaction with PCh residues present on the pneumococcal cell wall C-polysaccharide (PnC) (19, 20). In mice, however, CRP is only a minor acute phase protein; therefore, mice have been useful in investigating the functions of human CRP *in vivo* (21).

In murine models of pneumococcal infection, passively administered human CRP has been shown to be protective against lethal pneumococcal infection, that is, CRP decreases bacteremia and enhances survival of infected mice (1, 22–26). CRP-deficient mice are more susceptible to pneumococcal infection than are wild type mice, which indicates that the trace level of endogenous mouse CRP is capable of exerting anti-pneumococcal functions (27). Mice transgenic for human CRP are also protected against infection with *S. pneumoniae* (28). The mechanism of anti-pneumococcal action of CRP in mice, however, is unknown.

Current research on defining the mechanism of anti-pneumococcal actions of CRP benefited from a key finding made several decades ago using passive administration of purified human CRP into mice (29). CRP was protective when injected into mice 6 h before to 2 h after the administration of pneumococci. CRP was not protective when mice received CRP 24 h after infection, suggesting that CRP is protective during early stage infection but not in late stage infection. For early stage protection, it is believed that the mechanism of action of CRP involves the capability of CRP to bind to pneumococci through PCh groups present on their surfaces and subsequent activation of the classical complement pathway by pathogen-bound CRP. Obviously, this mechanism does not operate for late stage infection. A PCh-independent mechanism for anti-pneumococcal function of CRP has been proposed along with an explanation for the inability of CRP to be protective against late stage infection (1, 24–26). In this article, we review PCh-dependent, PCh-independent, and other proposed mechanisms for the anti-pneumococcal function of CRP during both early stage infection (when CRP and pneumococci are administered into mice 30 min apart) and late stage infection (when CRP and pneumococci are administered into mice 24 h apart).

PCh-DEPENDENT ANTI-PNEUMOCOCCAL FUNCTION OF CRP

In vivo experiments employing a CRP mutant incapable of binding to PCh, PnC, and whole pneumococci provided

results indicating that CRP-mediated protection of mice against infection is independent of binding of CRP to PCh; the CRP mutant was as effective as wild-type CRP in protecting mice against early stage infection (26). The PCh-binding mechanism, however, does contribute to the protection of mice during the early stage of infection (25, 26). The PCh-dependent mechanism contributes to the initial and immediate clearance of pneumococci as has been shown employing a variety of murine models of infection (26, 27). Overall, the combined data suggest that both PCh-dependent and PCh-independent mechanisms operate in the protection of mice against early stages of infection, although the PCh-dependent mechanism is not necessary (25, 26).

Indirect evidence has been presented to show the importance of the PCh-binding property of CRP and subsequent complement activation by CRP-complexes in protection from infection. It has been shown that CRP binds to gram negative bacterial lipopolysaccharide (LPS) if the LPS is modified by adding a few PCh residues to it. The binding of CRP to PCh-modified LPS increases based on the number of PCh residues added and subsequently affects the resistance of the organism to the killing effects of serum (30). Also, the pneumococcal surface protein PspA, which is a choline-binding protein, is known to bind to PCh. PspA thus competes and inhibits the binding of CRP to PCh on pneumococci and decreases complement activation (31). Similarly, pneumococci growing as a biofilm are avirulent due to a decrease in PnC production although with an increase in PCh expression, interference from pneumococcal surface protein PspC, reduced binding of C1q to CRP-PCh complexes, and subsequent failure to activate complement (32, 33). Biofilm formation in *S. pneumoniae* is an effective means of evading complement attack (33).

One study suggested that the property of CRP to activate the classical pathway of complement in human serum is irrelevant for the protective function of CRP in mice infected with *S. pneumoniae*, because human CRP does not activate murine complement via the classical pathway (23). Since complement-deficient mice do not show CRP-mediated protection to pneumococcal infection (34), it is possible that CRP-complexes are able to activate murine complement system via a pathway other than the classical pathway (1, 23). It has been proposed that human CRP-complexes are able to activate the lectin pathway in murine serum and are able to activate both the classical and lectin pathways in human serum (23). CRP has been shown to interact with both L-ficolin and M-ficolin and activate the lectin pathway of complement (35–39). The interaction between CRP and L-ficolin increases 100-fold under the conditions of slight acidosis and reduced calcium levels, and it has also been shown that the cross-talk between CRP and L-ficolin mediates killing of *Pseudomonas aeruginosa* in plasma (37). L-ficolin also recognizes PCh on pneumococcal strains and triggers activation of the lectin complement pathway (40). Lectin-like oxidized LDL receptor, LOX-1, can also recognize CRP and is involved in CRP-dependent complement activation (41, 42). CRP is a major hemolymph protein in the horseshoe crab *Carcinoscorpius rotundicauda*. When CRP is in the hemolymph, it binds to a range of bacteria through galactose-binding protein and ficolin. Accordingly, it has been proposed that CRP does not act

alone but collaborates with other plasma lectins to form stable pathogen recognition complexes when targeting a wide range of bacteria for destruction (35).

PCh-INDEPENDENT ANTI-PNEUMOCOCCAL FUNCTION OF CRP

Factor H, a regulator of complement activation, has been implicated in resistance of pneumococci to complement attack (43, 44). Factor H protects from complement attack by inhibiting the activation of the alternative pathway on host cells and on those pathogenic surfaces which are capable of recruiting factor H from the plasma. On the host cells, factor H binds to polyanionic structures and glycoproteins found on the cell surface (45). On *S. pneumoniae*, factor H binds to a surface protein called Hic (factor H-binding inhibitor of complement) which is a variant of PspC (46, 47). Thus, pneumococci use factor H to evade complement-mediated killing. The recruitment of factor H by pneumococci might be the reason why CRP does not protect mice from pneumococcal infections during late stage infection.

CRP does not bind to factor H under normal physiological conditions (48–52). Denaturation conditions for CRP enable CRP to bind to factor H (4, 48–51). For example, immobilization of CRP on to a surface enables CRP to bind to factor H (4, 53, 54). Monomeric CRP (mCRP) also binds to factor H, in a Ca^{2+}-independent manner (55). The Y384H polymorphism of factor H affects binding affinity for mCRP. CRP binds to factor H-Tyr^{384} more strongly compared to factor H-His^{384} which is the risk allele (56–60). PCh does not compete with factor H for binding to CRP (52). It has been suggested that when CRP immobilizes itself on *S. pneumoniae*, it limits excessive complement activation by recruiting factor H (61, 62). CRP has also been shown to modulate lectin pathway-dependent cytolysis by recruiting factor H (63, 64). When CRP binds to dead cells it does not recruit factor H (55). mCRP also binds to factor H-related proteins (FHR) FHR1 and FHR5 and to factor H like protein 1 (FHL-1) which inhibit subsequent recruitment of factor H (65–68). CRP has also been shown to recruit factor H on other cell types, for example, CRP recruits factor H after binding to collectin CL-P1 on the surface of placental cells (69, 70). Otherwise, the interaction of CRP with CL-P1 activates the classical complement pathway. The interaction of CL-P1 with factor H might be the key to prevent self-attack due to complement activation induced by the CL-P1 and CRP interaction (69, 70).

Based on results obtained from the experiments performed under defined conditions—native pentameric CRP does not bind to factor H while mCRP binds to factor H—it was hypothesized that a non-native pentameric CRP may also be able to bind to factor H (48). Indeed, the native pentameric structure of CRP could be modified *in vitro* to generate non-native pentameric CRP capable of binding to factor H (2, 48–50). Since non-native CRP and Hic can bind to factor H simultaneously, it is possible that non-native CRP can bind to factor H-coated pneumococci, cover the factor H-Hic complex formed on

bacteria and therefore eliminate the repressive effect of factor H on complement activation (71–73). Recently, a CRP mutant capable of binding to immobilized factor H was evaluated for its ability to protect against late stage pneumococcal infection. The CRP mutant protected mice against infection regardless of the time of administration into mice (71–73). These data lead to the proposal that the PCh-independent mechanism first involves a structural change in CRP which is then followed by the interaction between structurally altered CRP and factor H-bound pneumococci. Once factor H on pneumococci is bound to structurally altered CRP, such pneumococci may not be resistant to complement attack any longer (1, 71–73).

Besides, factor H, *S. pneumoniae* have also been shown to recruit another complement inhibitor, C4b-binding protein (C4BP) via Hic that also recruits factor H (74, 75). Pneumococci also use another cell surface protein, enolase, to recruit C4BP (75). By recruiting C4BP, pneumococci are able to evade complement attack. We hypothesize that non-native CRP may also be protective against those pathogens which recruit C4BP for complement evasion: non-native CRP could bind to factor H/C4BP-coated pneumococci, and then the complex formed by CRP, factor H/C4BP, and Hic could activate the lectin pathway of complement and trigger killing of the pneumococci. The possibility cannot be ruled out that the PCh-independent mechanism may involve the binding of non-native CRP to pneumococcal surface proteins, as CRP has been shown to interact with several choline-binding proteins found on pneumococci in a Ca^{2+}-independent manner (76).

CRP AS AN ANTI-BACTERIAL MOLECULE

CRP binds to several pathogenic serotypes of *S. pneumoniae* (77–79) and binds more avidly to those strains which contain PCh in both cell wall and capsular polysaccharides, such as type 27 (80). CRP, like lectins, also reacts with polysaccharides that do not contain PCh, such as depyruvylated type-IV capsular polysaccharide prepared from type 27, in the presence of calcium, and probably the reaction is due to N-acetylgalactosamine in the polysaccharide (81–84). CRP appears to have opsonin properties; it causes agglutination and lysis of gram positive bacteria *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes,* and *Streptococcus agalactiae* (77, 78).

The anti-bacterial action of CRP is not limited to gram positive bacteria only. CRP also protects mice from the early stages of infection with *Salmonella enterica* serovar Typhimurium, which is a gram negative bacterium and to which CRP does not bind *in vitro* (85). But CRP has been shown to bind to *S. enterica* in the presence of serum (35). CRP also binds to *Haemophilus influenzae* (86). *H. influenzae* undergoes phase variation in expression of the PCh on the cell surface-exposed outer core of the LPS. PCh-positive variants are more sensitive to the bactericidal activity of human serum which requires the binding of serum CRP to whole bacteria with subsequent activation of complement (86–88). The ability of *H. influenzae* to vary PCh expression to zero may relate to its ability to cause invasive infection by evading attack by CRP (86). Mouse

models of *H. influenzae* infection have not been established yet to determine whether CRP protects against infection with *H. influenzae* (27). CRP also binds to *Neisseriae spp.* in a Ca^{2+}-dependent manner (89–91). PCh is present on the LPS of several species of commensal *Neisseriae* and, like *H. influenzae*, *Neisseriae* also undergo phase variation in expression of the PCh on their LPS (91). Mouse protection experiments have not been performed for *Neisseriae* either, employing native or non-native pentameric CRP.

Some experiments suggest a role of CRP in protecting animals against lethal toxicity of LPS, although the subject has been controversial (92–96). In the hemolymph of horseshoe crab, *Carcinoscorpius rotundicauda*, CRP was identified as the major LPS-binding protein in infections with *Pseudomonas aeruginosa* (97). CRP bound to all bacteria tested in the horseshoe crab hemolymph (35). The binding of CRP to LPS is indirect; a third molecule called galactose-binding protein (GBP) participates in bridging CRP and LPS (98). Upon binding to LPS, GBP interacts with CRP to form a pathogen-recognition complex, which helps to eliminate invading microbes (35, 98). Combined data raise the possibility that CRP functions as a general anti-bacterial molecule; CRP may require a change in its pentameric conformation and also seek help from other serum proteins to form pathogen-recognition complexes.

CRP AS AN ANTI-INFLAMMATORY MOLECULE

Native pentameric CRP can dissociate into mCRP via an intermediate non-native pentameric structure (50, 99–101). All three forms, native pentameric, non-native pentameric, and mCRP display different ligand recognition functions *in vitro* (2, 102–104). Under conditions of low pH, reduced calcium levels and oxidation-reduction, CRP is converted to a non-native conformation but remains pentameric (48–50, 105–107). When non-native CRP binds to a non-PCh ligand, it denatures further to mCRP. Similarly, when CRP binds to cell membranes, liposomes, and cell-derived microvesicles, it undergoes a structural change which involves spatial separation of the monomers from each other without disrupting the pentameric symmetry to form a transitional state CRP (108). The mechanism by which CRP recognizes membrane lipids and binds in a Ca^{2+}-independent manner depends on the combination of protein form, lipid composition, and membrane shape (109, 110). Surface-immobilization of CRP generates a preservable intermediate with dual antigenicity expression of both CRP and mCRP. The intermediate exhibits modified bioactivities, such as a high affinity with solution-phase proteins (107). It has

been shown that mCRP but not CRP is the major isoform present in local inflammatory lesions (111). Since mCRP is insoluble, it is considered a tissue-bound form of CRP. Thus, an intermediate stage of CRP structure seems to be responsible for anti-inflammatory host defense functions of CRP *in vivo*. Structural changes *in vivo* may be converting CRP into an anti-inflammatory molecule assuming that the ultimate pro-inflammatory by-product, mCRP, is continuously being removed. An intrinsically disordered region of amino acid residues 35–47 in CRP is responsible for mediating the interactions of mCRP with diverse ligands (112), and possibly also responsible for mediating the interactions of non-native pentameric CRP with diverse ligands (48–50).

CONCLUSIONS

While native CRP is protective only against early stage infection, non-native pentameric CRP is protective against both early stage and late stage infections in murine models of pneumococcal infection. Because non-native pentameric CRP displays PCh-independent anti-pneumococcal activity, it is quite possible that CRP functions as a general anti-bacterial molecule. Thus, pentameric CRP is an anti-inflammatory molecule.

A long-term goal could be to focus on the discovery and design of small-molecule compounds to target CRP, a compound that can change the structure of endogenous CRP so that the structurally altered CRP is capable of binding to factor H-bound pneumococci. A recent study showed that injections of sub-inhibitory concentrations of antibiotics enhanced the binding of CRP to three antibiotic-resistant *S. pneumoniae* strains in serum and enhanced antibody-dependent complement activation (113). Based on these findings, another goal could be to investigate the effects of combinations of non-native pentameric CRP with various antibiotics in pre-clinical studies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Agrawal A, Suresh MV, Singh SK, Ferguson DA Jr. The protective function of human C-reactive protein in mouse models of *Streptococcus pneumoniae* infection. *Endocr Metab Immune*

Disord Drug Targets (2008) 8:231–7. doi: 10.2174/1871530087868 48321
2. Agrawal A, Gang TB, Rusiñol AE. Recognition functions of pentameric C-reactive protein in cardiovascular disease. *Mediators Inflamm.* (2014) 2014:1–6. doi: 10.1155/2014/319215

3. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* (2005) 23:337–66. doi: 10.1146/annurev.immunol.23.021704.115756

4. Vilahur G, Badimon L. Biological actions of pentraxins. *Vasc Pharmacol.* (2015) 73:38–44. doi: 10.1016/j.vph.2015.05.001

5. Shrive AK, Cheetham GM, Holden D, Myles DA, Turnell WG, Volanakis JE, et al. Three dimensional structure of human C-reactive protein. *Nat Struct Biol.* (1996) 3:346–54. doi: 10.1038/nsb0496-346

6. Lv JM, Lü SQ, Liu ZP, Zhang J, Gao BX, Yao ZY, et al. Conformational folding and disulfide bonding drive distinct stages of protein structure formation. *Sci Rep.* (2018) 8:1494. doi: 10.1038/s41598-018-20014-y

7. Agrawal A, Lee S, Carson M, Narayana SVL, Greenhough TJ, Volanakis JE. Site-directed mutagenesis of the phosphocholine-binding site of human C-reactive protein: Role of Thr[76] and Trp[67]. *J Immunol.* (1997) 158:345–50.

8. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* (1999) 7:169–77. doi: 10.1016/S0969-2126(99)80023-9

9. Agrawal A, Simpson MJ, Black S, Carey MP, Samols D. A C-reactive protein mutant that does not bind to phosphocholine and pneumococcal C-polysaccharide. *J Immunol.* (2002) 169:3217–22. doi: 10.4049/jimmunol.169.6.3217

10. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin, and sphingomyelin. *J Immunol.* (1974) 112:2135–47.

11. Agrawal A, Volanakis JE. Probing the C1q-binding site on human C-reactive protein by site-directed mutagenesis. *J Immunol.* (1994) 152:5404–10.

12. Agrawal A, Shrive AK, Greenhough TJ, Volanakis JE. Topology and structure of the C1q-binding site on C-reactive protein. *J Immunol* (2001) 166:3998–400. doi: 10.4049/jimmunol.166.6.3998

13. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. *N Engl J Med.* (1995) 332:1280–4. doi: 10.1056/NEJM199505113321907

14. Van der Poll T, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* (2009) 374:1543–56. doi: 10.1016/S0140-6736(09)61114-4

15. Malley R, Anderson PW. Serotype-independent pneumococcal experimental vaccines that induce cellular as well as humoral immunity. *Proc Natl Acad Sci USA.* (2012) 109:3623–27. doi: 10.1073/pnas.1121383109

16. Tillett WS, Francis T Jr. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *J Exp Med.* (1930) 52:561–71. doi: 10.1084/jem.52.4.561

17. Kushner I. The phenomenon of the acute phase response. *Ann NY Acad Sci USA.* (1982) 389:39–48. doi: 10.1111/j.1749-6632.1982.tb22124.x

18. Song JY, Eun BW, Nahm MH. Diagnosis of pneumococcal pneumonia: current pitfalls and the way forward. *Infect Chemother.* (2013) 45:351–66. doi: 10.3947/ic.2013.45.4.351

19. Abernethy TJ, Avery OT. The occurrence during acute infections of a protein not normally present in the blood: I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C polysaccharide of pneumococcus. *J Exp Med.* (1941) 73:173–82.

20. Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med.* (1971) 136:612–4. doi: 10.3181/00379727-136-35323

21. Whitehead AS, Zahedi K, Rits M, Mortensen RF, Lelias JM. Mouse C-reactive protein: generation of cDNA clones, structural analysis, and induction of mRNA during inflammation. *Biochem J.* (1990) 266:283–90. doi: 10.1042/bj2660283

22. Yother J, Volanakis JE, Briles DE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in mice. *J Immunol.* (1982) 128:2374–6.

23. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. Role of the property of C-reactive protein to activate the classical pathway of complement in protecting mice from pneumococcal infection. *J Immunol.* (2006) 176:4369–74. doi: 10.4049/jimmunol.176.7.4369

24. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. Human C-reactive protein protects mice from *Streptococcus pneumoniae* infection without

binding to pneumococcal C-polysaccharide. *J Immunol.* (2007) 178:1158–63. doi: 10.4049/jimmunol.178.2.1158

25. Gang TB, Hammond DJ Jr, Singh SK, Ferguson DA Jr, Mishra VK, Agrawal A. The phosphocholine-binding pocket on C-reactive protein is necessary for initial protection of mice against pneumococcal infection. *J Biol Chem.* (2012) 287:43116–25. doi: 10.1074/jbc.M112.427310

26. Gang TB, Hanley GA, Agrawal A. C-reactive protein protects mice against pneumococcal infection via both phosphocholine-dependent and phosphocholine-independent mechanisms. *Infect Immun.* (2015) 83:1845–52. doi: 10.1128/IAI.03058-14

27. Simons JP, Loeffler JM, Al-Shawi R, Ellmerich S, Hutchinson WL, Tennent GA, et al. C-reactive protein is essential for innate resistance to pneumococcal infection. *Immunology* (2014) 142:414–20. doi: 10.1111/imm.12266

28. Szalai AJ, Briles DE, Volanakis JE. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in transgenic mice. *J Immunol.* (1995) 155:2557–63.

29. Nakayama S, Gewurz H, Holzer T, Du Clos TW, Mold C. The role of the spleen in the protective effect of C-reactive protein in *Streptococcus pneumoniae* infection. *Clin Exp Immunol.* (1983) 54:319–26.

30. Fox KL, Li J, Schweda EKH, Vitiazeva V, Makepeace K, Jennings MP, et al. Duplicate copies of lic1 direct the addition of multiple phosphocholine residues in the lipopolysaccharide of *Haemophilus influenzae. Infect Immun.* (2008) 76:588–600. doi: 10.1128/IAI.00748-07

31. Mukerji R, Mirza S, Roche AM, Widener RW, Croney CM, Rhee D-K, et al. Pneumococcal surface protein A inhibits complement deposition on the pneumococcal surface by competing with the binding of C-reactive protein to cell-surface phosphocholine. *J Immunol.* (2012) 189:5327–35. doi: 10.4049/jimmunol.1201967

32. Sanchez CJ, Kumar N, Lizcano A, Shivshankar P, Dunning Hotopp JC, Jorgensen JH, et al. *Streptococcus pneumoniae* in biofilms are unable to cause invasive disease due to altered virulence determinant production. *PLoS ONE* (2011) 6:e28738. doi: 10.1371/journal.pone.0028738

33. Domenech M, Ramos-Sevillano E, García E, Moscoso M, Yuste J. Biofilm formation avoids complement immunity and phagocytosis of *Streptococcus pneumoniae. Infect Immun.* (2013) 81:2606–15. doi: 10.1128/IAI.00491-13

34. Mold C, Rodic-Polic B, Du Clos TW. Protection from *Streptococcus pneumoniae* infection by C-reactive protein and natural antibody requires complement but not Fc gamma receptors. *J Immunol.* (2002) 168:6375–81. doi: 10.4049/jimmunol.168.12.6375

35. Ng PML, Le Saux A, Lee CM, Tan NS, Lu J, Thiel S, et al. C-reactive protein collaborates with plasma lectins to boost immune response against bacteria. *EMBO J.* (2007) 26:3431–40. doi: 10.1038/sj.emboj.7601762

36. Tanio M, Wakamatsu K, Kohno T. Binding site of C-reactive protein on M-ficolin. *Mol Immunol.* (2009) 47:215–21. doi: 10.1016/j.molimm.2009.09.032

37. Zhang J, Koh J, Lu J, Thiel S, Leong BSH, Sethi S, et al. Local inflammation induces complement crosstalk which amplifies the antimicrobial response. *PLoS Pathog.* (2009) 5:e1000282. doi: 10.1371/journal.ppat.1000282

38. Zhang J, Yang L, Ang Z, Yoong SL, Tran TTT, Anand GS, et al. Secreted M-ficolin anchors onto monocyte transmembrane G protein-coupled receptor 43 and cross talks with plasma C-reactive protein to mediate immune signaling and regulate host defense. *J Immunol.* (2010) 185:6899–910. doi: 10.4049/jimmunol.1001225

39. Ma YJ, Lee BL, Garred P. An overview of the synergy and crosstalk between pentraxins and collectins/ficolins: their functional relevance in complement activation. *Exp Mol Med.* (2017) 49:e320. doi: 10.1038/emm.2017.51

40. Vassal-Stermann E, Lacroix M, Gout E, Laffly E, Pedersen CM, Martin L, et al. Human L-ficolin recognizes phosphocholine moieties of pneumococcal teichoic acid. *J Immunol.* (2014) 193:5699–708. doi: 10.4049/jimmunol.1400127

41. Yoshimoto R, Fujita Y, Kakino A, Iwamoto S, Takaya T, Sawamura T. The discovery of LOX-1, its ligands and clinical significance. *Cardiovasc Drugs Ther.* (2011) 25:379–91. doi: 10.1007/s10557-011-6324-6

42. Fujita Y, Yamaguchi S, Kakino A, Iwamoto S, Yoshimoto R, Sawamura T. Lectin-like oxidized LDL receptor 1 is involved in CRP-mediated complement activation. *Clin Chem.* (2011) 57:1398–405. doi: 10.1373/clinchem.2011.168625

43. Zipfel PF, Hallström T, Hammerschmidt S, Skerka C. The complement fitness factor H: role in human diseases and for immune escape of pathogens, like pneumococci. *Vaccine* (2008) 26:I67–74. doi: 10.1016/j.vaccine.2008.11.015

44. Kopp A, Hebecker M, Svobodová E, Józsi M. Factor H: a complement regulator in health and disease, and a mediator of cellular interactions. *Biomolecules* (2012) 2:46–75. doi: 10.3390/biom2010046

45. Meri S, Pangburn MK. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. *Proc Natl Acad Sci USA.* (1990) 87:3982–6. doi: 10.1073/pnas.87.10.3982

46. Janulczyk R, Iannelli F, Sjoholm AG, Pozzi G, Bjorck L. Hic, a novel surface protein of *Streptococcus pneumoniae* that interferes with complement function. *J Biol Chem.* (2000) 275:37257–63. doi: 10.1074/jbc.M004572200

47. Jarva H, Janulczyk R, Hellwage J, Zipfel PF, Björck L, Meri S. *Streptococcus pneumoniae* evades complement attack and opsonophagocytosis by expressing the *pspC* locus-encoded Hic protein that binds to short consensus repeats 8-11 of factor H. *J Immunol.* (2002) 168:1886–94. doi: 10.4049/jimmunol.168.4.1886

48. Hammond DJ Jr, Singh SK, Thompson JA, Beeler BW, Rusiñol AE, Pangburn MK, et al. Identification of acidic pH-dependent ligands of pentameric C-reactive protein. *J Biol Chem.* (2010) 285:36235–44. doi: 10.1074/jbc.M110.142026

49. Singh SK, Thirumalai A, Hammond DJ Jr, Pangburn MK, Mishra VK, Johnson DA, et al. Exposing a hidden functional site of C-reactive protein by site-directed mutagenesis. *J Biol Chem.* (2012) 287:3550–8. doi: 10.1074/jbc.M111.310011

50. Singh SK, Thirumalai A, Pathak A, Ngwa DN, Agrawal A. Functional transformation of C-reactive protein by hydrogen peroxide. *J Biol Chem.* (2017) 292:3129–36. doi: 10.1074/jbc.M116.773176

51. Hakobyan S, Harris CL, van den Berg CW, Fernandez-Alonso MC, de Jorge EG, de Cordoba SR, et al. Complement factor H binds to denatured rather than to native pentameric C-reactive protein. *J Biol Chem.* (2008) 283:30451–60. doi: 10.1074/jbc.M803648200

52. Okemefuna AI, Nan R, Miller A, Gor J, Perkins SJ. Complement factor H binds at two independent sites to C-reactive protein in acute phase concentrations. *J Biol Chem.* (2010) 285:1053–65. doi: 10.1074/jbc.M109.044529

53. Jarva H, Jokiranta TS, Hellwage J, Zipfel PF, Meri S. Regulation of complement activation by C-reactive protein: targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8-11. *J Immunol* (1999) 163:3957–62.

54. Sjöberg AP, Trouw LA, Clark SJ, Sjölander J, Heinegård D, Sim RB, et al. The factor H variant associated with age-related macular degeneration (His-384) and the non-disease-associated form bind differentially to C-reactive protein, fibromodulin, DNA, and necrotic cells. *J Biol Chem.* (2007) 282:10894–900. doi: 10.1074/jbc.M610256200

55. Mihlan M, Stippa S, Józsi M, Zipfel PF. Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death Differ.* (2009) 16:1630–40. doi: 10.1038/cdd.2009.103

56. Clark SJ, Higman VA, Mulloy B, Perkins SJ, Lea SM, Sim RB, et al. His-384 allotypic variant of factor H associated with age-related macular degeneration has different heparin binding properties from the non-disease-associated form. *J Biol Chem.* (2006) 281:24713–20. doi: 10.1074/jbc.M605083200

57. Laine M, Jarva H, Seitsonen S, Haapasalo K, Lehtinen MJ, Lindeman N, et al. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. *J Immunol.* (2007) 178:3831–6. doi: 10.4049/jimmunol.178.6.3831

58. Herbert AP, Deakin JA, Schmidt CQ, Blaum BS, Egan C, Ferreira VP, et al. Structure shows that a glycosaminoglycan and protein recognition site in factor H is perturbed by age-related macular degeneration-linked single nucleotide polymorphism. *J Biol Chem.* (2007) 282:18960–8. doi: 10.1074/jbc.M609636200

59. Lauer N, Mihlan M, Hartmann A, Schlötzer-Schrehardt U, Keilhauer C, Scholl HPN, et al. Complement regulation at necrotic cell lesions is impaired by the age-related macular degeneration-associated factor-H His[402] risk variant. *J Immunol.* (2011) 187:4374–83. doi: 10.4049/jimmunol.1002488

60. Molins B, Fuentes-Prior P, Adán A, Antón R, Arostegui JI, Yagüe J, et al. Complement factor H binding of monomeric C-reactive protein downregulates proinflammatory activity and is impaired with at risk polymorphic CFH variants. *Sci Rep.* (2016) 6:22889. doi: 10.1038/srep22889

61. Mold C, Gewurz H. Inhibitory effect of C-reactive protein on alternative C pathway activation by liposomes and *Streptococcus pneumoniae. J Immunol.* (1981) 127:2089–92.

62. Mold C, Kingzette M, Gewurz H. C-reactive protein inhibits pneumococcal activation of the alternative pathway by increasing the interaction between factor H and C3b. *J Immunol.* (1984) 133:882–5.

63. Suankratay C, Mold C, Zhang Y, Potempa LA, Lint TF, Gewurz H. Complement regulation in innate immunity and the acute phase response: inhibition of mannan-binding lectin-initiated complement cytolysis by C-reactive protein (CRP). *Clin Exp Immunol.* (1998) 113:353–9. doi: 10.1046/j.1365-2249.1998.00663.x

64. Zhang Y, Suankratay C, Zhang XH, Lint TF, Gewurz H. Lysis via the lectin pathway of complement activation: minireview and lectin pathway enhancement of endotoxin-initiated hemolysis. *Immunopharmacology* (1999) 42:81–90. doi: 10.1016/S0162-3109(99)00029-6

65. Hebecker M, Okemefuna AI, Perkins SJ, Mihlan M, Huber-Lang M, Józsi M. Molecular basis of C-reactive protein binding and modulation of complement activation by factor H-related protein 4. *Mol Immunol.* (2010) 47:1347–55. doi: 10.1016/j.molimm.2009.12.005

66. Csincsi ÁI, Kopp A, Zöldi M, Bánlaki Z, Uzonyi B, Hebecker M, et al. Factor H–related protein 5 interacts with pentraxin 3 and the extracellular matrix and modulates complement activation. *J Immunol* (2015) 194:4963–73. doi: 10.4049/jimmunol.1403121

67. Csincsi ÁI, Szabó Z, Bánlaki Z, Uzonyi B, Cserhalmi M, Kárpáti É, et al. FHR-1 binds to C-reactive protein and enhances rather than inhibits complement activation. *J Immunol.* (2017) 199:292–303. doi: 10.4049/jimmunol.1600483

68. Swinkels M, Zhang JH, Tilakaratna V, Black G, Perveen R, McHarg S, et al. C-reactive protein and pentraxin-3 binding of factor H-like protein 1 differs from complement factor H: implications for retinal inflammation. *Sci Rep.* (2018) 8:1643. doi: 10.1038/s41598-017-18395-7

69. Roy N, Ohtani K, Matsuda Y, Mori K, Hwang I, Suzuki Y, et al. Collectin CL-P1 utilizes C-reactive protein for complement activation. *Biochim Biophys Acta* (2016) 1860:1118–28. doi: 10.1016/j.bbagen.2016.02.012

70. Roy N, Ohtani K, Hidaka Y, Amano Y, Matsuda Y, Mori K, et al. Three pentraxins C-reactive protein, serum amyloid P component and pentraxin 3 mediate complement activation using collectin CL-P1. *Biochim Biophys Acta* (2017) 1861:1–14. doi: 10.1016/j.bbagen.2016.11.023

71. Ngwa DN. *Comparison of Anti-pneumococcal Functions of Native and Modified Forms of C-Reactive Protein.* Dissertation/Master's thesis. Johnson City, TN: East Tennessee States University (2016).

72. Ngwa DN, Gang TB, Singh SK, Agrawal A. Engineered C-reactive protein with improved protective action against pneumococcal infection. *J Immunol.* (2016) 196:63.

73. Ngwa DN, Singh SK, Gang TB, Agrawal A. A C-reactive protein-based strategy to reduce antibiotic dosing. *J Immunol.* (2018) 200:170.

74. Sjöberg AP, Trouw LA, McGrath FDG, Hack CE, Blom AM. Regulation of complement activation by C-reactive protein: targeting of the inhibitory activity of C4b-binding protein. *J Immunol.* (2006) 176:7612–20. doi: 10.4049/jimmunol.176.12.7612

75. Agarwal V, Hammerschmidt S, Malm S, Bergmann S, Riesbeck K, Blom AM. Enolase of *Streptococcus pneumoniae* binds human complement inhibitor C4b-binding protein and contributes to complement evasion. *J Immunol.* (2012) 189:3575–84. doi: 10.4049/jimmunol.1102934

76. Frolet C, Beniazza M, Roux L, Gallet B, Noirclerc-Savoye M, Vernet T, et al. New adhesin functions of surface-exposed pneumococcal proteins. *BMC Microbiol.* (2010) 10:190. doi: 10.1186/1471-2180-10-190

77. Kindmark CO. *In vitro* binding of human C-reactive protein by some pathogenic bacteria and zymosan. *Clin Exp Immunol.* (1972) 11:283–9.

78. Mold C, Rodgers CP, Kaplan RL, Gewurz H. Binding of human C-reactive protein to bacteria. *Infect Immun.* (1982) 38:392–5.

79. De Beaufort AJ, Langermans JAM, Matze-van der Lans AM, Hiemstra PS, Vossen JM, van Furth R. Difference in binding of killed and live *Streptococcus pneumoniae* serotypes by C-reactive protein. *Scand J Immunol.* (1997) 46:597–600. doi: 10.1046/j.1365-3083.1997.d01-171.x

80. Edwards KM, Gewurz H, Lint TF Mold C. A role for C-reactive protein in the complement-mediated stimulation of human neutrophils by type 27 Streptococcus pneumoniae. *J Immunol.* (1982) 128:2493–6.

81. Higginbotham J, Heidelberger M, Gotschlich E. Degradation of a pneumococcal type-specific polysaccharide with exposure of group specificity. *Proc Natl Acad Sci USA.* (1970) 67:138–42. doi: 10.1073/pnas.67.1.138

82. Heidelberger M, Gotschlich EC, Higginbotham JD. Inhibition experiments with pneumococcal C and depyruvylated type-IV polysaccharides. *Carbohydr Res.* (1972) 22:1–4. doi: 10.1016/S0008-6215(00)85719-5

83. Köttgen E, Hell B, Kage A, Tauber R. Lectin specificity and binding characteristics of human C-reactive protein. *J Immunol.* (1992) 149:445–53.

84. Lee RT, Lee YC. Carbohydrate ligands of human C-reactive protein: binding of neoglycoproteins containing galactose-6-phosphate and galactose-terminated disaccharide. *Glycoconj J.* (2006) 23:317–27. doi: 10.1007/s10719-006-6173-x

85. Szalai AJ, VanCott JL, McGhee JR, Volanakis JE, Benjamin WH Jr. Human C-reactive protein is protective against fatal *Salmonella enterica* serovar typhimurium infection in transgenic mice. *Infect Immun.* (2000) 68:5652–6. doi: 10.1128/IAI.68.10.5652-5656.2000

86. Weiser JN, Pan N, McGowan KL, Musher D, Martin A, Richards J. Phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. *J Exp Med.* (1998) 187:631–40. doi: 10.1084/jem.187.4.631

87. Lysenko E, Richards JC, Cox AD, Stewart A, Martin A, Kapoor M, et al. The position of phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* affects binding and sensitivity to C-reactive protein-mediated killing. *Mol Microbiol.* (2000) 35:234–45. doi: 10.1046/j.1365-2958.2000.01707.x

88. Weiser JN, Pan N. Adaptation of *Haemophilus influenzae* to acquired and innate humoral immunity based on phase variation of lipopolysaccharide. *Mol Microbiol.* (1998) 30:767–75. doi: 10.1046/j.1365-2958.1998.01108.x

89. Serino L, Virji M. Genetic and functional analysis of the phosphorylcholine moiety of commensal *Neisseria* lipopolysaccharide. *Mol Microbiol.* (2002) 43:437–48. doi: 10.1046/j.1365-2958.2002.02755.x

90. Casey R, Newcombe J, McFadden J, Bodman-Smith KB. The acute-phase reactant C-reactive protein binds to phosphorylcholine-expressing *Neisseria meningitidis* and increases uptake by human phagocytes. *Infect Immun.* (2008) 76:1298–304. doi: 10.1128/IAI.00741-07

91. Serino L, Virji M. Phosphorylcholine decoration of lipopolysaccharide differentiates commensal *Neisseriae* from pathogenic strains: identification of licA-type genes in commensal *Neisseriae. Mol Microbiol.* (2000) 35:1550–9. doi: 10.1046/j.1365-2958.2000.01825.x

92. Xia D, Samols D. Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. *Proc Natl Acad Sci USA.* (1997) 94:2575–80. doi: 10.1073/pnas.94.6.2575

93. Noursadeghi M, Bickerstaff MCM, Gallimore JR, Herbert J, Cohen J, Pepys MB. Role of serum amyloid P component in bacterial infection: Protection of the host or protection of the pathogen. *Proc Natl Acad Sci USA.* (2000) 97:14584–9. doi: 10.1073/pnas.97.26.14584

94. Chae MR, Park BH, Kim JS, Rho HW, Park JW, Kim HR. Protective effect of C-reactive protein against the lethality induced by *Vibrio vulnificus* lipopolysaccharide. *Microbiol Immunol.* (2000) 44:335–40. doi: 10.1111/j.1348-0421.2000.tb02503.x

95. Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with FcγR. *J Immunol.* (2002) 169:7019–25. doi: 10.4049/jimmunol.169.12.7019

96. Hirschfield GM, Herbert J, Kahan MC, Pepys MB. Human C-reactive protein does not protect against acute lipopolysaccharide challenge in mice. *J Immunol.* (2003) 171:6046–51. doi: 10.4049/jimmunol.171.11.6046

97. Ng PML, Jin Z, Tan SSH, Ho B, Ding JL. C-reactive protein: a predominant LPS-binding acute phase protein responsive to *Pseudomonas* infection. *J Endotoxin Res.* (2004) 10:163–74. doi: 10.1177/09680519040100030301

98. Low DHP, Frecer V, Le Saux A, Srinivasan GA, Ho B, Chen J, et al. Molecular interfaces of the galactose-binding protein tectonin domains in host-pathogen interaction. *J Biol Chem.* (2010) 285:9898–907. doi: 10.1074/jbc.M109.059774

99. Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, et al. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: *in vivo* proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation* (2014) 130:35–50. doi: 10.1161/CIRCULATIONAHA.113.007124

100. Braig D, Nero TL, Koch HG, Kaiser B, Wang X, Thiele JR, et al. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. *Nat Commun.* (2017) 8:14188. doi: 10.1038/ncomms14188

101. McFadyen JD, Kiefer J, Braig D, Loseff-Silver J, Potempa LA, Eisenhardt SU, et al. Dissociation of C-reactive protein localizes and amplifies inflammation: evidence for a direct biological role of C-reactive protein and its conformational changes. *Front Immunol.* (2018) 9:1351. doi: 10.3389/fimmu.2018.01351

102. Suresh MV, Singh SK, Agrawal A. Interaction of calcium-bound C-reactive protein with fibronectin is controlled by pH: *in vivo* implications. *J Biol Chem.* (2004) 279:52552–57. doi: 10.1074/jbc.M409054200

103. Salazar J, Martínez MS, Chávez-Castillo M, Núñez V, Añez R, Torres Y, et al. C-reactive protein: an in-depth look into structure, function, and regulation. *Int Sch Res Not.* (2014) 2014:653045. doi: 10.1155/2014/653045

104. Wu Y, Potempa LA, El Kebir D, Filep JG. C-reactive protein and inflammation: conformational changes affect function. *Biol Chem.* (2015) 396:1181–97. doi: 10.1515/hsz-2015-0149

105. Wang MY, Ji SR, Bai CJ, El Kebir D, Li HY, Shi JM, et al. A redox switch in C-reactive protein modulates activation of endothelial cells. *FASEB J.* (2011) 25:3186–96. doi: 10.1096/fj.11-182741

106. Kenawy HI, Boral I, Bevington A. Complement-coagulation cross-talk: a potential mediator of the physiological activation of complement by low pH. *Front Immunol.* (2015) 6:1–10. doi: 10.3389/fimmu.2015.00215

107. Lv JM, Wang MY. *In vitro* generation and bioactivity evaluation of C-reactive protein intermediate. *PLoS ONE* (2018) 13:e0198375. doi: 10.1371/journal.pone.0198375

108. Ji SR, Wu Y, Zhu L, Potempa LA, Sheng FL, Lu W, et al. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP(m). *FASEB J.* (2007) 21:284–94. doi: 10.1096/fj.06-6722com

109. Alnaas AA, Moon CL, Alton M, Reed SM, Knowles MK. Conformational changes in C-reactive protein affect binding to curved membranes in a lipid bilayer model of the apoptotic cell surface. *J Phys Chem B.* (2017) 121:2631–9. doi: 10.1021/acs.jpcb.6b11505

110. Goda T, Miyahara Y. Specific binding of human C-reactive protein towards supported monolayers of binary and engineered phospholipids. *Colloids Surfaces B Biointerfaces* (2018) 161:662–9. doi: 10.1016/j.colsurfb.2017.11.036

111. Shi P, Li X-X, Zhu W, Yang H, Dong C, Li X-M. Immunohistochemical staining reveals C-reactive protein existing predominantly as altered conformation forms in inflammatory lesions. *Acta Biol Hung.* (2014) 65:265–73. doi: 10.1556/ABiol.65.2014.3.3

112. Li HY, Wang J, Meng F, Jia ZK, Su Y, Bai QF, et al. An intrinsically disordered motif mediates diverse actions of monomeric C-reactive protein. *J Biol Chem.* (2016) 291:8795–804. doi: 10.1074/jbc.M115.695023

113. Ramos-Sevillano E, Rodríguez-Sosa C, Cafini F, Giménez MJ, Navarro A, Sevillano D, et al. Cefditoren and ceftriaxone enhance complement-mediated immunity in the presence of specific antibodies against antibiotic-resistant pneumococcal strains. *PLoS ONE* (2012) 7:e44135. doi: 10.1371/journal.pone.0044135