Published in final edited form as:
Endocr Metab Immune Disord Drug Targets. 2008 December ; 8(4): 231–237.

The Protective Function of Human C-reactive Protein in Mouse Models of *Streptococcus pneumoniae* Infection

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Abstract

Human C-reactive protein (CRP), injected intravenously into mice or produced inside mice by a human transgene, protects mice from death following administration of lethal numbers of *Streptococcus pneumoniae*. The protective effect of CRP is due to reduction in the concentration of bacteria in the blood. The exact mechanism of CRP-dependent killing of pneumococci and the partners of CRP in this process are yet to be defined. The current efforts to determine the mechanism of action of CRP in mice are directed by four known *in vitro* functions of CRP: 1. the ability of pneumococcal C-polysaccharide-complexed CRP to activate complement pathways, 2. the ability of CRP to bind to Fcγ receptors on phagocytic cells, 3. the ability of CRP to bind to immobilized complement regulator protein factor H which can also be present on pneumococci, and, 4. the ability of CRP to interact with dendritic cells. CRP-treated dendritic cells may well be as host-defensive as CRP alone. An interesting condition for the protective function of CRP is that CRP must be given to mice within a few hours of the administration of pneumococci. CRP does not protect mice if given later, suggesting that CRP works prophylactically but not as a treatment for infection. However, full knowledge of CRP may lead to the development of CRP-based treatment strategies to control pneumococcal infection. Also, because CRP deficiency in humans has not yet been reported, it becomes important to investigate the deficiency of the mechanism of action of CRP in CRP-positive individuals.

Keywords

C-reactive protein; complement system; dendritic cells; factor H; Fcγ receptors; lectin pathway; phagocytosis; phosphocholine; pneumococci

Introduction

C-reactive protein (CRP) was discovered in the serum of a patient infected with *Streptococcus pneumoniae*. In 1920s, researchers in Oswald Theodore Avery's laboratory were fractionating the materials which they had isolated from *S. pneumoniae*. They found that a substance present in fraction C reacted with and precipitated a protein present in sera obtained from pneumonia patients during acute illness. The reactive substance in fraction C was subsequently named

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pneumococcal C-polysaccharide (PnC) and the reactive protein in the patients'sera was named CRP [1-3].

S. pneumoniae is a Gram-positive bacterium that colonizes the upper respiratory tract. It is the most common pathogen that causes community-acquired pneumonia and is also a significant cause of septicemia and meningitis [4-9]. Systemic pneumococcal infection raises serum CRP level by upto 1000-fold [1-3]. The increase in the concentration of CRP in the serum is due to increased biosynthesis of CRP in the hepatocytes in response to inflammatory cytokines [10-12]. The serum CRP level is considered a useful marker of pneumonia [13,14]. Despite the observations that pneumococcal infection raises serum CRP levels, that CRP appears in the blood before the induction of a primary antibody response, and that CRP binds to PnC, it is still unclear what *in vivo* CRP does to pneumococci in humans. In mouse models of pneumococcal infection, CRP has been shown to be protective, that is, CRP decreases bacteremia and increases survival of infected mice. Here, we review the various directions taken to explore the mechanism of the protective function of CRP against pneumococcal infection, the biological implications of knowing the mechanism of action of CRP, and the limitations of using mice as an animal model to understand the functions of CRP in humans [also see some previous CRP reviews: 15-23].

Binding of CRP to Pneumococci

CRP binds to several serotypes of *S. pneumoniae* including pathogenic strains, such as types 3, 4, 6, 14, 19, 23 and 27, and the nonpathogenic strain R36a. CRP binds to both heat-killed and live virulent pneumococci. Using type 3 pneumococci, it has been shown that CRP binds to pneumococci *in vitro* in both human and mouse sera [24-29]. The best characterized ligand of CRP in pneumococci is PnC which is a component of the pneumococcal cell wall. The binding of CRP to PnC occurs through phosphocholine (PCh) residues which are present in each repeating pentasaccharide unit of PnC [30,31]. CRP binds more avidly to pneumococcal strains which contain PCh in both cell wall and capsular polysaccharides, such as type 27 [27,32]. CRP has lectin-like properties because it also reacts with polysaccharides that do not contain PCh, such as depyruvylated capsular polysaccharide prepared from type 27, and it binds to polysaccharides containing only galactose or N-acetylglucosamine [33-37]. In addition, CRP has also been shown to bind to phosphoethanolamine-containing substances [38-41]. The binding of CRP to PCh, PnC, phosphoethanolamine, carbohydrates or whole pneumococci requires calcium and occurs in both calcium-containing buffers and growth media [3,29,41-43]. These recognition functions of CRP are relevant because it is believed that the protective function of CRP against pneumococcal infection in experimental mice begins with the binding of CRP to pneumococci.

CRP is a homopentameric protein with a PCh-binding site located on each subunit. The crystal structure of the CRP-PCh complex and the mutational analyses of CRP have revealed the amino acids in CRP which connect CRP to PCh. The amino acid Glu81 in the PCh-binding hydrophobic pocket of CRP interacts with the positively charged nitrogen atom of choline in PCh. The amino acid Phe66 provides hydrophobic interactions with the three methyl groups of choline in PCh. The amino acid Thr76 is critical for creating the appropriately sized pocket to accommodate PCh. The phosphate group of PCh directly coordinates with the two calcium ions bound to CRP. Recently, a CRP mutant, called F66A/E81A, in which both Phe66 and Glu81 are mutated to Ala, has been engineered. The F66A/E81A CRP does not bind to immobilized PCh, PnC or live pneumococci *in vitro* and has been used in the experiments aimed at determining the functions of CRP in a mouse model of pneumococcal infection [29, 40,44-46].

The Fate of CRP-Complexed Pneumococci

CRP-PnC complexes activate the complement system *in vitro* in both human and mouse sera. The fate of CRP-complexed pneumococci *in vivo* is thought to depend upon the ability of the CRP-complexed pneumococci to activate complement. The activated complement participates in the elimination of bacteria. There are three pathways of complement activation: classical pathway, alternative pathway, and lectin pathway [47]. The classical pathway is activated by antigen-antibody complexes and also by pathogen-bound lectin known as SIGN-R1 which is expressed on marginal zone macrophages within the spleen. Thus, the classical pathway can be activated in both antibody-dependent and antibody-independent manners. The alternative pathway is activated by the pathogens themselves. The lectin pathway is activated by mannan-bearing pathogens after they bind to mannose-binding lectin (MBL) in the serum.

In human serum, like antigen-antibody complexes, PnC-complexed CRP binds to C1q, the first component of the classical pathway, and activates the classical pathway of complement [48-50]. CRP bound to heat-killed pneumococci types 3, 6 and 27, and R36a has also been shown to activate the classical pathway [26]. The C1q-binding site on CRP and the CRP-binding site on C1q have been reported. It is the globular region of C1q that participates in CRP-C1q interaction [51-56]. The most critical amino acid in the C1q-binding site of CRP for CRP-C1q interaction is Tyr175 [57,58]. CRP-mediated activation of the classical pathway generates opsonins which target the CRP-bound substances for opsonophagocytosis [59,60]. In addition to activating the classical pathway, CRP can also activate the lectin pathway. It has been shown that CRP can bind to ficolins and activate the lectin pathway of complement [61].

In mouse serum, unlike in human serum, the activation of mouse complement by PnC-bound human CRP is not through the classical pathway, because human CRP does not bind to mouse C1q [58]. The pathway through which human CRP activates mouse complement is under investigation but it could be *via* the lectin pathway. The activation of different complement pathways by human CRP in human and mouse sera indicates species-specificity of CRP. However, the complement activation is the only known species-specific difference related to the effector functions of CRP. Nevertheless, the mouse model is not suitable for investigating any function of CRP that may depend upon CRP-mediated activation of the classical complement pathway in humans. The species-specific observations also provide a lesson concerning the problem of extrapolating *in vitro* properties of CRP to *in vivo* effects. It is important to establish the *in vitro* properties of CRP using mouse materials so that experiments to determine *in vivo* functions of CRP can be interpreted in mice.

Besides complement-dependent opsonophagocytosis of CRP-bound ligands, CRP has also been shown to promote complement-independent phagocytosis of pneumococci by leukocytes. Such phagocytosis-inducing activity of CRP was also thought to determine the fate of CRP-complexed pneumococci *in vivo.* However, it remains controversial that CRP alone is capable of promoting phagocytosis without a requirement for complement. It has been shown in one study that CRP is capable of promoting phagocytosis of those pneumococci to which it binds, such as the PCh-expressing pneumococci. CRP did not mediate phagocytosis of those pathogenic strains which had no PCh in their capsule. Prior binding of CRP to pneumococci seems to be a requirement for CRP-mediated enhancement of phagocytosis because just the addition of CRP to whole heparinized blood had little effect on the extent of phagocytosis. In another study, only in the presence of complement was CRP an effective opsonin for *S. pneumoniae* type 27 [26,28,32,62-66]. It is important to note, however, that complement-deficient sera have not yet been used in the phagocytosis assays. Neither mouse sera have been used in such assays. As discussed below, in addition to the activities of PnC-bound CRP, many

PnC-independent activities of CRP have also been implicated in determining the fate of CRP-bound pneumococci *in vivo*.

CRP-Mediated Protection of Mice from Infection

CRP is an evolutionarily conserved protein; it is present in all vertebrates and some invertebrates [67-70]. However, CRP exhibits species-specificity in being a component of the acute phase response. In contrast to human CRP, mouse CRP is just a trace serum protein and not an acute phase protein. Therefore, from this angle, mouse is a convenient species to be used to investigate the *in vivo* functions of passively administered purified human CRP.

If an administered dose of pneumococci is not lethal in mice, then it is not lethal because mice can protect themselves from infection against a certain number of injected bacteria by using several components of the innate immune system. The components of innate immunity include the recognition of PnC by naturally occurring anti-pneumococcal IgM antibodies and the binding of capsular polysaccharide to the lectin SIGN-R1 expressed on splenic macrophages, which then lead to the activation of the classical complement pathway to provide defense against pneumococci. Even in the absence of specific acquired antibodies, the classical pathway has been shown to be the most important pathway for innate immunity to pneumococci [5,9, 47,71-76]. Thus, complement plays an essential role in controlling replication of pneumococci in the circulation.

On the other hand, if an administered dose of pneumococci is going to be a lethal dose, then mice require CRP as an additional host-defense component. Two types of mouse models have been used to investigate host-defense functions of CRP related to the protection of mice from pneumococcal infection. One model involved the intravenous administration of pure human CRP into mice. The other model was the mouse made transgenic for human CRP. In the first model, passively administered human CRP has been shown to be protective against lethal infection with virulent *S. pneumoniae* type 3 and 4 as determined by increased survival of and decreased bacteremia in infected mice. The protective activity of CRP can be demonstrated even in the immunodeficient *xid* mouse which has virtually no naturally occurring anti-pneumococcal antibodies. The minimum amount of CRP required to protect the mice, however, varied in different laboratories, probably reflecting differences in mouse strain and in the virulence of various pneumococcal preparations. Mice transgenic for human CRP were also protected from lethal pneumococcal infection and showed both decreased bacteremia and increased survival [29,58,77-79]. There are two routes of experimental pneumococcal infection in mice. Intranasal infection (pneumonia model) with pneumococci results in adhesion of the bacteria to the mucosa, a process that is dependent upon PCh present in PnC. Intravenous infection (septicemia or endotoxic shock model) is dependent upon the capsular polysaccharide. CRP has been shown to be protective against both intravenous and intranasal infections. However, it has not yet been elucidated whether the mechanism of action of CRP in protection against infection depends on the route of administration of pneumococci.

CRP protects mice from infection only when injected within the range of 6 h before to 2 h after injecting pneumococci into mice. Although protection requires the presence of CRP in the early stages of infection, the action of CRP is slow; the number of bacteria in the blood does not start decreasing until after CRP is cleared from the circulation. The effect of CRP is usually measurable only after a day of infection, although in one study, a much earlier effect of CRP on bacteremia was shown. The protective function of CRP was not observed when mice received CRP 36 h post-infection [29,58,77-81]. These findings suggest that CRP protects mice from being infected but does not cure mice with existing infection.

Mechanism of Protective Function of CRP in Mice: Requirement of Partners

The increased resistance to infection in CRP-treated mice is associated with reduction of bacteremia and the maintenance of reduced bacteremia. The mechanisms by which CRP reduces bacteremia remain undefined. How does CRP, directly or indirectly, act on the bacterial surfaces to kill them? Who may be the partners of CRP in carrying out the protective role? The current efforts to find answers to these questions are directed by the following four known *in vitro* functions of CRP: 1. the ability of PnC-complexed CRP to activate complement pathways, 2. the ability of CRP to bind to Fcγ receptors (FcγR) on phagocytic cells, 3. the ability of CRP to bind to immobilized complement regulator protein factor H which can also be present on pneumococci, and, 4. the ability of CRP to interact with dendritic cells (DC). Thus, the possible partners of CRP in executing the protective function of CRP are complement pathways, FcγRs, factor H and DCs, as discussed below.

Role of Complement

Employing complement component C3 knockout and cobra venom factor-treated decomplemented mice, it has been shown that a functioning complement system is required for full protective activity of CRP in mice against intravenous *S. pneumoniae* infection [72, 80-82]. Data obtained from complement C4 knockout mice indicated that the classical pathway or lectin pathway or both contribute to CRP-mediated protection. CRP, however, was capable of affording some degree of protection by itself. Based on the *in vitro* properties of CRP mentioned earlier, it was assumed that in mice, CRP was protective through a pathway in which CRP binds to exposed PnC on the bacterial surface; the liganded CRP activates complement through the classical pathway and bacteremia is reduced through complement-dependent opsonophagocytosis [58,80-82]. This assumption turned out to be incorrect because human CRP does not bind murine C1q and therefore cannot activate the mouse classical pathway. The characteristic of CRP to activate the classical complement pathway in human serum does not constitute the mechanism of CRP-mediated protection of mice from infection. Because ligand-complexed human CRP does activate mouse complement, although not through the classical pathway, it is possible that complement may be partnering with CRP in protecting mice from infection.

Other findings indicate that CRP-mediated protection of mice from infection may be totally independent of the ability of CRP to activate complement. First, the F66A/E81A mutant CRP, incapable of binding to *S. pneumoniae in vitro* and hence incapable of activating complement, has been used in the protection experiments [29,58]. Surprisingly, this mutant CRP also protected mice from infection just like the wild-type CRP did. Assuming that this mutant CRP did not bind to bacteria *in vivo*, then it means that CRP-mediated decrease in bacteremia and the resulting protection of mice do not need complement activated by CRP-complexes. Second, considering that the half-life of injected CRP in mice is only approximately 4 hours [83], it is evident that after a day of infection when the protective effect of CRP was measurable, CRP may not be present in the blood, again suggesting that the activation of complement by CRP-complexes may not be responsible for the protection of mice from infection.

As mentioned earlier, pneumococci can themselves activate complement: they activate the alternative pathway, they activate the classical pathway through SIGN-R1, and they also activate the classical pathway through antibodies if the antibodies are present. Pneumococci do not need CRP to activate complement. Then why should CRP work through complement? Why should CRP bind to pneumococci only for the purposes of activating complement? It has been hypothesized previously that complement serves as the first line of defense and that CRP protects mice from those bacteria that escape complement attack [29,58]. A definitive answer to these questions on the role of the property of pathogen-bound CRP to activate complement

in protection against pneumococcal infection in mice can be found by using a mutant CRP that does not activate mouse complement. Such a CRP mutant is not yet available.

Role of Fcγ Receptors

CRP binds to phagocytic cells, neutrophils, endothelial cells and DCs through the IgG receptors FcγRI (CD64) and FcγRIIa (CD32) expressed on their surface, and changes the functions of those cells [84-96]. CRP binds to both human and mouse FcγRs [97,98]. Thus, in addition to opsonization by activated complement fragments, CRP can also mediate direct phagocytosis of bacteria, by binding to FcγRs on phagocytic cells [21,92,96,98]. Many FcγR-mediated functions of CRP have been reported recently [99-102]. CRP has been shown to enhance cytokine responses to pneumococci through interactions with FcγRs [103]. However, the binding of CRP to FcγRs has been controversial for some time [104-106]. The exact physiological conditions in which this interaction can occur *in vivo* are unclear. The questions whether CRP is on the surface of FcγR-bearing cells *in vivo* or not, and whether the aggregation or modification of CRP is required for binding of CRP to FcγR-bearing cells or not, remain unanswered.

It has been shown that FcγR does not play a role in CRP-mediated protection of mice from pneumococcal infection. CRP decreases bacteremia in FcγR-deficient mice also and protects these mice against pneumococcal infection [19,72]. Another approach to determine the role of CRP-FcγR interaction in the protection of mice from pneumococcal infection is to use a CRP mutant incapable of binding to FcγRs. Using FcγRI, it has been shown that the region on the CRP molecule that binds C1q also provides the contact amino acids for FcγRs. These amino acids in CRP are Thr173, Asn186, Lys114, and Leu176. The binding sites on CRP for FcγR and for C1q are discrete but overlapping [107]. A CRP mutant incapable of binding to mouse FcγR has not yet been used in mouse studies.

Role of Factor H

Factor H is a regulator of the alternative pathway of complement. It protects host cells from complement attack by inhibiting the activation of the alternative pathway on host cells and on those pathogenic surfaces which are capable of binding to factor H [108]. CRP binds to factor H *in vitro* when either one is in the immobilized phase [59,109-114]. This interaction neither requires nor is inhibited by calcium. It has been suggested that CRP first binds to targets such as *S. pneumoniae* and subsequently limits excessive alternative pathway activation by recruiting factor H [115-117]. CRP has also been shown to modulate lectin pathway-dependent cytolysis by recruiting factor H [59,118,119],

The binding of CRP to factor H may contribute to the protective action of CRP in mice against pneumococcal infection. Initially, factor H binds to a protein called Hic (factor H-binding inhibitor of complement) present on the pneumococcal cell wall leading to inhibition of activation of the alternative pathway on the pneumococcal cell surface [120]. Thus, pneumococci use factor H to evade self-activated complement-mediated killing. Since factor H also binds CRP and since both CRP and Hic can bind to factor H simultaneously, it is possible that CRP may prevent bacteria from escaping attack by the alternative complement pathway. 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 activation of the alternative pathway. Alternatively, CRP could bind to factor H-coated pneumococci, and then the complex formed by CRP, factor H, and Hic could activate the lectin pathway of complement and trigger killing of the pneumococci. A factor H-binding site on CRP has been located which is different from the C1q-binding site [59,112]. Based on this information, we are generating a CRP mutant incapable of binding to factor H to examine the role of factor H in CRP-dependent protection of mice with pneumococcal infection.

It has been shown recently that the CRP mutant, F66A/E81A, which does not bind PnC or live virulent *S. pneumoniae in vitro*, also reduces bacteremia and protects mice against infection, indicating that the binding of CRP to pneumococci is not required for the protective effects of CRP [29,58]. However, it cannot yet be ruled out that mutant CRP may bind to pneumococci *in vivo*. The mutant CRP may bind to pneumococci indirectly through an intermediate (not directly through PnC) such as factor H in the mouse circulation and then protect mice from infection by the mechanism mentioned above. The ability of CRP to bind pneumococci through PnC may be important for preventing colonization of bacteria in the intranasal infection model. CRP has been shown to block the attachment of PCh-expressing bacteria to platelet-activating factor (PAF) receptors on human pharyngeal epithelial cells [7,121,122]. The PCh-binding ability of CRP was also found to be necessary for protection of mice from PAF challenge [123].

CRP was not found to be protective against pneumococcal infection in mice deficient in complement factor B, a component of the alternative pathway, indicating that the CRP-mediated protection *in vivo* requires the participation of the alternative pathway also [19]. Alternatively, the finding that CRP was not protective in factor B knockout mice also suggests that the pneumococci which are otherwise killed by the alternative pathway were not killed by CRP. This interpretation, in turn, supports the notion that CRP acts on only a certain variant or specific population of bacteria *in vivo*. Indeed, it has been shown that pneumococci undergo spontaneous phase variation between a transparent and an opaque colony phenotype; the latter being more virulent in a murine model of sepsis [124].

Role of Dendritic Cells

CRP binds to DCs [96,125-127]. As a result, CRP may alter differentiation, maturation, and functions of DCs such as the DC-induced activities of T cells. CRP-treated DCs have been found to have different functions than that of untreated DCs. It is not known, however, whether CRP is internalized by DCs as it has been reported for monocytic cells and endothelial cells. Upon binding to monocytic and endothelial cells, CRP is internalized and, in case of monocytic cells, it is also degraded [128,129]. It has recently been shown that CRP enhances uptake and presentation of pneumococcal antigens through FcγRs on DCs and stimulates protective adaptive immunity [96]. As reviewed previously, much evidence also indicates that CRP provides a link between innate and adaptive immunity and can modulate the functioning of the immune system [29,58,130-138]. For example, upon induction of CRP in mice transgenic for human CRP, T cells have been found that recognize CRP epitopes on thymic and splenic antigen-presenting cells (APC) [135]. It has also been shown that the depletion of Treg cells in MRL-lpr mice abrogates the protective effect of CRP on autoimmune nephritis indicating that CRP might interact with Treg cells which are known to influence the functions of DCs [136].

A new concept that has emerged recently is that CRP may contribute to the protection of mice from infection by acting directly on the effector cells of the immune system [29]. This concept is based on the findings that the protective effect of CRP in mice is a slow process [29], that a single early injection of CRP into mice is sufficient to protect mice from infection [80], that the complement pathways may not be acting with CRP as discussed above, and that the effect of CRP is measurable at a time when there is little CRP in the circulation. The spleen is known to be involved in the clearance of bacteria and is the possible site for the opsonic functions of CRP *in vivo* [47,80,139]. Thus, it has been proposed that APCs in the spleen take up CRP and present it in processed form on their surfaces. The APC exposing CRP peptides may recognize pathogenic bacterial polysaccharides (not necessarily only PCh) and act as a pattern-recognition protein for pathogen-associated molecular patterns and enhance cell-mediated cytotoxicity to reduce bacteremia, as described previously for toll-like receptors [140].

Adoptive transfer experiments using CRP-treated DCs need to be performed to investigate their effect in protecting mice against pneumococcal infection.

Conclusions and Future Plans

In addition to protecting mice from pneumococcal infection, CRP has also been shown to protect mice from infection with *Salmonella typhimurium*, a pathogen to which CRP binds in the presence of serum [60,141]. CRP also binds to *Haemophilus influenzae* and *Neisseriae spp*. [142,143]. More mouse protection experiments need to be performed to determine the range of pathogens against which CRP is protective. CRP has been shown to be an anti-atherosclerosis agent and it appears that CRP is also a general anti-microbial agent [144].

The mechanism of the protective function of CRP in mouse models of pneumococcal infection is not known. Originally, the complement-activating and the phagocytosis-stimulating properties of ligand-bound CRP were thought to determine the fate of CRP-complexed pneumococci *in vivo*. Recent data also suggest the participation of factor H-binding and DC-binding properties of CRP in mediating the *in vivo* host-defense functions of CRP. The generation of CRP mutants for use as investigative tools to explore the mechanism of action of CRP in mice is in progress in our laboratory. We seek a CRP mutant that does not activate mouse complement, a mutant that does not bind factor H, and a mutant that does not bind FcγRs. The knowledge of CRP mechanisms of action may lead us to develop CRP-based vaccination strategies to prevent, or even to treat, pneumococcal infection in humans.

Humans deficient in the ability to synthesize CRP have not been reported nor have mutations in the gene that would alter the amino acid sequence of the protein. CRP is present in everybody. However, it is not necessary that CRP functions similarly in every individual. Once the mechanism of action of CRP and the partners of CRP are known, then it would be important to focus on investigating the presence of the partners required for the actions of CRP in CRP-positive individuals and confirm that the CRP-dependent mechanisms operate equally in each individual.

Acknowledgments

This work was supported by a grant (R01HL071233) from the National Institutes of Health.

References

1. Tillett WS, Francis T Jr. J Exp Med 1930;52:561–571.
2. Tillett WS, Goebel WF, Avery OT. J Exp Med 1930;52:895–900.
3. Abernethy TJ, Avery OT. J Exp Med 1941;73:173–182.
4. Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS Jr, Swartz MN. N Engl J Med 1993;328:21–28. [PubMed: 8416268]
5. Tuomanen EI, Austrian R, Masure HR. N Engl J Med 1995;332:1280–1284. [PubMed: 7708073]
6. Lim WS, Macfarlane JT, Boswell TC, Harrison TG, Rose D, Leinonen M, Saikku P. Thorax 2001;56:296–301. [PubMed: 11254821]
7. Gould JM, Weiser JN. J Infect Dis 2002;186:361–371. [PubMed: 12134232]
8. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, Ngetsa C, Slack MP, Njenga S, Hart CA, Maitland K, English M, Marsh K, Scott JA. N Engl J Med 2005;352:39–47. [PubMed: 15635111]
9. Yuste J, Botto M, Bottoms SE, Brown JS. PLoS Pathog 2007;3:e120.doi:10.1371/journal.ppat.0030120
10. Voleti B, Agrawal A. J Immunol 2005;175:3386–3390. [PubMed: 16116232]
11. Singh PP, Voleti B, Agrawal A. J Immunol 2007;178:7302–7309. [PubMed: 17513780]

12. Cha-Molstad H, Young DP, Kushner I, Samols D. Mol Immunol 2007;44:2933–2942. [PubMed: 17335903]
13. Luna CM. Chest 2004;125:1192–1195. [PubMed: 15078723]
14. Chalmers JD, Singanayagam A, Hill AT. Am J Med 2008;121:219–225. [PubMed: 18328306]
15. Agrawal A. Mol Immunol 2005;42:927–930. [PubMed: 15829284]
16. Agrawal, A.; Singh, PP.; Bottazzi, B.; Garlanda, C.; Mantovani, A. Target Pattern Recognition in Innate Immunity. Kishore, U., editor. Landes Bioscience/Eurekah; USA: 2006. www.landesbioscience.com/iu/outputisbn.php?isbn=1-58706-199-6
17. Mortensen RF, Zhong W. J Leukoc Biol 2000;67:495–500. [PubMed: 10770281]
18. Volanakis JE. Mol Immunol 2001;38:189–197. [PubMed: 11532280]
19. Szalai AJ. Microbes Infect 2002;4:201–205. [PubMed: 11880053]
20. Black S, Kushner I, Samols D. J Biol Chem 2004;279:48487–48490. [PubMed: 15337754]
21. Marnell L, Mold C, Du Clos TW. Clin Immunol 2005;117:104–111. [PubMed: 16214080]
22. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Annu Rev Immunol 2005;23:337–366. [PubMed: 15771574]
23. Pepys MB. Clin Med 2007;7:562–578. [PubMed: 18193704]
24. Löfström G. Br J Exp Pathol 1944;25:21–26.
25. Kindmark CO. Clin Exp Immunol 1972;11:283–289. [PubMed: 4402700]
26. Mold C, Edwards KM, Gewurz H. Infect Immun 1982;37:987–992. [PubMed: 7129640]
27. Mold C, Rodgers CP, Kaplan RL, Gewurz H. Infect Immun 1982;38:392–395. [PubMed: 6815093]
28. De Beaufort AJ, Langermans JAM, Matze-van der Lans AM, Hiemstra PS, Vossen JM, Van Furth R. Scand J Immunol 1997;46:597–600. [PubMed: 9420623]
29. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. J Immunol 2007;178:1158–1163. [PubMed: 17202380]
30. Jennings HJ, Lugowski C, Young NM. Biochemistry 1980;19:4712–4719. [PubMed: 7426624]
31. Volanakis JE, Kaplan MH. Proc Soc Exp Biol Med 1971;136:612–614. [PubMed: 4395924]
32. Edwards KM, Gewurz H, Lint TF, Mold C. J Immunol 1982;128:2493–2496. [PubMed: 7077077]
33. Higginbotham JD, Heidelberger M, Gotschlich EC. Proc Natl Acad Sci USA 1970;67:138–142. [PubMed: 4394061]
34. Heidelberger M, Gotschlich EC, Higginbotham JD. Carbohyd Res 1972;22:1–4.
35. Volanakis JE, Narkates AJ. J Immunol 1981;126:1820–1825. [PubMed: 7217669]
36. Köttgen E, Hell B, Kage A, Tauber R. J Immunol 1992;149:445–453. [PubMed: 1624792]
37. Lee RT, Lee YC. Glycoconj J 2006;23:317–327. [PubMed: 16897175]
38. Oliveira EB, Gotschlich EC, Liu TY. J Immunol 1980;124:1396–1402. [PubMed: 6766973]
39. Schwalbe RA, Dahlbäck B, Coe JE, Nelsestuen GL. Biochemistry 1992;31:4907–4915. [PubMed: 1375509]
40. Agrawal A, Simpson MJ, Black S, Carey MP, Samols D. J Immunol 2002;169:3217–3222. [PubMed: 12218140]
41. Singh SK, Suresh MV, Prayther DC, Moorman JP, Rusinol AE, Agrawal A. J Immunol 2008;180:4316–4322. [PubMed: 18322245]
42. Mullenix MC, Mortensen RF. Mol Immunol 1994;8:615–622. [PubMed: 8196672]
43. Suresh MV, Singh SK, Agrawal A. J Biol Chem 2004;279:52552–52557. [PubMed: 15456743]
44. Shrive AK, Cheetham GMT, Holden D, Myles DAA, Turnell WG, Volanakis JE, Pepys MB, Bloomer AC, Greenhough TJ. Nature Struct Biol 1996;3:346–354. [PubMed: 8599761]
45. Thompson D, Pepys MB, Wood S. Structure 1999;7:169–177. [PubMed: 10368284]
46. Agrawal A, Lee S, Carson M, Narayana SVL, Greenhough TJ, Volanakis JE. J Immunol 1997;158:345–350. [PubMed: 8977209]
47. Roozendaal R, Carroll MC. Cell 2006;125:29–32. [PubMed: 16615887]
48. Kaplan MH, Volanakis JE. J Immunol 1974;112:2135–2147. [PubMed: 4151108]
49. Siegel J, Rent R, Gewurz H. J Exp Med 1974;140:631–647. [PubMed: 4472155]

50. Claus DR, Siegel J, Petras K, Osmand AP, Gewurz H. J Immunol 1977;119:187–192. [PubMed: 17637]
51. Agrawal A, Shrive AK, Greenhough TJ, Volanakis JE. J Immunol 2001;166:3998–4004. [PubMed: 11238646]
52. Gaboriaud C, Juanhuix J, Gruez A, Lacroix M, Darnault C, Pignol D, Verger D, Fontecilla-Camps JC, Arlaud GJ. J Biol Chem 2003;278:46974–46982. [PubMed: 12960167]
53. Kishore U, Ghai R, Greenhough TJ, Shrive AK, Bonifati DM, Gadjeva M, Waters P, Kojouharova MS, Chakraborty T, Agrawal A. Immunol Lett 2004;95:113–128. [PubMed: 15388251]
54. McGrath FDG, Brouwer MC, Arlaud GJ, Daha MR, Hack CE, Roos A. J Immunol 2006;176:2950–2957. [PubMed: 16493053]
55. Roumenina LT, Russeva MM, Zlatarova A, Ghai R, Kolev M, Olova N, Gadjeva M, Agrawal A, Bottazzi B, Mantovani A, Reid KBM, Kishore U, Kojouharova MS. Biochemistry 2006;45:4093–4104. [PubMed: 16566583]
56. Sjowall C, Wettero J, Bengtsson T, Askendal A, Almroth G, Skogh T, Tengvall P. Biochem Biophys Res Comm 2007;352:251–258. [PubMed: 17113035]
57. Agrawal A, Volanakis JE. J Immunol 1994;152:5404–5410. [PubMed: 8189060]
58. Suresh MV, Singh SK, Ferguson DA Jr, Agrawal A. J Immunol 2006;176:4369–4374. [PubMed: 16547275]
59. Berman S, Gewurz H, Mold C. J Immunol 1986;136:1354–1359. [PubMed: 3944459]
60. Mold C, Gewurz H, Du Clos TW. Immunopharmacology 1999;42:23–30. [PubMed: 10408362]
61. Ng PML, Le Saux A, Lee CM, Tan NS, Lu J, Thiel S, Ho B, Ding JL. EMBO J 2007;26:3431–3440. [PubMed: 17581635]
62. Hokama Y, Coleman MK, Riley RF. J Bacteriol 1962;83:1017–1024. [PubMed: 13908279]
63. Williams RC, Quie PG. J Immunol 1968;101:426–432. [PubMed: 4175475]
64. Kindmark CO. Clin Exp Immunol 1971;8:941–948. [PubMed: 4397484]
65. Mortensen RF, Osmand AP, Lint TF, Gewurz H. J Immunol 1976;117:774–781. [PubMed: 60448]
66. Mortensen RF, Duszkiewicz JA. J Immunol 1977;119:1611–1616. [PubMed: 915271]
67. Agrawal A, Mitra S, Ghosh N, Bhattacharya S. Indian J Exp Biol 1990;28:788–789. [PubMed: 2253974]
68. Kushner I. Ann N Y Acad Sci 1982;389:39–48. [PubMed: 7046585]
69. Pepys MB. Immunology 1979;37:637–641. [PubMed: 500121]
70. Whitehead AS, Zahedi K, Rits M, Mortensen RF, Lelias JM. Biochem J 1990;266:283–290. [PubMed: 2310378]
71. Briles DE, Nahm M, Schroer K, Davie J, Baker P, Kearney J, Barletta R. J Exp Med 1981;153:694–705. [PubMed: 7252411]
72. Mold C, Rodic-Polic B, Du Clos TW. J Immunol 2002;168:6375–6381. [PubMed: 12055255]
73. Brown JS, Hussell T, Gilliland SM, Holden DW, Paton JC, Ehrenstein MR, Walport MJ, Botto M. Proc Natl Acad Sci USA 2002;99:16969–16974. [PubMed: 12477926]
74. Kerr AR, Paterson GK, Riboldi-Tunnicliffe A, Mitchell TJ. Infect Immun 2005;73:4245–4252. [PubMed: 15972516]
75. Kang YS, Do Y, Lee HK, Park SH, Cheong C, Lynch RM, Loeffler JM, Steinman RM, Park CG. Cell 2006;125:47–58. [PubMed: 16615889]
76. Brouwer N, Dolman KM, Van Houdt M, Sta M, Roos D, Kuijpers TW. J Immunol 2008;180:4124–4132. [PubMed: 18322223]
77. Yother J, Volanakis JE, Briles DE. J Immunol 1982;128:2374–2376. [PubMed: 7061864]
78. Mold C, Nakayama S, Holzer TJ, Gewurz H, Du Clos TW. J Exp Med 1981;154:1703–1708. [PubMed: 7299351]
79. Szalai AJ, Briles DE, Volanakis JE. J Immunol 1995;155:2557–2563. [PubMed: 7650385]
80. Nakayama S, Gewurz H, Holzer T, Du Clos TW, Mold C. Clin Exp Immunol 1983;54:319–326. [PubMed: 6652963]
81. Horowitz J, Volanakis JE, Briles DE. J Immunol 1987;138:2598–2603. [PubMed: 3559209]
82. Szalai AJ, Briles DE, Volanakis JE. Infect Immun 1996;64:4850–4853. [PubMed: 8890251]

83. Motie M, Schaul KW, Potempa LA. Drug Metab Disp 1998;26:977–981.
84. Müller H, Fehr J. J Immunol 1986;136:2202–2207. [PubMed: 3950412]
85. Zeller JM, Landay AL, Lint TF, Gewurz H. J Lab Clin Med 1986;108:567–576. [PubMed: 2946790]
86. Zahedi K, Tebo JM, Siripont J, Klimo GF, Mortensen RF. J Immunol 1989;142:2384–2392. [PubMed: 2926136]
87. Crowell RE, Du Clos TW, Montoya G, Heaphy E, Mold C. J Immunol 1991;147:3445–3451. [PubMed: 1834740]
88. Marnell LL, Mold C, Volzer MA, Burlingame RW, Du Clos TW. J Immunol 1995;155:2185–2193. [PubMed: 7636267]
89. Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW. J Exp Med 1999;190:585–590. [PubMed: 10449529]
90. Chi M, Tridandapani S, Zhong W, Coggeshall KM, Mortensen RF. J Immunol 2002;168:1413–1418. [PubMed: 11801683]
91. Bodman-Smith KB, Melendez AJ, Campbell I, Harrison PT, Allen JM, Raynes JG. Immunology 2002;107:252–260. [PubMed: 12383205]
92. Bodman-Smith KB, Gregory RE, Harrison PT, Raynes JG. J Leukoc Biol 2004;75:1029–1035. [PubMed: 15075346]
93. Rodriguez JA, Bodman-Smith KB, Raynes JG. Clin Exp Immunol 2004;138:271–277. [PubMed: 15498037]
94. Heuertz RM, Schneider GP, Potempa LA, Webster RO. Int J Biochem Cell Biol 2005;37:320–335. [PubMed: 15474978]
95. Escribano-Burgos M, Lopez-Farre A, Mar Gonzalez MD, Macaya C, Garcia-Mendez A, Mateos-Caceres PJ, Alonso-Orgaz S, Carrasco C, Rico LA, Porres Cubero JC. Clin Sci 2005;108:85–91. [PubMed: 15387799]
96. Thomas-Rudolf D, Du Clos TW, Snapper CM, Mold C. J Immunol 2007;178:7283–7291. [PubMed: 17513778]
97. Stein MP, Mold C, Du Clos TW. J Immunol 2000;164:1514–1520. [PubMed: 10640769]
98. Mold C, Gresham HD, Du Clos TW. J Immunol 2001;166:1200–1205. [PubMed: 11145702]
99. Rocker C, Manolov DE, Kuzmenkina EV, Tron K, Slatosch H, Torzewski J, Nienhaus GU. Am J Pathol 2007;170:755–763. [PubMed: 17255341]
100. Ryu J, Lee CW, Shin JA, Park CS, Kim JJ, Park SJ, Han KH. Cardiovasc Res 2007;75:555–565. [PubMed: 17531211]
101. Yang J, Wezeman M, Zhang X, Lin P, Wang M, Qian J, Wan B, Kwak LW, Yu L, Yi Q. Cancer Cell 2007;12:252–265. [PubMed: 17785206]
102. Hanriot D, Bello G, Ropars A, Seguin-Devaux C, Poitevin G, Grosjean S, Latger-Cannard V, Devaux Y, Zannad F, Regnault V, Lacolley P, Mertes PM, Hess K, Longrois D. Thromb Haemost 2008;99:558–569. [PubMed: 18327405]
103. Mold C, Du Clos TW. J Immunol 2006;176:7598–7604. [PubMed: 16751406]
104. Hundt M, Zielinska-Skowronek M, Schmidt RE. Eur J Immunol 2001;31:3475–3483. [PubMed: 11745367]
105. Du Clos TW, Mold C, Edberg JE, Kimberly RP. J Clin Invest 2001;107:643. [PubMed: 11238567]
106. Saeland E, Van Royen A, Hendriksen K, Vilé-Weekhout H, Rijkers GT, Sanders LAM, Van de Winkel JGJ. J Clin Invest 2001;107:641–642. [PubMed: 11238565]
107. Bang R, Marnell L, Mold C, Stein MP, Du Clos KT, Chivington-Buck C, Du Clos TW. J Biol Chem 2005;280:25095–25102. [PubMed: 15878871]
108. Pangburn MK. Immunopharmacology 2000;49:149–157. [PubMed: 10904114]
109. Jarva H, Jokiranta TS, Hellwage J, Zipfel PF, Meri S. J Immunol 1999;163:3957–3962. [PubMed: 10490997]
110. Giannakis E, Jokiranta TS, Male DA, Ranganathan S, Ormsby RJ, Fischetti VA, Mold C, Gordon DL. Eur J Immunol 2003;33:962–969. [PubMed: 12672062]
111. Sjoberg AP, Trouw LA, Clark SJ, Sjolander J, Heinegard D, Sim RB, Day AJ, Blom AM. J Biol Chem 2007;282:10894–10900. [PubMed: 17293598]

112. Biro A, Rovo Z, Papp D, Cervenak L, Varga L, Fust G, Thielens NM, Arlaud GJ, Prohaszka Z. Immunology 2007;121:40–50. [PubMed: 17244159]
113. Laine M, Jarva H, Seitsonen S, Haapasalo K, Lehtinen MJ, Lindeman N, Anderson DH, Johnson PT, Jarvela I, Jokiranta TS, Hageman GS, Immonen I, Meri S. J Immunol 2007;178:3831–3836. [PubMed: 17339482]
114. Skerka C, Lauer N, Weinberger AAWA, Keilhauer CN, Suhnel J, Smith R, Schlotzer-Schrehardt U, Fritsche L, Heinen S, Hartmann A, Weber BHF, Zipfel PF. Mol Immunol 2007;44:3398–3406. [PubMed: 17399790]
115. Sjoberg AP, Trouw LA, McGrath FDG, Hack CE, Blom AM. J Immunol 2006;176:7612–7620. [PubMed: 16751408]
116. Mold C, Kingzette M, Gewurz H. J Immunol 1984;133:882–885. [PubMed: 6234363]
117. Mold C, Gewurz H. J Immunol 1981;127:2089–2092. [PubMed: 6913608]
118. Suankratay C, Mold C, Zhang Y, Potempa LA, Lint TF, Gewurz H. Clin Exp Immunol 1998;113:353–359. [PubMed: 9737662]
119. Zhang Y, Suankratay C, Jhang XH, Lint TF, Gewurz H. Immunopharmacology 1999;42:81–90. [PubMed: 10408369]
120. Jarva H, Janullczyk R, Hellwage J, Zipfel PF, Björck L, Meri S. J Immunol 2002;168:1886–1894. [PubMed: 11823523]
121. Gould JM, Weiser JN. Infect Immun 2001;69:1747–1754. [PubMed: 11179352]
122. Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. Nature 1995;377:435–438. [PubMed: 7566121]
123. Black S, Wilson A, Samols D. J Immunol 2005;175:1192–1196. [PubMed: 16002722]
124. Kim JO, Romero-Steiner S, Sørensen UBS, Blom J, Carvalho M, Barnard S, Carlone G, Weiser JN. Infect Immun 1999;67:2427–2333.
125. Zhang R, Becnel L, Li M, Chen C, Yao Q. Eur J Immunol 2006;36:2993–3006. [PubMed: 17051617]
126. Tobiasova-Czetoova Z, Palmborg A, Lundqvist A, Karlsson G, Adamson L, Bartunkova J, Masucci G, Pisa P. Immunol Lett 2005;100:113–119. [PubMed: 16154491]
127. Van Vre EA, Bult H, Hoymans VY, Van Tendeloo VFI, Vrints CJ, Bosmans JM. Atheroscler Thromb Vasc Biol 2008;28:511–518.
128. Tebo JM, Mortensen RF. Biochim Biophys Acta 1991;1095:210–216. [PubMed: 1958695]
129. Devaraj S, Du Clos TW, Jialal I. Atheroscler Thromb Vasc Biol 2005;25:1359–1367.
130. Rodriguez W, Mold C, Kataranovski M, Hutt JA, Marnell LL, Verbeek JS, Du Clos TW. J Immunol 2006;178:530–538. [PubMed: 17182593]
131. Jiang S, Xia D, Samols D. Scand J Rheumatol 2006;35:351–355. [PubMed: 17062433]
132. Bell SA, Faust H, Schmid A, Meurer M. Clin Exp Immunol 1998;113:327–332. [PubMed: 9737658]
133. Sjowall C, Bengtsson AA, Sturfelt G, Skogh T. Arthrit Res Ther 2004;6:R87–R94.
134. Sjowall C, Wettero J. Clin Chim Acta 2007;378:13–23. [PubMed: 17239838]
135. Klein TC, Döffinger R, Pepys MB, Rüther U, Kyewski B. Eur J Immunol 1995;25:3489–3495. [PubMed: 8566042]
136. Rodriguez W, Mold C, Marnell LL, Hutt J, Silverman GJ, Tran D, Du Clos TW. Arthrit Rheumat 2006;54:325–335.
137. Baum LL, James KK, Glaviano RR, Gewurz H. J Exp Med 1983;157:301–311. [PubMed: 6848619]
138. Vetter ML, Gewurz H, Baum LL. J Leukoc Biol 1986;39:13–25. [PubMed: 3455709]
139. Brown EJ, Hosea SW, Frank MM. J Clin Invest 1981;67:975–982. [PubMed: 7204580]
140. Padovan E, Landmann RM, De Libero G. Trends Immunol 2007;28:308–314. [PubMed: 17537673]
141. Szalai AJ, VanCott JL, McGhee JR, Volanakis JE, Benjamin WH Jr. Infect Immun 2000;68:5652–5656. [PubMed: 10992466]
142. Lysenko E, Richards JC, Cox AD, Stewart A, Martin A, Kapoor M, Weiser JN. Mol Microbiol 2000;35:234–245. [PubMed: 10632893]
143. Serino L, Virji M. Mol Microbiol 2002;43:437–448. [PubMed: 11985720]
144. Singh SK, Suresh MV, Voleti B, Agrawal A. Ann Med 2008;40:110–120. [PubMed: 18293141]

Abbreviations

CRP
C-reactive protein

DC
Dendritic cell

FcγR
Fcγ receptor

MBL
Mannose-binding lectin

PCh
Phosphocholine

PnC
Pneumococcal C-polysaccharide

S. pneumoniae
Streptococcus pneumoniae