

**C-REACTIVE PROTEIN IS PROTECTIVE AGAINST
STREPTOCOCCUS PNEUMONIAE INFECTION IN MICE***

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C-reactive protein (CRP)¹ is an acute phase serum protein produced rapidly in response to inflammatory stimuli. The binding and functional characteristics of CRP suggest that it may play a role in host defense against infection. CRP has binding specificity for the phosphocholine (PC) determinant of cell wall C-polysaccharide (PnC) from *Streptococcus pneumoniae* (1, 2). The binding of CRP to PnC can lead to C activation in human serum by the classical pathway (3). We have found CRP binding and C activation by intact *S. pneumoniae* of several different serotypes (unpublished observations).

CRP in the presence of human C has been shown to opsonize PnC-sensitized erythrocytes (E-PnC) for phagocytosis by human monocytes (4) and mouse macrophages (5). We have extended these studies by demonstrating opsonization of mouse E-PnC by CRP in a mouse in vivo clearance model (6). Opsonization in this system resulted in increased splenic localization of E-PnC and required the presence of both CRP and mouse C. Despite these findings with E-PnC, the effectiveness of CRP as a bacterial opsonin has been uncertain. Earlier in vitro studies of phagocytosis have been conflicting (7-9). In an in vivo study, an association was reported between elevated levels of CRP and increased resistance to *Staphylococcus aureus* infection in endotoxin-treated mice (10). We have found an enhanced phagocytic response by human neutrophils to CRP- and C-treated *S. pneumoniae* type 27 that has capsular as well as cell wall PC determinants.²

Recently Briles et al. (11) reported that IgM antibody to PC can protect CBA/N mice against intravenous challenge with type 3 *S. pneumoniae* (Pn3). Since CRP shares the binding specificity and opsonic activity of IgM antibody to PC, it might be expected to provide similar protection. We have found that pretreatment with CRP can increase survival of mice infected with Pn3 or type 4 *S. pneumoniae* (Pn4). This effect was evident in normal BALB/c mice and in mice unable to produce antibody to PC because of neonatal tolerance induction. This study provides direct evidence of the effectiveness of CRP in host defense against pneumococcal infection.

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¹ Abbreviations used in this paper: α T15, A-HeJ antiserum against HOPC-8 myeloma protein; CFU, colony-forming units; CRP, C-reactive protein; E-PnC, erythrocytes coated with pneumococcal C-polysaccharide; PC, phosphocholine; Pn3, Pn4, serotypes 3 and 4 of *Streptococcus pneumoniae*; PnC, pneumococcal C-polysaccharide.

² Edwards, K., H. Gewurz, T. F. Lint, and C. Mold. A role for C-reactive protein in the complement-mediated opsonization of type 27 *Streptococcus pneumoniae*. Manuscript in preparation.

Materials and Methods

Animals. BALB/c mice were purchased from Cumberland View Farms (Clinton, Tenn.). Newborn BALB/c mice were obtained from females that were pregnant when received. A/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, Maine).

Bacteria. Pn4 and the nonencapsulated strain R36a were obtained from the American Type Cell Culture Collection (Rockville, Md.). Pn3 was a clinical isolate from the Vanderbilt Vaccine Clinic provided by Dr. Kathryn Edwards (Vanderbilt University, Nashville, Tenn.). Bacteria were maintained on sheep blood agar (BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, Md.). Pn3 and Pn4 were passaged through mice before use in these studies. For inoculation of mice, log phase cultures of Pn3 or Pn4 in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) were collected by centrifugation, washed, and resuspended in saline.

Antigens and Antisera. PnC was isolated from R36a by the method of Liu and Gotschlich (12). The PC-binding myeloma protein, HOPC-8, was prepared from ascites in tumor-bearing BALB/c mice and purified by methods published previously (13). Antibody to the T15 idiotype of HOPC-8 (α T15) was raised by immunizing A/HeJ mice with HOPC-8 as described (14).

Neonatal Suppression. BALB/c mice were injected intraperitoneally within 2 d after birth with either 0.1 ml of 10 μ g/ml PnC or 0.1 ml of α T15 serum. Mice treated in these ways have been shown to exhibit tolerance to PC antigens as adults (14). Neonatally suppressed mice were used at 8–12 wk of age.

Measurement of Antibody to PnC. Antibody titers to PnC in normal and suppressed mice were measured by hemagglutination of sheep E coated with PnC (14).

CRP. Human CRP was purified from pleural and peritoneal fluids as previously described (15).

Protection of Mice by CRP. Mice were injected intravenously with 150 or 200 μ g of CRP in 0.1 ml. After 30 min mice were injected intravenously with 0.1 ml bacteria. The number of viable bacteria injected was determined from plate counts of each inoculum. Results were analyzed by the χ^2 test.

Results

Tolerance to PC antigens was induced by injecting mice as neonates with either PnC or α T15. Antibody titers to PnC were determined in serum samples from suppressed and control mice at 8–12 wk of age (Table I). Titers were also determined in surviving mice on day 3 after intravenous injection with 6×10^4 colony-forming units (CFU) of Pn3. The effectiveness of the tolerance induction was shown by the low titers of antibody in suppressed mice (highest titer 2^4) compared with control mice (titers ranging from 2^7 to 2^{10}). Neonatal suppression of mice with PnC also increased their susceptibility to Pn3 infection, decreasing the LD₅₀ from 5×10^4 CFU in normal mice to $<5 \times 10^3$ CFU in PnC-suppressed mice.

Mice suppressed by α T15 treatment were injected with 200 μ g CRP and challenged with 5×10^4 or 1×10^5 CFU Pn3 (Fig. 1). For the lower inoculum, CRP treatment

TABLE I
Antibody to PnC in Normal and Neonatally Suppressed BALB/c Mice

Neonatal treatment*	Antibody to PnC (log ₂ serum titer)					
	Preinfection‡			Postinfection‡		
	n§	mean	range	n	mean	range
Saline	5	9.0	9–9			
None	5	9.2	9–10	9	7.8	7–9
PnC	24	1.5	0–3	11	2.6	2–4
α T15	22	0.5	0–3	9	2.6	2–4

* Mice were injected within 2 d after birth with 0.1 ml saline, 10 μ g/ml PnC, or α T15 serum i.p.

‡ Serum antibody was measured in 8–12 wk-old mice and in surviving mice 3 d after injection of Pn3.

§ Number of mice tested.

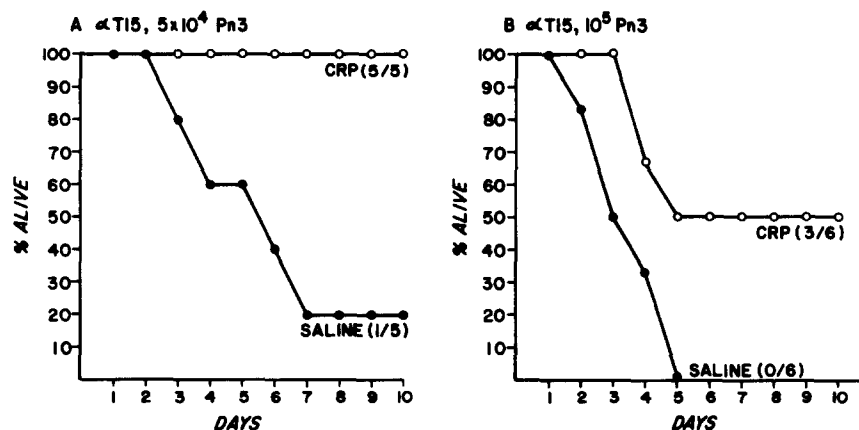


FIG. 1. Effect of CRP treatment on survival of suppressed mice infected with Pn3. Mice treated as neonates with α T15 were treated with 200 μ g CRP in 0.1 ml or 0.1 ml saline i.v. 30 min before intravenous injection of 5×10^4 CFU (panel A) or 10^5 CFU (panel B) of Pn3. Number of surviving mice are shown in parentheses.

TABLE II
Effect of CRP Treatment on Survival of Normal BALB/c Mice
Infected with Pn3

Inoculum (CFU Pn3)	Treatment*	n‡	Percent alive§
2×10^4	CRP	5	100
2×10^4	Saline	5	60
8×10^4	CRP	8	88
8×10^4	Saline	8	25
5×10^5	CRP	5	20
5×10^5	Saline	5	0

* Mice were injected with 200 μ g CRP in 0.1 ml or with 0.1 ml saline i.v. 30 min before intravenous injection of Pn3.

‡ Number of mice tested.

§ Survival 10 d postinfection. Deaths occurred between 2 and 7 d after infection.

increased survival from 20 to 100% ($P < 0.01$). CRP was also protective with the higher inoculum, increasing survival from 0 to 50% ($P < 0.05$). Similar results were observed with PnC-suppressed mice (not shown).

Next we tested the effect of CRP in normal mice challenged with Pn3 (Table II). Mice pretreated with CRP showed an increase in the percent surviving at every dose tested. The difference in mortality between CRP-treated and control mice was highly significant ($P < 0.005$) at an inoculum of 8×10^4 CFU. CRP treatment of normal mice increased the LD₅₀ from 4×10^4 to 2×10^5 CFU Pn3.

CRP is not known to react with the type-specific capsular polysaccharide of Pn3 (16). Therefore we felt that its ability to protect mice against Pn3 infection was related to its binding to PnC in the cell wall. To test this hypothesis, we assessed the ability of CRP to protect mice from infection with Pn4 (Fig. 2). The Pn4 strain used was highly virulent with an LD₅₀ of $\sim 10^2$ CFU in normal mice. CRP increased survival of mice infected with 10^3 CFU Pn4 from 33 to 82% ($P < 0.025$).

Discussion

The experiments presented here define a mouse model in which human CRP provides protection against pneumococcal infection. Previous studies on the opsonic

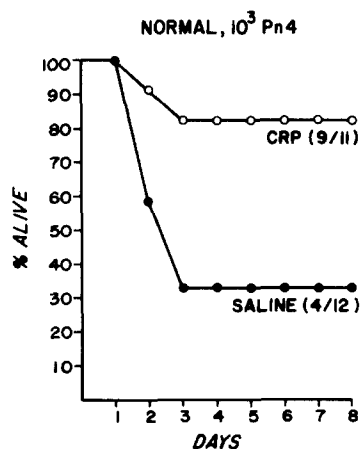


FIG. 2. Effect of CRP treatment on survival of normal mice injected with Pn4. Mice were treated with 150 μ g CRP in 0.1 ml or 0.1 ml saline i.v. 30 min before intravenous injection of 10^3 CFU of Pn4. Number of surviving mice are shown in parentheses.

properties of CRP have shown species compatibility between human CRP and mouse macrophages (5), and between human CRP and mouse C (6). We therefore felt that the mouse represented a reasonable model with which to study the effectiveness of CRP as a bacterial opsonin.

In BALB/c mice, CRP is a trace serum protein that does not elevate beyond 2 μ g/ml (17). These mice have high titers of antibody to PC before immunization. This antibody is predominantly of the T15 idotype and may be suppressed by injection of α T15 or PnC early in life (14). Thus, neonatally suppressed BALB/c mice lack both CRP and antibody to PC. We have shown here that such mice are more susceptible to infection with Pn3 than normal BALB/c mice. This supports the findings of Briles et al. (11) who reported a protective role for antibody to PC in CBA/N mice infected with Pn3.

CRP injected intravenously to attain levels similar to those in acute phase serum protected suppressed mice against Pn3 infection. Such mice lacked antibody to PnC both before and after infection whether or not they were treated with CRP. Injection of CRP was effective even in the presence of natural antibody to PnC, because it increased survival of normal mice infected with Pn3. CRP also protected mice against Pn4 infection, reflecting its reactivity with the group-specific polysaccharide. Since CRP lacks reactivity with the capsular polysaccharides of Pn3 and Pn4 (16, 18), its protective ability is apparently related to its PnC specificity.

In previous experiments (6) CRP has been found to increase the splenic localization of E-PnC in mice. Since the spleen has been shown to be particularly important in protection against pneumococcal infections (19, 20), alteration of clearance patterns of *S. pneumoniae* in the presence of CRP may be responsible for protection from lethality.

These experiments demonstrate a role for CRP in host defense against pneumococcal infection. The early production of CRP in response to infection may provide group-specific protection against *S. pneumoniae* in the presence or absence of an antibody response.

Summary

C-reactive protein (CRP) has several properties that suggest that it may function as a bacterial opsonin. CRP shows binding reactivity with pneumococcal C-polysaccharide, the cell wall carbohydrate of *Streptococcus pneumoniae*. In this study we have demonstrated protection of mice against serotypes 3 and 4 of *S. pneumoniae* infection by a single prior injection of CRP. This effect was seen both in mice that lacked antibody to phosphocholine and in normal mice. Thus the opsonic properties of CRP previously described may be related to protection against pneumococcal infection.

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