Association of C-reactive Protein and Circulating Leukocytes with Resistance to *Staphylococcus aureus* Infection in Endotoxin-treated Mice and Rabbits

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The response of rabbits and mice to treatment with *Escherichia coli* endotoxin, as measured by C-reactive protein (CRP) and leukocyte levels, and resistance to *Staphylococcus aureus* infection was studied to evaluate the significance of these responses and their associations. In both species, there was an initial leukopenia without early recovery of normal lymphocyte levels. This was followed by an increase in polymorphonuclear leukocytes and a return to near the normal range. The CRP level was slightly altered during the stage of decreased resistance and increased throughout the remainder of the period of observation. The resistance level was decreased initially, recovered to normal levels, and continued to increase. The changes in CRP and resistance levels were closely associated. It would appear that this association between CRP and resistance, the antibacterial activity of CRP, and its action on the polysaccharides obtained from bacterial cell walls are evidence for the participation of CRP in nonspecific resistance to infection.

The physiological and immunological responses of experimental animals to endotoxins of gram-negative bacteria have been extensively investigated in recent years. An intriguing characteristic often associated with these lipopolysaccharides is their ability to induce an increased resistance to infection with gram-negative bacteria (15). This nonspecific enhancement of resistance has been proposed to involve increased levels or activities of a variety of different factors including: properdin (16), reticuloendothelial system (4), enzymatic activity (17), and specific antibody (20).

Patterson and Mora (13) observed that enhanced resistance of chickens to intra-articular infection with *Staphylococcus aureus* was associated with an increase in serum levels of C-reactive protein (CRP) and that isolated preparations of this protein had antibacterial activity. Treatment of animals with endotoxin can also induce enhanced resistance to infection with this gram-positive organism. In another study (12) it was observed that the resistance of endotoxin-treated mice to infection with this pathogen is associated with changes in levels of serum CRP and that the CRP of this species also has antibacterial properties. In this regard, Ekstedt (6) suggested that vaccine-enhanced resistance to infection with *S. aureus* may depend, in part, on the stimulation of nonspecific defense mechanisms.

Mulholland and Cluff (10) noted that endotoxin-induced changes in resistance of rabbits to local infection with *S. aureus* may be closely correlated with the circulating levels of leukocytes in the blood of treated animals. In this study, endotoxin-induced changes in both serum CRP and circulating leukocytes were examined to evaluate further the significance of the association of these host responses with resistance of mice and of rabbits to infection with *S. aureus* by intraperitoneal and intracutaneous routes, respectively. The results suggest that the induced resistance was closely paralleled by the CRP response and, to a lesser extent, by the leukocyte response.

**Materials and Methods**

Mouse studies. Male mice of the Fairfield-Webster strain, weighing 18 to 22 g, were obtained from...
Euer’s Farm, Austin, Texas. Upon arrival, the animals were placed in an air-conditioned room, given food and water ad libitum, and rested for a period of 6 to 8 days prior to use. Endotoxin treatment, preparation of bacterial cells, and challenge of animals were essentially the same as those previously described (12). Thus, groups of mice were given a single intravenous (tail vein) injection of 0.25 ml of saline containing 100 μg of Escherichia coli endotoxin (O55:B5, Difco) at 2, 6, 12, or 24 hr prior to challenge with the infectious agent. Zero-time animals were not injected and served as normal controls. Each of these groups was subdivided into five smaller groups, four of which were challenged by intraperitoneal injection with 0.25 ml of saline containing one of a series of four 0.5-log10 dilutions of the Fritchie strain of S. aureus. The largest dose employed contained approximately 3.2 × 10^8 CFU (colony-forming units) of the pathogen. The fifth subgroup in each of the major groups was utilized as a source of blood (obtained from the orbital plexus) for the various tests. Hematocrit values were determined by the microhematocrit technique in heparinized capillary tubes. Total and differential leukocyte levels were determined by conventional techniques with duplicate counts on each sample. Smears were stained with May-Grunwald-Giemsa. CRP titers were determined by the latex rapid plate test using antihuman CRP.

Rabbit studies. New Zealand white rabbits of mixed sexes, weighing 1.8 to 2.3 kg, were obtained from La Marque Rabbitsry, La Marque, Texas. The animals were caged individually, given food and water ad libitum, and maintained in an air-conditioned environment throughout the period of study. At 24 to 48 hr prior to endotoxin treatment, hair was clipped from an area approximately 10 cm in diameter on the back and sides of each animal. Rabbits were given a single intravenous injection (ear vein) of 1.0 ml of saline containing 10 μg of the E. coli endotoxin at 4, 12 or 24 hr prior to challenge with the infectious agent. The pathogen employed was the JH strain of S. aureus and was prepared for use by the methods described for the Fritchie strain (12). Rabbits were challenged by intracutaneous injection with 0.1 ml of saline containing 10^8 CFU of this organism on the right side and 10^9 CFU on the left side of the mid-dorsal line. After 24 to 48 hr, fur remaining at the site of injection was removed with a depilatory (Nair). Resistance to infection was estimated by determining the diameter of the necrotic area surrounding the site of injection with the aid of plastic overlay graduated in the range of 0.25 to 7.0 cm. The data obtained were used for calculating size of the lesion area. Although lesions in both control and 4-hr endotoxin-treated rabbits often exhibited additional areas of spread by gravity drainage, this portion of the lesion was routinely excluded from measurements of lesion size. Blood was collected from veins of the noninjected ear at the time of challenge. Both blood and serum samples were tested by methods described for mice, with the exception that the CRP level was estimated by the capillary precipitin test as described by Anderson and McCarty (1). Temperature changes were determined by using a telethermometer and a small animal probe.

Antisera used in this study were obtained from Hyland Laboratories, Los Angeles, Calif. Endotoxin was obtained from Difco Laboratories, Detroit, Mich.

**RESULTS**

The influence of endotoxin on resistance to infection was evaluated in four groups of 25 mice each, which were injected with 0.25 ml of saline containing 100 μg of E. coli endotoxin at 2, 6, 12, or 24 hr prior to challenge with the Fritchie strain of S. aureus. A similar number of mice, not treated with endotoxin, were included in the experiment as a control group. Five animals per group were bled from the orbital sinus for hematocrit and CRP determinations. The remaining mice in each group were challenged with one of a series of graded amounts of S. aureus to obtain the median lethal dose (LD50) for the respective groups (14). The experiment was

**TABLE 1. Effects of endotoxin on C-reactive protein (CRP), leukocytes, and resistance to the lethal effects of Staphylococcus aureus infection in mice**

<table>
<thead>
<tr>
<th>Time of challenge</th>
<th>log10 CRP</th>
<th>Leukocytes</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td>× 10^8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (control)</td>
<td>3.5</td>
<td>8.8</td>
<td>8,948 (2,075-13,850)</td>
<td>2,605 (539-5,886)</td>
<td>6,342 (1,535-9,972)</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>1,806e (448-2,745)</td>
<td>1,806e (448-2,745)</td>
<td>2,902 (1,209-4,865)</td>
<td>1,953 (265-4,920)</td>
</tr>
<tr>
<td>6</td>
<td>7.7</td>
<td>2,902 (1,209-4,865)</td>
<td>2,902 (1,209-4,865)</td>
<td>7,676 (5,481-10,578)</td>
<td>2,104e (1,216-4,448)</td>
</tr>
<tr>
<td>12</td>
<td>13.5</td>
<td>7,676 (5,481-10,578)</td>
<td>7,676 (5,481-10,578)</td>
<td>16,750 (10,786-24,672)</td>
<td>16,750 (10,786-24,672)</td>
</tr>
<tr>
<td>24</td>
<td>20.8d</td>
<td>16,750 (10,786-24,672)</td>
<td>16,750 (10,786-24,672)</td>
<td>16,750 (10,786-24,672)</td>
<td>16,750 (10,786-24,672)</td>
</tr>
</tbody>
</table>

* After endotoxin.
* Values in parentheses represent ranges, other values are averages.
* P = 0.05 when compared to controls (unpaired rank test).
* P = 0.05 when compared to controls (ChiP test).
repeated with similar results, data for each group were pooled, and the average results for each are presented in Table 1. As noted previously, treatment with endotoxin induced a biphasic effect on resistance to this challenge, with the negative phase being most pronounced by the 2-hr period. During the 2- to 12-hr period, there was progressive recovery to the control level. From the 12- to 24-hr period, resistance continued to increase to a level approximately four times that of the control. It should be noted that the initial period of negative phase of resistance was associated with a transitory, but definite, increase in hematocrit and thus coincided with the period of endotoxic shock. CRP levels also declined during the initial period, after which there was a progressive increase in concentration of this protein in the sera to a maximal level at 24 hr, equivalent to approximately 2.5 times that of the control. This relationship of the CRP response to the changes in resistance is shown more clearly in Fig. 1 and 2.

The total leukocyte count also decreased by the 2-hr period, but remained at this minimum level through the 6-hr period, and, although it rose to control level by the 12-hr period, was again depressed at the time of maximal resistance at the 24-hr period (Fig. 1). This leukocyte response to endotoxin reflects similar changes in the levels of circulating neutrophils, which is associated with a pronounced lymphopenia persisting throughout the observation period. It is evident from the data presented in Table 1 and Fig. 1 that, although the initial phase of negative resistance involved measurable changes in all of the parameters evaluated, only the CRP response paralleled the changes occurring in both negative and positive phases of resistance.

Rabbit studies. Similar studies were conducted with the rabbit used as the experimental animal. In these experiments, groups of three rabbits each were injected intravenously with 10 μg of E. coli endotoxin at 4, 12, or 24 hr prior to intracutaneous challenge with the JH strain of S. aureus. A similar number of rabbits, not treated with endotoxin, was included in the experiment as a control group. Resistance was determined on the basis of size of the resulting lesion, and the results obtained from the various groups were compared in terms of a "resistance index," which is the ratio of lesion areas of control to test group times 10. The experiment was repeated. Endotoxin treatment also induced a biphasic effect on resistance to local infection (Table 2). The negative phase of resistance was apparent at 4 hr, and resistance increased to levels two and three times that of the control by the 12- and 24-hr periods, respectively. The negative phase in resistance was associated with a transient pyrogenic response and a measurable increase in hematocrit, which was still evident at the 12- but not at the 24-hr period. CRP levels, however, showed little response to treatment during the first 4 hr, but, as indicated in Fig. 3, rose steadily thereafter to levels two and one-half and four and one-half times that of the control by the 12- and 24-hr periods, respectively.

The leukocyte response to endotoxin was characterized by an initial leukopenia during the negative phase of resistance, followed by a marked leukocytosis at the 12-hr period during the positive phase, with a return to control level at the period of maximal resistance at the 24-hr period. As noted in the mouse, this leukocyte response was largely a reflection of changes in the level of circulating heterophils, and was accompanied by a definite lymphopenia during
**TABLE 2. Effects of endotoxin on Cx-reactive protein (CxRP), leukocytes, and resistance to intradermal Staphylococcus aureus infection in rabbits**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Time of challenge after endotoxin (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 4 12 24</td>
</tr>
<tr>
<td>Resistance index .............</td>
<td>10 (1.8-16.4)* 3.17 (1.8-7.0) 21.86 (14.0-53.2) 31.78 (23.7-96.1)</td>
</tr>
<tr>
<td>CxRP .......................</td>
<td>0.8 (0.5-0.1) 0.8 (0.5-1.0) 2 3.7 (3-4)</td>
</tr>
<tr>
<td>Leukocytes (total) ...........</td>
<td>14,933 6,617 24,750 (22,100-29,000) 16,892 (15,700-17,600)</td>
</tr>
<tr>
<td>(3,125-9,600) (13,750-17,100)</td>
<td></td>
</tr>
<tr>
<td>Heterophils .................</td>
<td>4,118 1,377 19,008 6,879 (5,734-8,478)</td>
</tr>
<tr>
<td>(3,627-4,189) (219-2,736)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes .................</td>
<td>10,511 5,092 5,380 9,430 (6,437-11,294)</td>
</tr>
<tr>
<td>(9,419-12,141) (2,859-6,576)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit .................</td>
<td>39.3 (38-40) 41.7 (36-44) 42 (40-44) 37 (37-38)</td>
</tr>
<tr>
<td>(22,100-29,000) (219-2,736)</td>
<td></td>
</tr>
<tr>
<td>Body temperature (F) .......</td>
<td>103.0 104.7 (104-105) 103.3 (103-104) 103</td>
</tr>
</tbody>
</table>

* Values in parentheses represent ranges; other values are averages.

The injection of endotoxin into animals stimulates a variety of both cellular and humoral responses including activation of the reticuloendothelial system (4), production of adrenal cortical steroids (9), and secretion of interferon (11, 18). In view of the diverse nature of the factors involved in the response to endotoxins, it would appear that the characteristics of the challenge agent employed would determine the relative importance of any particular factor in endotoxin-enhanced resistance. It has been reported that leukocytes may play an important role in resistance to infection with S. aureus. The studies reported by Mulholland and Cluff (10) suggested that endotoxin-induced changes in resistance of rabbits to local infection with S. aureus are closely associated with leukocyte changes after this treatment. Particularly striking was the observation that resistance was greatly decreased when leukocyte levels reached a critical low level.

It has been reported that changes in leukocyte levels also occur in mice treated with endotoxin (2). Similar effects were noted in the present study. The changes in total leukocyte levels reflected...
the lymphocyte reduction without early recovery. The heterophile count increased with early recovery of resistance but did not continue to increase as did resistance.

The CRP changes were similar in both mice and rabbits and were closely associated with the changes in resistance.

It has been reported that endotoxin is removed from the blood by the liver (5) and interferes with certain of its functions (e.g., tryptophan pyrrolase; reference 3). Inasmuch as CRP is synthesized by the liver (8), it would appear that CRP levels may be a good index of the effect of endotoxin on the animal.

Although specific functions of CRP have not been proven, sufficient evidence is available to permit drawing logical conclusions concerning its role. CRP is characterized by its reaction with the C-polysaccharide of Diplococcus pneumoniae (19), which consists of mucopentide and teichoic acid (7). This complex is similar to that found in other gram-positive bacteria and might, therefore, provide a common substrate for CRP attack on many infectious organisms. CRP was reported to cause the lysis of S. aureus and other gram-positive bacteria (L. T. Patterson, J. M. Harper, and R. D. Higginbothom, Federation Proc., p. 699, 1965) and to cause agglutination of several species under conditions unsuitable for extensive lysis (12). It would appear that CRP may function as an opsonin or bacteriolytic agent, thereby contributing to resistance to infection.

**Literature Cited**


