Listeria monocytogenes Cell Walls Induce Decreased Resistance to Infection

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Received for publication 8 August 1977

A significant decrease in murine resistance to Listeria monocytogenes was induced by using crude Listeria cell wall fraction (LCWF) and purified Listeria cell walls (PF). When equal amounts of these materials were injected, PF was more effective than LCWF in decreasing resistance. The PF effect was dose dependent when measured either as a decrease in 50% lethal dose of the Listeria challenge or as a decrease in survival time of the infected mice. PF apparently does not act directly on the Listeria since it (i) did not cause a change in in vitro growth of Listeria and (ii) did not increase the virulence of Listeria passaged in vivo or in vitro. The greatest decrease in resistance was observed when both PF and the Listeria challenge were injected intraperitoneally, which may suggest a localized effect. A decrease in resistance was seen when PF was given as early as 3 days before challenge. There was little or no decrease in resistance when PF was given 2 days after the Listeria challenge. Mice previously immunized with live Listeria were immune to Listeria challenge. However, after PF injection the immune mice showed a decreased resistance which was of the same order of magnitude as that seen in unimmunized mice. The effect of PF seems to be at least partially nonspecific, since a decrease in resistance to Salmonella typhimurium could also be demonstrated in PF-treated mice. Phagocytosis of Listeria both in vivo and in vitro did not appear to be inhibited by PF, although the ability of PF-treated mice to kill Listeria in the peritoneal cavity was inhibited.

A crude cell wall preparation (LCWF) from the gram-positive bacterium Listeria monocytogenes has been shown to possess mitogenic (4) and adjuvant (2, 3) activities and to induce specific resistance to listeriosis (18). Recently, LCWF and purified L. monocytogenes cell walls (PF) derived from LCWF have been shown to activate complement and to generate factors from serum chemotactic for polymorphonuclear neutrophils (1). Since cell walls are among the first bacterial components encountered by the host after invasion by pathogenic bacteria, it is not surprising that the host should respond to these components in an attempt to defend itself. Conversely, the cell wall or outer envelope would be a most advantageous location for bacterial factors that could thwart host defense systems. Antiphagocytic materials are often located on or incorporated in the outer envelopes of bacteria (5, 12, 15). The chemical composition of some bacteria may make them resistant to killing by normal phagocytes (6, 11), and some pathogens may contain substances in or on their outer envelopes that inhibit phagosome-lysosome fusion (7, 11). These components all tend to increase the pathogenicity of the organisms involved by decreasing the effectiveness of the host defenses.

There have been previous reports that factors from L. monocytogenes can reduce host resistance to listerial infection. Patocka et al. (16) described an "endotoxin-like," ether-extractable component of L. monocytogenes that had some resistance-decreasing properties. In addition, Silverman et al. (19) showed that a heat-stable filtrate of sonically treated L. monocytogenes decreased the resistance of mice to Listeria infection. However, neither these factors nor the decreased resistance they induce has been well characterized.

Data reported in this paper describe the effect of Listeria cell walls on host resistance to Listeria infection in an effort to increase understanding of events involved in host-bacterium interactions during infection with intracellular bacteria.

MATERIALS AND METHODS

Animals. For the experiments reported in this paper, 9- to 12-week-old (C57BL/6 × DBA/2)F1 (BDF1) mice were used. In each experiment, mice were of the same age and sex. Many of the experiments reported...
The cells/ml were determined by Muench (17). The deviation of the LD₅₀ was 1.4 x 10⁵ bacteria during the course of this study. Salmonella typhimurium, ATCC 14028, was originally purchased from Difco Laboratories (Detroit, Mich.) in lyophilized form and was stored at -70°C suspended in Trypticase soy broth. For injection, a tube of stock suspension was thawed, passed once in Trypticase soy broth, and diluted in saline for injection. The intraperitoneal (i.p.) 50% lethal dose (LD₅₀) in BDF₁ mice was routinely 4 x 10⁵ bacteria. For injection, a tube of stock suspension was thawed, passed once in Trypticase soy broth, and diluted in saline. The i.p. LD₅₀ for this strain was less than 10² organisms in BDF₁ mice.

Cell wall preparations. The preparation of LCWF has been described (18). Briefly, washed L. monocytogenes cells were sonically disrupted and centrifuged at 2,000 x g for 15 min. The resulting supernatant was centrifuged at 12,000 x g for 45 min. This pellet, rich in cell walls, was called LCWF. For injections, LCWF was sterilized by autoclaving.

PF was prepared from LCWF as described previously (1). To do this, 10 g of LCWF was autoclaved and treated with 10 mg of deoxyribonuclease II, 10 mg of ribonuclease A (Sigma Chemical Co., St. Louis, Mo.), and 100 mg of trypsin (specific activity 10,000 N-benzoyl-L-arginine ethyl ester U/mg; Schwarz/Mann, Orangeburg, N.Y.) at 37°C for 48 h. The remaining insoluble material was washed and treated with 100 mg of Pronase (type V from Sigma) at 37°C for 48 h. The insoluble residue, which represented 45 to 50% of the original dry weight of LCWF, was washed extensively with sterile, pyrogen-free distilled water. The purified cell wall suspension thus obtained was adjusted to 10 mg (dry weight)/ml, autoclaved, and stored at -20°C.

Determination of decrease in resistance. Decreased resistance induced by Listeria cell walls was determined by an increase in mortality as indicated by the number of mice dead at each challenge dose per number of mice challenged. Control groups with saline instead of cell walls were included in each experiment so that the degree of decrease in resistance could be seen. In some experiments the mean day of death ± standard deviation was also determined; survivors were not included in calculation of the mean. The LD₅₀ was determined by the method of Reed and Muench (17).

Peritoneal cells. To obtain peritoneal cells, mice were killed with chloroform and injected i.p. with 5 ml of balanced salt solution containing 5 U of heparin per ml. The peritoneal cavity was agitated, and cells and fluid were then removed with a needle and syringe. The peritoneal cells were collected by centrifugation at 1,000 rpm for 10 min and resuspended to 2 x 10⁶ cells/ml in RPMI 1640 with 10% heat-inactivated fetal bovine serum. The cells were then placed in Lab-Tek four-chamber culture slides (Lab-Tek Products, Naperville, Ill.), 1 ml/chamber, and incubated for 1 h at 37°C in an atmosphere of 8% O₂, 12% CO₂, and 80% N₂. The nonadherent cells were then removed by two rinses with warm, sterile balanced salt solution, and 1 ml of RPMI 1640 with 10% fetal bovine serum and additional glutamine was added back to each chamber for reincubation.

These adherent cell cultures are referred to as macrophage monolayers; however, we recognize that other cell types may also have been present.

In vitro phagocytosis. After the macrophage monolayers were cultured for 18 h, PF and Listeria (10 Listeria/macrophase) were added to the appropriate chambers. After mixing with a Pasteur pipette, the cultures were incubated for 12 to 15 min to allow phagocytosis. This short interval was used to prevent significant increase in numbers of bacteria. After phagocytosis, the chambers were rinsed twice with warm, sterile balanced salt solution, air dried, and stained with Wright stain followed by Giemsa stain. The plastic chambers were removed from the slides, and the stained monolayers were examined microscopically. At least 15 oil immersion fields from each chamber were counted to determine the number of macrophages per field, the percentage of macrophages containing Listeria, and the number of Listeria per macrophage.

RESULTS

Effect of Listeria cell wall fractions on resistance. When LCWF was injected i.p. into mice at the same time or within a few hours of an i.p. Listeria challenge, a marked decrease in LD₅₀ was noted. LCWF and a PF preparation derived from LCWF both could decrease the LD₅₀ of L. monocytogenes (Table 1). The decrease in LD₅₀ was greater with PF than with LCWF when equal amounts were injected. Subsequent experiments were performed only with PF because it was more pure and more active on a dry-weight basis.

Effect of PF on in vitro growth of Listeria. Although it seemed likely that the decrease in LD₅₀ observed with LCWF and PF was due to a decrease in host resistance, it was possible that the cell wall preparations were affecting the bacteria directly rather than the host. To examine this possibility, Listeria were cultured in the presence and absence of PF. A typical growth curve of L. monocytogenes with and without PF is shown in Fig. 1. The addition of 100 µg of PF per ml to the cultures caused an initial increase of approximately 0.02 optical density (600 nm) unit. When this was taken into account, no significant difference in growth rate or yield of Listeria was observed in the presence of PF. There was also no significant change in the LD₅₀ of cultures containing PF (Fig. 1).

Experiments were then conducted to determine whether Listeria recovered from PF-in-
**TABLE 1. Decrease in resistance to L. monocytogenes induced by PF and LCWF**

<table>
<thead>
<tr>
<th>Material injected</th>
<th>1.1 x 10^6</th>
<th>1.1 x 10^5</th>
<th>1.1 x 10^4</th>
<th>1.1 x 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF, 50 µg</td>
<td>—</td>
<td>—</td>
<td>6/6 (2.8 ± 0.5)</td>
<td>6/6 (5.2 ± 1.7)</td>
</tr>
<tr>
<td>PF, 5 µg</td>
<td>—</td>
<td>6/6 (2.8 ± 0.5)</td>
<td>6/6 (3.3 ± 0.2)</td>
<td>4/6 (7.0 ± 2.0)</td>
</tr>
<tr>
<td>LCWF, 50 µg</td>
<td>—</td>
<td>—</td>
<td>4/6 (5.1 ± 1.2)</td>
<td>0/6</td>
</tr>
<tr>
<td>LCWF, 5 µg</td>
<td>—</td>
<td>6/6 (3.5 ± 0.2)</td>
<td>1/6 (7.0 ± 0)</td>
<td>1/6 (7.0 ± 0)</td>
</tr>
<tr>
<td>Saline</td>
<td>6/6 (4.1 ± 0.7)</td>
<td>1/6 (5.0 ± 0)</td>
<td>0/6</td>
<td>—</td>
</tr>
</tbody>
</table>

*Ten-week-old male BDF mice were injected i.p. with 0.5 ml of LCWF, PF, or saline and challenged i.p. within 1 h with 0.5 ml of Listeria.*

b Results are expressed as the number of mice dead/number of mice challenged in each group. The mean day of death ± standard deviation is given in parentheses.

c —, Not done.

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**Fig. 1. In vitro growth of L. monocytogenes in tryptose phosphate broth (TPB) with (○) and without (●) 100 µg of PF/ml. The initial inoculum was 1 x 10^6 L. monocytogenes per ml. Samples of cultures were taken near the end of log-phase growth (6 h), and LD₅₀ was determined by i.p. challenge of normal mice.**

Injected mice were more virulent than Listeria recovered from normal mice. Mice were injected with 1 x 10^6 Listeria and 200 µg of PF, and Listeria were recovered from the peritoneal cavity 24 h later. The organisms recovered from PF-treated mice were injected i.p. into normal mice, and the LD₅₀ was determined. The LD₅₀ of Listeria from PF-treated mice was 5 x 10^5, whereas the LD₅₀ of the Listeria before passage in PF-treated mice was 4.8 x 10^5. These experiments suggested that PF had no direct effect on the virulence of Listeria grown either in vivo or in vitro.

**Effective dose of PF.** Experiments were conducted to determine the effective doses of PF for inducing decreased resistance. Even the lowest dose used, 5 µg of PF, caused a 700-fold decrease in the LD₅₀ (Table 2). Furthermore, the dose response was more consistent when expressed as the mean day of death rather than as decrease in LD₅₀. In most of the following experiments, 100 to 200 µg of PF was used to ensure a decrease of at least 1,000-fold in the LD₅₀.

Since 500 µg of PF had decreased the LD₅₀ to less than 100 Listeria (Table 2), it was of interest to determine how few Listeria could kill PF-treated mice. As few as four organisms, and probably only one, could kill mice treated with 500 µg of PF. Therefore, when high doses of PF were used, there was essentially no host resistance to Listeria infection. Loss of resistance was not due to any toxic effects of PF itself (Table 3). As much as 1 mg of PF could be injected into mice i.p. with no observable adverse effects.

**Effect of the route of injection of PF on decrease in resistance.** Thus far, PF and challenge doses of Listeria were both administered i.p. because we assumed that the extreme insolubility of PF might produce a localized rather than a systemic effect. To determine whether the decrease in resistance was in fact localized, mice were injected with 200 µg of PF by various routes and challenged i.p. with Listeria. The results (Table 4) indicated that the effect of PF is primarily localized, since a significant decrease in resistance was observed after i.p. challenge only if PF had also been given i.p. Moreover, if the Listeria challenge was given intravenously (i.v.) rather than i.p., there was only a slight decrease in resistance regardless of the route of PF injection (Table 5). Although a slight decrease in resistance to i.v. challenge was induced by i.v. or i.p. PF, the greatest decrease in resistance was seen when both PF and challenge were given i.p.
Effect of PF on resistance of immune mice. In an attempt to lessen the decrease in resistance caused by PF, mice were immunized with live *L. monocytogenes*, and the susceptibility of these immune mice to the resistance-decreasing effects of PF was determined. To do this, mice were immunized i.p. with 5 × 10⁶ live

Effect of time of PF injection on decrease in resistance. To determine at what time intervals before or after *Listeria* challenge injection of PF was effective, several timing experi-
ments, such as the one presented in Table 7, were performed. The greatest decrease in resistance was always obtained when PF was given the same day as the challenge. This was particularly evident in terms of the mean day of death. The effect of PF dropped drastically when given 1 day after challenge, and PF was essentially ineffective when given 2 days after challenge. However, PF was still partially effective when given 3 days before challenge. Other experiments (data not shown) indicate that PF was also partially effective as early as 5 days before challenge but completely ineffective when given 7 days before challenge. Consistently strong decreases in resistance were seen only when PF was given on day -2, day -1, and the day of the challenge.

Specificity of the decrease in resistance induced by PF. Experiments conducted to determine whether the decreased resistance induced by PF was specific for *L. monocytogenes* or whether PF could also decrease resistance to *S. typhimurium* infection indicated that PF treatment did decrease resistance to *S. typhimurium* (Table 8). The effect of PF on *S. typhimurium* infection appeared to be much less than the effect on *L. monocytogenes* infection, although the two organisms were not compared in the same experiment. This difference may have been due to the greater virulence of the *Salmonella* strain used, to differences in the mechanisms of resistance to *Salmonella* and *Listeria*, or to the effect of PF being at least partially specific for *Listeria*.

**In vivo growth of *Listeria*.** The early deaths of PF-treated mice suggested that the in vivo growth of *Listeria* in these mice was more rapid than in untreated mice. To examine this possibility, the number of *Listeria* in peritoneal washes from i.p.-challenged mice was determined at various times after challenge. Large challenge doses (more than 10⁶ organisms) were used in order to recover detectable numbers of *Listeria* at all time periods. However, large challenge doses killed both PF-treated and untreated mice. Therefore, mice previously immunized with live *L. monocytogenes* were used in these experiments so that mice not given PF would survive the high challenge doses. The immune mice were injected i.p. with 5 × 10⁶ *Listeria* with or without 100 µg of PF. At various times after injection, mice from each group were sacrificed and peritoneal washes were obtained. The peritoneal washes were centrifuged in the cold at 800 rpm for 5 min, and the supernatants were removed, diluted, and cultured on blood agar plates to determine the numbers of free *Listeria* present. For a crude determination of the numbers of cell-associated *Listeria*, the pellets were resuspended to 1.0 ml, and 0.1 ml of these suspensions was added to 0.9 ml of 2% saponin and

### Table 6. Decrease in resistance induced by PF in normal and immune mice

| Challenge dose | No. of mice dead/no. challenged | Normal mice | Immune mice
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Saline</td>
<td>PF</td>
</tr>
<tr>
<td>1.5 × 10⁷</td>
<td>—</td>
<td>—</td>
<td>1/6</td>
</tr>
<tr>
<td>1.5 × 10⁸</td>
<td>6/6</td>
<td>0/6</td>
<td>6/6</td>
</tr>
<tr>
<td>1.5 × 10⁹</td>
<td>5/5</td>
<td>6/6</td>
<td>5/6</td>
</tr>
<tr>
<td>1.5 × 10⁴</td>
<td>0/6</td>
<td>6/6</td>
<td>—</td>
</tr>
<tr>
<td>1.5 × 10⁵</td>
<td>—</td>
<td>6/6</td>
<td>—</td>
</tr>
<tr>
<td>1.5 × 10⁶</td>
<td>—</td>
<td>6/6</td>
<td>—</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>5.9 × 10⁶</td>
<td>&lt;10⁸</td>
<td>&gt;10⁷</td>
</tr>
</tbody>
</table>

* Groups of 6 BDF₁ mice were injected i.p. with 0.5 ml of saline or PF suspension (200 µg) and challenged i.p. within 1 h with 0.5 ml of *Listeria*.

* Mice were immunized by injecting 5 × 10⁹ live *L. monocytogenes* i.p. at 4, 3, and 1 week before challenge.

* Not done.

* Calculated by the method of Reed and Muench (17).

### Table 7. Kinetics of the decrease in resistance induced by PF

<table>
<thead>
<tr>
<th>Day of PF injection</th>
<th>1.55 × 10⁶</th>
<th>1.55 × 10⁷</th>
<th>1.55 × 10⁸</th>
<th>1.55 × 10⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5/5 (4.5 ± 0.2)</td>
<td>0/5</td>
<td>0/5</td>
<td>—</td>
</tr>
<tr>
<td>-3</td>
<td>4/5 (5.1 ± 2.0)</td>
<td>5/5 (4.8 ± 0.6)</td>
<td>2/5 (6.5 ± 1.1)</td>
<td>—</td>
</tr>
<tr>
<td>-2</td>
<td>5/5 (3.7 ± 0.7)</td>
<td>5/5 (4.4 ± 0.4)</td>
<td>4/5 (6.2 ± 1.3)</td>
<td>—</td>
</tr>
<tr>
<td>-1</td>
<td>5/5 (3.9 ± 1.1)</td>
<td>5/5 (3.0 ± 0)</td>
<td>5/5 (4.5 ± 0.7)</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>5/5 (2.6 ± 0.4)</td>
<td>1/5 (9.0 ± 0)</td>
<td>0/5</td>
<td>—</td>
</tr>
<tr>
<td>+1</td>
<td>5/5 (7.4 ± 1.3)</td>
<td>0/5</td>
<td>0/5</td>
<td>—</td>
</tr>
<tr>
<td>+2</td>
<td>1/5 (7.0 ± 0)</td>
<td>0/5</td>
<td>0/5</td>
<td>—</td>
</tr>
</tbody>
</table>

* Ten-week-old male BDF₁ mice were injected i.p. with 100 µg of PF on the days indicated and on day zero were challenged i.p. with the number of *L. monocytogenes* indicated. The day zero PF dose was given approximately 1 h before challenge.

* Results are expressed as the number of mice dead/number of mice challenged in each group. The mean day of death ± standard deviation is given in parentheses.

* — Not done.
incubated at 37°C for 20 min. The resulting lysates were diluted in saline and cultured on blood agar plates. The total number of peritoneal cells recovered was also established by cell counts. In addition, to determine the number of adherent cells present, aliquots of each cell suspension were placed in Lab-Tek four-chamber slides. After incubation at 37°C for 30 min to allow adherence, the nonadherent cells were rinsed off, the slides were stained, and the cells were counted. *Listeria* grew better in peritoneal cavities of PF-treated mice than in untreated mice, and PF-treated mice were all dead 48 h after challenge, whereas the untreated mice not only survived the challenge but showed no overt signs of illness (Fig. 2). Phagocytosis was apparently not impaired by PF, since at 15 min after challenge the number of cell-associated *Listeria* in PF-treated mice was equal to or greater than the number of cell-associated *Listeria* in untreated mice. There was a significant increase in the total number of peritoneal cells which was of the same magnitude in both groups of mice. A peak recovery of 15 × 10⁶ peritoneal cells was reached 5 h after injection. However, as early as 1 h after challenge, the peritoneal cavities of PF-treated mice contained fewer adherent cells than the peritoneal cavities of mice receiving only *Listeria*. This decrease in adherent cells was apparently not due to any direct toxic effect of PF, since control mice injected with PF alone did not show any decrease in adherent cells.

**In vitro phagocytosis of *Listeria***. Experiments were then conducted to examine directly the effect of PF on phagocytosis of *Listeria*. Normal peritoneal macrophage monolayers were cultured for 18 h and then challenged with 10⁶ *Listeria* with or without PF. In one such experiment in which duplicate monolayers were assayed, there was no significant difference in phagocytosis of *Listeria* with or without PF (Table 9). In other experiments not shown, a slight but possibly significant increase in phagocytosis was observed in the presence of PF. These results indicate that in vitro phagocytosis of *Listeria* is not inhibited by PF.

Figure 3 shows the effect of PF on in vitro macrophage monolayers. Macrophages exposed to PF for 24 h (Fig. 3b) were more rounded and had a more dense cytoplasm than untreated
TABLE 9. Effect of PF on in vitro phagocytosis of L. monocytogenes by mouse peritoneal macrophages*

<table>
<thead>
<tr>
<th>PF (µg/ml)</th>
<th>Percent phagocytosis*</th>
<th>Listeria/macrophage</th>
<th>Macrophages/field</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>37.6 ± 8.2</td>
<td>2.5 ± 1.2</td>
<td>17.6 ± 2.7</td>
</tr>
<tr>
<td>None</td>
<td>39.9 ± 13.0</td>
<td>2.8 ± 1.7</td>
<td>16.6 ± 3.4</td>
</tr>
<tr>
<td>10</td>
<td>47.1 ± 13.5</td>
<td>2.6 ± 1.2</td>
<td>16.2 ± 4.7</td>
</tr>
<tr>
<td>10</td>
<td>38.9 ± 8.0</td>
<td>2.9 ± 1.9</td>
<td>16.1 ± 5.1</td>
</tr>
<tr>
<td>50</td>
<td>36.5 ± 12.0</td>
<td>2.7 ± 1.3</td>
<td>16.9 ± 3.5</td>
</tr>
<tr>
<td>50</td>
<td>34.3 ± 10.4</td>
<td>2.9 ± 2.1</td>
<td>16.3 ± 4.3</td>
</tr>
</tbody>
</table>

* Mouse peritoneal macrophages were cultured for 18 h in RPMI 1640 with 10% heat-inactivated fetal bovine serum in Lab-Tek chamber slides, 10⁶ macrophages/ml per chamber. Duplicate chambers were then challenged with 10 Listeria per macrophage with or without PF as indicated. The slides were incubated for 12 min, rinsed twice vigorously with warm, sterile balanced salt solution, air dried, and stained. The results are presented as the mean standard deviation of at least 15 oil immersion fields per chamber.

b (Number of macrophages per field containing Listeria/total number of macrophages per field) × 100.

c Mean number of Listeria per macrophage in macrophages with ingested bacteria.

d Mean number of macrophages per field in 15 fields.

macrophages (Fig. 3a). Nevertheless, the treated macrophages were still viable and could still phagocytose Listeria.

DISCUSSION

The data presented here show that Listeria cell wall preparations can decrease murine resistance to L. monocytogenes infection. The cell wall preparations apparently act on the host rather than directly on the Listeria, since PF did not change the in vitro growth of Listeria or increase the virulence of Listeria passaged either in vitro or in vivo. PF was most effective at decreasing resistance when injected on the same day and by the same route as the Listeria challenge. However, some decrease in resistance was seen when PF was injected up to 3 to 5 days before challenge, but not when it was injected after challenge. These results suggest that PF must be present when and where the Listeria infection first occurs and that the effect is on an early step in the infectious process. The PF-induced decrease in resistance was also observed
in mice immunized against \textit{Listeria}. Although resistance was not decreased to the same LD$_{50}$ in immune mice as in nonimmune mice, the decrease in resistance was of approximately the same order of magnitude in both cases. The decrease in resistance was at least partially nonspecific, since PF also decreased resistance to infection with \textit{S. typhimurium}.

Although the mechanism by which PF decreases resistance is not yet clear, the failure of PF-treated mice to control even the early growth of i.p.-injected \textit{Listeria} suggests that some early killing event is inhibited by PF. Although inhibition of phagocytosis seemed to be a likely candidate for the mechanism of action of PF, PF did not decrease the number of cell-associated \textit{Listeria} in the peritoneal cavities of mice or inhibit phagocytosis of \textit{Listeria} by macrophage monolayers. These results suggest that it is a post-phagocytic event that is inhibited by PF. PF did induce morphological changes in mouse peritoneal macrophages in vitro, but direct effects of PF on macrophage function other than phagocytosis were not examined.

Other materials have been shown to decrease resistance to bacterial infection. Silverman \textit{et al.} (19) described a mortality-enhancing factor (MEF) obtained from filtrates of sonically treated \textit{L. monocytogenes}. MEF could decrease resistance to \textit{Listeria} and several other pathogens, but the material itself was not characterized except that it is heat stable and of relatively small size, based on its ability to pass through ultrafine glass filters. Whether MEF is the same as PF cannot be determined without knowing more about the actual composition of both materials. In addition to these \textit{Listeria} components, \textit{Klebsiella pneumoniae} capsular polysaccharides (13) and the nonbacterial chemical dextran sulfate 500 (9) have been shown to reduce resistance to bacterial infection. The actual mechanisms by which these various materials decrease resistance to infection have not been determined, but indirect evidence suggests that at least in some cases phagocytic cells may be involved (10, 14).

However, other mechanisms may also be important. For instance, \textit{Listeria} cell walls can activate complement by the alternative pathway (1), with PF being more active than LCWF. As reported here, PF likewise has greater activity than LCWF in decreasing resistance to infection. This observed correlation plus the fact that dextran sulfate 500 also activates complement (8) suggests indirectly that complement depletion may be involved in the decrease in resistance seen with these two materials. This possibility as well as possible inhibition of phagocyte function by PF is now being investigated. These studies should provide information on mechanisms of resistance to intracellular pathogens and may also clarify whether the phenomenon of decreased resistance induced by \textit{Listeria} cell walls as described in this paper plays a role in pathogenesis by \textit{L. monocytogenes}.

\textbf{ACKNOWLEDGMENTS}

We thank J. E. Caldwell and A. L. Hartman for their excellent technical assistance.

This study was supported in part by Public Health Service grant AI-11240 from the National Institute of Allergy and Infectious Diseases. L.A.B. is the recipient of Public Health Service research fellowship AI-06111 from the same Institute.

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