Reversion of Dextran Sulfate-Induced Loss of Antibacterial Resistance by *Bordetella pertussis*

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Parenteral injection of dextran sulfate 500 (DS 500; 50 mg/kg of body weight) into mice caused a complete loss of resistance to a sublethal (2×10^3 to 5×10^3) infection with *Listeria monocytogenes*. Such loss could be prevented by pretreatment of animals with 3×10^9 heat-killed *Bordetella pertussis* organisms (PO) 5 to 30 days before the administration of DS 500. The increased phagocytic capacity induced by PO was only exhausted when a fourfold dose of DS 500, effecting complete loss of antibacterial resistance (50 mg/kg of body weight), was administered. Listeriosis in mice treated with DS 500 is characterized by rapid-progressive necro-purulent inflammation of liver and spleen, lack of mononuclear phagocyte response, and 100% lethality within 72 h after infection. In contrast, the time course, extent, and morphological characteristics of listeriosis in animals pretreated with PO before the DS 500 application were not significantly different from those of nonpretreated controls. Evidence is presented that the protective effect of PO is due to activation of the mononuclear phagocyte system, which without such treatment is blocked by the DS 500 administration. The data presented indicate that the protective effect of PO is due only in part to the endotoxic moiety of these bacteria. Differences in the course and morphology of listeriosis in animals with dysfunction of the mononuclear phagocyte system and in animals with deficiency of the cellular immune system are discussed.

Immunity to infection with *Listeria monocytogenes*, a facultative intracellular parasite, is based on cellular immune mechanisms. Immunity to this pathogen can only be transferred by specifically sensitized lymphocytes, not by serum (19, 20, 21). In addition to these thymus-derived (T) lymphocytes, activated macrophages are required for effective killing of *L. monocytogenes* (20, 23, 24, 30). Surprisingly, nude mice with congenital dysplasia of the thymus and complete lack of T lymphocytes have recently been found to possess an enhanced bactericidal capacity for these organisms during the initial phase of experimental listeriosis. This resistance is evidently nonspecific and due to the presence of relatively large numbers of activated macrophages in such animals (4, 6, 7, 16). On the other hand, the injection of dextran sulfate 500 (DS 500) into euthymic normal mice causes loss of antibacterial resistance, due to selective damage of mononuclear phagocytes (7, 13, 14).

Adjuvant activity of *Bordetella pertussis* organisms (PO) is assumed to be mediated in part by increased activity of macrophages (27). Moreover, it has been found recently that cell-me-

diated resistance to infection with *L. monocytogenes* can be significantly increased by pretreatment of mice with *B. pertussis* vaccine (H. Hof, P. Emmerling, H. Finger, and C.-H. Wirsing, submitted for publication). Since this bacterial adjuvant is able to overcome the suppression of antibody formation induced by various agents (cf. 8), it appeared meaningful to investigate whether or not PO are likewise capable of overcoming DS 500-mediated loss of antibacterial resistance.

MATERIALS AND METHODS

Mice. Young adult female NMRI mice (specific pathogen free) weighing 24 to 28 g were obtained from the Central Institute for Laboratory Animals, Hannover, Germany.

Infection. A mouse-virulent strain of *L. monocytogenes*, serotype 4b, cultured on tryptose agar, was employed. The virulence of the bacteria used was maintained by continuous passage in mice. The animals were infected with a sublethal dose of 2×10^3 to 5×10^3 viable bacteria, injected intravenously (i.v.) in a constant volume of 0.2 ml of 0.15 M saline.

Enumeration of bacteria in spleens of infected mice. The evaluation of resistance to infection with

L. monocytogenes was made by determining the number of viable organisms present in the spleen at different intervals postinfection (p.i.). For this purpose, the spleens were removed and homogenized in an Omni-Mixer (Sorvall, Newton, Conn.). The homogenate was gradually diluted (10-fold dilution). Each dilution was plated on two replicate dishes with tryptose agar. Colony counts were made after 18 h of incubation at 37°C, and the number of bacteria per spleen was calculated. The significance of the difference of mean values was determined by Student's *t* test.

DS 500. DS 500 (molecular weight, 500,000), purchased from Pharmacia, Uppsala, Sweden, was injected at a dosage of either 50, 100, or 200 mg/kg (body weight). All DS 500 injections were done intraperitoneally (i.p.) in 0.2 ml of 0.15 M saline 24 h before infection with *L. monocytogenes*.

Adjuvants. *B. pertussis* vaccine (OP no. 1207, Behring-Werke, Marburg, Germany) and two preparations of bacterial endotoxins were used as adjuvants. The *B. pertussis* vaccine contained 3×10^{10} PO per ml (phase 1, not adsorbed, killed at 56°C for 30 min in Merthiolate 1:10,000). Immediately before injection, the vaccine was diluted 1:2 with 0.15 M sterile saline. A constant volume of 0.2 ml containing 3×10^9 PO was given at different intervals before infection with *L. monocytogenes*.

Both endotoxins used were injected by the i.p. route 5 days before infection with *L. monocytogenes*. A dose of either 5 or 50 μ g in 0.2 ml of 0.15 M sterile saline was employed. One endotoxin sample was a commercial preparation from *Salmonella typhosa* 0901 (Difco, code 3124) (ST-ET); the other endotoxin was a preparation extracted from *B. pertussis* (OP no. 1207, Behring-Werke, Marburg, Germany) (PO-ET) according to the phenol-water method (29), as described previously (11). The endotoxin content of PO was found to comprise 2.57% of the PO dry weight, a figure comparable to that of other gram-negative bacteria (1.5 to 2.5%). Thus, the PO dose employed in these studies (3×10^9 PO) was calculated to contain 2.3 μ g of endotoxin. Chemical and ultrastructural analysis as well as tests for biological activity revealed that the PO-ET used did not differ significantly from endotoxin preparations of other gram-negative bacteria (11).

Morphological studies. At autopsy, the liver, spleen, pancreas, kidneys, and omentum of all experimental animals were removed, fixed in 4% buffered Formalin, and embedded in paraffin. Histological sections of each of these organs were stained with hematoxylin-eosin (25) and examined. In addition, to follow the fate of DS 500 in vivo, sections of liver and spleen were stained with toluidine blue (25), permitting the detection of DS 500 due to its metachromatic reaction. To compare the extent of the inflammatory processes developing in the four experimental groups, lesions present in liver and spleen were evaluated semiquantitatively, as previously described (15).

RESULTS

Experiment A. Three groups of mice were infected i.v. with 4×10^3 viable *L. monocytogenes* organisms at day 0. The animals of group

I, serving as the controls, received no further injection, whereas the other groups received 24 h before infection an i.p. injection of either 50 mg of DS 500 per kg (group II) or a simultaneous i.p. injection of 50 mg of DS 500 per kg and 3×10^9 PO (group III).

The control mice (group I) showed the typical course of experimental listeriosis (18, 19), with the maximum number of viable listeriae in the spleens on day 4. Thereafter, a rapid decline occurred. In contrast, all animals of the DS 500-pretreated mouse group II died between 24 and 48 h p.i. As compared with mice of group I, the spleens of animals of group II contained significantly ($P < 0.001$) increased numbers of viable listeriae at day 1. The additional injection of PO (group III) did not influence the DS 500-induced increase in susceptibility to infection with *L. monocytogenes*.

Experiment B. In this experiment, three groups of mice were infected i.v. with 2×10^3 listeriae on day 0. Experiment A differed in that animals of group III received the i.p. injection of PO 4 days before treatment with DS 500, i.e., 5 days before infection with *L. monocytogenes*.

All animals of the DS 500-treated group II died within the initial 3-day period p.i. (Fig. 1). In contrast, all mice of group III, pretreated with 3×10^9 PO 4 days before the injection of DS 500, survived. The time course of the infection, as revealed by the number of viable listeriae detectable in the spleen at specific intervals p.i., was very similar to that of the control group I (Fig. 1). This evidently shows that DS 500-induced loss of antibacterial resistance can be completely overcome by pretreatment with PO 4 days before DS 500 application.

FIG. 1. Overcoming of DS 500-mediated loss of antibacterial resistance in mice by PO when given 4 days before DS 500. Five to six mice were used per point.

Experiment C. To elucidate the morphological events underlying the protective effect of PO, as described in experiment B, 96 mice were divided into four equal experimental groups. All animals were infected by the i.v. route with a sublethal dose of 4.5×10^3 viable *L. monocytogenes* organisms. Mice of group I received additionally an i.p. injection of 3×10^9 PO 5 days before infection, and an i.p. injection of 50 mg of DS 500 per kg of body weight 1 day before infection. Mice of group II were pretreated only with an i.p. injection of 50 mg of DS 500 per kg of body weight 1 day before infection, and animals of group III received an i.p. injection of 3×10^9 PO 5 days before infection. Mice of group IV received neither DS 500 nor PO and served as controls. Out of the four experimental groups, three to six mice from each group were sacrificed before infection and 5, 24, 48, and 168 h after infection. *Listeria*-induced morphological changes in mice of group II could not be studied later than 48 h p.i., since 100% of animals were dead 72 h p.i. In contrast, only 10% of mice of group IV died spontaneously within the period of observation, and all animals of groups I and III survived.

Morphological changes prior to infection with *L. monocytogenes*. In contrast to the nonpretreated controls (group IV), animals that had received heat-killed PO (groups I and III) exhibited strong activation of mononuclear phagocytes, such as Kupffer cells, in the liver,

mild diffuse damage of hepatocytes, increased liver cell regeneration, slight follicular hyperplasia of the spleen, and intense granulocytic as well as lymphocytic infiltration of the red pulp. In addition, mice pretreated with PO (i.p.) disclosed considerable fibrinous-purulent peritonitis and pancreatitis, persisting over the whole period of observation. Such changes were not seen in the other experimental animal groups that had not received PO. Histological sections of liver and spleen from mice pretreated with DS 500 (groups I and II), showed most prominently abundant numbers of large mononuclear phagocytes characterized by a foamy vacuolar cytoplasm. Toluidine blue staining revealed that these cells were completely filled with a granular metachromatic material corresponding to DS 500 (Fig. 2). The amount of DS 500 detectable in livers and spleens of mice of group II over the whole period of observation considerably exceeded that in animals of group I, although both had received the same dose of DS 500.

Morphological changes subsequent to infection with *L. monocytogenes*. As evident from the aforementioned data, the internal organs of the four experimental animal groups, following pretreatment with PO and/or DS 500, showed slight but characteristic morphological differences. However, such differences were much more pronounced after infection with *L. monocytogenes*. A general survey of the time course and extent of the *Listeria*-induced in-

FIG. 2. (A) Liver of a mouse 24 h after i.p. injection of DS 500. Enlarged Kupffer cells with vacuolar cytoplasm containing granular metachromatic material. Toluidine blue staining. $\times 640$. (B) Spleen of a mouse 24 h after i.p. injection of DS 500. Numerous large mononuclear phagocytes with foamy cytoplasm. Hematoxylin-eosin staining. $\times 400$.

flammatory processes in liver and spleen of the different experimental animal groups is given in Fig. 3 and 4. Data presented are based on a semiquantitative evaluation of morphological changes, irrespective of their histological character. As evident, the extent and the intensity of lesions in mice pretreated with DS 500 (group II) strikingly exceeded those of the other animal

groups. This was true for both liver (Fig. 3) and spleen (Fig. 4). None of these animals survived more than 72 h. In contrast, all mice that had received PO before the DS 500 application (group I) survived and disclosed lesions equal to or only slightly exceeding those in the controls. The least morphological changes in both liver and spleen were observed (Fig. 3 and 4) in ani-

FIG. 3. Time course and extent of inflammatory lesions in liver of NMRI mice infected with *L. monocytogenes* after pretreatment with PO and/or DS 500. Symbols: ○, ●, △, □: intravenous infection with 4.5×10^3 viable listeriae at day 0; ●, △: intraperitoneal injection of 50 mg of DS 500 per kg at day -1; △, □: intraperitoneal injection of 3×10^6 PO at day -5. Each point represents the mean of results from three to six experimental animals.

FIG. 4. Time course and extent of inflammatory lesions in spleen of NMRI mice infected with *L. monocytogenes* after pretreatment with PO and/or DS 500. Symbols: ○, ●, △, □: intravenous infection with 4.5×10^3 viable listeriae at day 0; ●, △: intraperitoneal injection of 50 mg of DS 500 per kg at day -1; △, □: intraperitoneal injection of 3×10^6 PO at day -5. Each point represents the mean of results from three to six experimental animals.

mals pretreated with PO only (group III). This indicates a protective effect of PO.

In addition to the profound differences in the extent of the inflammatory processes induced by infection with *L. monocytogenes*, significant differences were observed in the histological nature of lesions. Whereas the livers of mice pretreated with DS 500 (group II) 48 h p.i. disclosed innumerable blurred abscesses with perifocal necro-

sis of hepatocytes, the livers of animals that had received PO in addition to DS 500 (group I) at the same time showed only a few sharply demarcated purulent foci (Fig. 5). Furthermore, whereas almost no mononuclear phagocyte response was present in mice pretreated with DS 500 only, significant phagocyte activation was evident in animals pretreated with PO and DS 500. Seven days after infection, purulent lesions

FIG. 5. (A) Liver of a mouse (pretreated with DS 500) 2 days after infection with *L. monocytogenes*. Numerous abscesses with perifocal necrosis of hepatocytes. Weak mononuclear phagocyte response. (B) Liver of a mouse (pretreated with killed PO and DS 500) 2 days after infection with *L. monocytogenes*. Single small purulent lesions. Intense mononuclear phagocyte response. (A) and (B) hematoxylin-eosin staining. $\times 160$, inset $\times 640$.

present in mice of groups I, III, and IV within the first 48 h p.i. were replaced by granulomata primarily made up of macrophages, small numbers of lymphocytes, and single granulocytes (Fig. 6). Basically, the histological character of these granulomata corresponded to that previously described (16).

Morphological differences between the various experimental animal groups were even more pronounced in the spleen than in the liver. Whereas the spleens of mice pretreated with DS 500 (group II) 48 h p.i. disclosed diffuse necropurulent inflammation with severe tissue destruction, this organ in animals that had received PO before the DS 500 application (group I) showed only slight granulocytic infiltration and single small lesions within the red pulp (Fig. 7). In addition, the spleens of all experimental animals exhibited a variable degree of follicular hyperplasia, and the spleens of mice that had received PO exhibited focal accumulations of blastlike cells. None of the animals employed in these studies revealed any pathological changes in the kidneys.

Experiment D. This experiment was performed to determine how long PO were effective. For this purpose, five groups of mice were infected i.v. with 4×10^3 viable listeriae at day 0. Again, the animals of group I served as the controls and received no further treatment. In contrast, mice of groups II to V were pretreated

by an i.p. injection of 50 mg of DS 500 per kg at day -1. In addition, mice of groups III to V had received an i.p. injection of 3×10^8 PO, either 5 (group III), 10 (group IV), or 15 days (group V) before infection with *L. monocytogenes*.

As expected, all mice of the DS 500-treated group II died within the initial 3-day period p.i. (Fig. 8). In contrast, only one mouse in each of groups I, IV, and V died 4 to 8 days p.i. Data presented in Fig. 8 indicate that the numbers of viable listeriae present in the spleens of the PO-treated groups III to V were even reduced, as compared with the control group I. The most pronounced reduction in numbers of listeriae was achieved in mice of group IV, which had received PO 9 days before DS 500, i.e., 10 days prior to infection. In a supplementary experiment, the time interval of pretreatment with PO was extended up to 45 days, showing that the protective effect of PO was still demonstrable when administered as early as 29 days before DS 500 pretreatment, i.e. 30 days prior to infection.

Experiment E. Another point which needed clarification was the question of whether or not pretreatment with PO was still effective when larger doses of DS 500 were injected. This was analyzed by the following experiments employing five groups of mice. Animals of all groups were infected i.v. with 3×10^3 viable *Listeriae* at day 0. Mice of group I received no further

FIG. 6. (A) Liver of a mouse (pretreated with killed PO and DS 500) 7 days after infection with *L. monocytogenes*. Granuloma composed of macrophages and single lymphocytes. (B) Liver of a (nonpretreated) control mouse 7 days after infection with *L. monocytogenes*. Granuloma composed of macrophages, lymphocytes, and single granulocytes. (A) and (B) hematoxylin-eosin staining. $\times 640$.

FIG. 7. (A) Spleen of a mouse (pretreated with DS 500) 2 days after infection with *L. monocytogenes*. Severe necro-purulent inflammation with destruction of red pulp. Slight follicular hyperplasia. (B) Spleen of a mouse (pretreated with killed PO and DS 500) 2 days after infection with *L. monocytogenes*. Mild granulocytic infiltration of red pulp. Slight follicular hyperplasia. (A) and (B) hematoxylin-eosin staining. $\times 400$.

treatment, while the other experimental animal groups were given a single dose of either 50 (groups II and III), 100 (group IV), or 200 mg (group V) of DS 500 per kg by the i.p. route 24 h before infection. In addition, the mice of groups III to V were pretreated by a single i.p. injection of 3×10^9 PO 5 days before infection.

The findings summarized in Fig. 9 clearly indicate that pretreatment of mice with PO was still effective in overcoming the DS 500-induced loss of antibacterial resistance when the dose of DS 500 was increased to 100 mg/kg.

Experiment F. To find out whether or not PO can substitute for bacterial endotoxin, two

sets of experiments were carried out. In the first series, five groups of mice were infected i.v. with 4.5×10^3 viable listeriae at day 0. While animals of group I remained without further treatment, mice of groups II to V were given an i.p. injection of 50 mg of DS 500 per kg 24 h before infection with *L. monocytogenes*. Animals of groups III to V were pretreated additionally with either 50 μ g of ST-ET (group III), 50 μ g of PO-ET (group IV), or 3×10^9 PO (group V) by the i.p. route at day -5.

The schedule of the second test series differed from that of the first series only in that the infective dose comprised 5×10^3 viable listeriae instead of 4.5×10^3 and that mice of groups III and IV were pretreated with 5 μ g of ST-ET (group III) or 5 μ g of PO-ET (group IV) instead of 50- μ g doses.

Pretreatment of mice with 3×10^9 PO again completely neutralized the DS 500-induced loss of antibacterial resistance (Fig. 10). In contrast, 50- μ g doses of both the endotoxin preparations used were effective only to a lesser degree. Numerous mice of groups III and IV succumbed after infection with *L. monocytogenes* (Fig. 10). As indicated by both the mortality rates and the numbers of viable listeriae in the spleens, the capacity of ST-ET to counteract the DS 500 effect was greater than that of PO-ET (Fig. 10). Similar findings were obtained when the endotoxins were administered at a dosage of 5 μ g instead of the 50- μ g dose previously employed. In this instance, PO-ET (group IV) produced

FIG. 9. Influence of PO on the loss of antibacterial resistance produced by different amounts of DS 500 in mice. Symbols: $\circ$, $\bullet$, Δ , $\square$: i.v. infection with 3×10^9 viable listeriae at day 0; $\bullet$, Δ , $\square$: i.p. injection of either 50 ($\bullet$, Δ), 100 (Δ), or 200 ($\square$) mg of DS 500 per kg at day -1; Δ , Δ , $\square$: i.p. injection of 3×10^9 PO at day -5. Five to six mice were used per point.

only a slight prolongation of survival times of the animals. In contrast, the majority of the animals of group III, pretreated with 5 μ g of ST-ET, survived. Taking into consideration that the endotoxin contents of 3×10^9 PO were calculated to comprise 2.3 μ g, the efficiency of 3×10^9 whole PO was significantly greater than that of an equivalent amount of PO-ET. This might indicate that the capacity of intact PO to overcome DS 500-induced loss of antibacterial resistance is not solely due to the endotoxic moiety of the bacteria.

DISCUSSION

Resistance to infection with *L. monocytogenes*, a facultative intracellular parasite, is assumed to be mediated by specifically sensitized T cells and expressed nonspecifically by activated macrophages possessing increased capacity to ingest and kill bacteria (2, 20, 30). Therefore, it may be expected that any reduction of the functional activity of either T cells or macrophages will result in enhanced susceptibility to infection. However, there is evidence that dysfunction of T cells has less dramatic consequences than dysfunction of macrophages. Nude mice with congenital dysplasia of the thymus and complete lack of T lymphocytes possess enhanced bactericidal activity for listeriae during the initial phase of experimental listeriosis (4, 6). This is followed by a state of balance between host and parasite, with survival of the host for at least several weeks (6, 7, 16). Thus, nude mice exhibit signs of a chronic infection, in

FIG. 8. Overcoming of DS 500-mediated loss of antibacterial resistance in mice by PO when administered at different times before DS 500. Symbols: $\circ$, $\bullet$, Δ , $\square$: i.v. infection with 4×10^3 viable listeriae at day 0; $\bullet$, Δ , $\square$: i.p. injection of 50 mg of DS 500 per kg at day -1; Δ , Δ , $\square$: i.p. injection of 3×10^9 PO administered either at day -5 (Δ), day -10 (Δ), or day -15 ($\square$). Five to six mice were used per point.

FIG. 10. Influence of 50- μ g doses of ST-ET and PO-ET on the DS 500-mediated loss of antibacterial resistance. Symbols: ○, ●, △, ▲, □: i.v. infection with 4.5×10^8 viable *Listeria* at day 0; ●, △, ▲, □: i.p. injection of 50 mg of DS 500 per kg at day -1; △, ▲, □: i.p. injection of either 50 μ g of ST-ET (□), 50 μ g of PO-ET (▲), or 3×10^6 PO (△) at day -5. Five to six mice were used per point.

contrast to the characteristic short-term listeriosis developing in phenotypically normal control animals.

On the other hand, blockade of the mononuclear phagocyte system by DS 500 virtually eliminates the mechanisms of defense to infection by *L. monocytogenes* (7, 13, 14, 28), resulting in overwhelming necro-purulent inflammatory processes and death of experimental animals. However, such lethal effects of DS 500 can be completely abolished by pretreatment of animals with PO. This not only increases survival rates from 0 to 100% but also reduces morphological changes to a level more or less identical to that of the controls.

It is well documented (26) that listeriosis in animals possessing an intact mononuclear phagocyte and cellular immune system develops in two distinct phases: an initial phase characterized by logarithmic growth of *Listeria* and presence of polymorphonuclear leukocytes within lesions, and a subsequent phase characterized by killing of bacteria and a predominance of macrophages. The second phase is accompanied by formation of a potent cell-mediated immunity to *Listeria*. In contrast, listeriosis in mice pretreated with DS 500 shows persistence of the logarithmic growth phase, persistence of puru-

lent inflammatory processes (until death), and a lack of mononuclear phagocyte response. The latter appears to be the primary dysfunction induced by DS 500. However, whether this dysfunction of mononuclear phagocytes is simply due to oversaturation with DS 500 or due to damage of these cells by DS 500 (14) remains to be determined. As far as the present studies are concerned, both mechanisms would ultimately produce the same effect: elimination of a cell system that plays a fundamental role in the defense of intracellular parasites such as *L. monocytogenes* (17, 19-21).

As shown in these investigations, DS 500-mediated loss of antibacterial resistance could be completely abolished by pretreatment of animals with PO. The injection of killed PO in humans and animals produces numerous biological phenomena. In mice, leukocytosis, splenomegaly, increase in sensitivity to agents such as histamine or serotonin, induction of nonspecific resistance to infection, potentiation of tumor defense mechanisms, and adjuvant activity are the most prominent features (8, 22). Recent studies (H. Hof et al., submitted for publication) have shown that the time interval between the PO injection and the infection with *L. monocytogenes* is of crucial significance for increased

resistance to *L. monocytogenes*. The optimal interval was found to be 5 to 15 days, although a significant degree of protection was still detectable when PO were given as early as 45 days before infection. In contrast, the simultaneous administration of PO (i.p.) and *Listeria* (i.v.) did not promote increased resistance. Instead, significantly reduced resistance was observed under such conditions (H. Hof et al., submitted for publication). Taking into account that the adjuvant effect of PO is maximum when PO is applied simultaneously with antigen and that the primary immune response is only slightly increased when the injection of PO is separated from the primary antigenic stimulus by more than 24 h (9, 10), one may conclude that the PO-induced resistance to infection and the immunopotentiating activity of PO are based on different mechanisms. There is increasing evidence that adjuvancy is related to or based on cytotoxic activity initiating a nonspecific proliferative impulse (1, 5, 8). Therefore, it is assumed that the recruitment of additional responding cells represents a prerequisite for the adjuvant activity of PO. Although the present studies do not provide direct information on the mechanisms leading to increased antibacterial resistance, there are good reasons to believe that the mononuclear phagocyte might be responsible for the increased resistance to *Listeria* by PO (H. Hof et al., submitted for publication). This assumption is substantiated by the observation that the amount of DS 500 detectable in histological sections of liver and spleen of mice injected with PO in addition to DS 500 was significantly smaller than that in animals receiving DS 500 only. The most plausible explanation for this finding is that PO increases the capacity of the mononuclear phagocyte system, thereby reducing the relative amounts of DS 500 detectable in tissue. This, as well as earlier evidence obtained in nude (4, 6) and T cell-deprived (3) mice, suggests that certain types of antibacterial resistance do not depend on specific cell-mediated immunity.

Furthermore, data presented here indicate that the capacity of PO to overcome the DS 500-induced loss of antibacterial resistance is only in part due to the endotoxin moiety of PO. The endotoxin content of 3×10^9 PO, constantly used throughout these experiments, was calculated to comprise $2.3 \mu\text{g}$ (11). Since the activity of 3×10^9 PO was significantly more pronounced than that of $50 \mu\text{g}$ of endotoxin from either *B. pertussis* or *S. typhosa* (Fig. 10), PO appears to possess further structural components that are active. This is supported by the finding that *B. pertussis* vaccine heated at 80°C for 30 min had significantly reduced protective activity in comparison

with the nonheated *B. pertussis* vaccine, when given 5 days before treatment with DS 500 (i.e., 6 days before infection with *L. monocytogenes*; C.-H. Wirsing and H. Finger, unpublished data). Likewise, the adjuvant activity of whole *B. pertussis* cells was greater than an amount of endotoxin equivalent to the endotoxin contents of those cells (11, 12; M. Pittman, Fed. Proc. 16: 867, 1957). Certainly, further studies are needed to characterize the *B. pertussis* components conferring antibacterial resistance.

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