Biological properties of lipopolysaccharides from *Bordetella* species

MITSURU WATANABE,¹ HIROAKI TAKIMOTO,¹ YOSHIO KUMAZAWA² and KEN-ICHI AMANO³*†

¹The Kitasato Institute and ²School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan
³Department of Bacteriology, Hirosaki University School of Medicine, Hirosaki 036, Japan

(Received 25 July 1989; revised 30 October 1989; accepted 28 November 1989)

Biological activities of lipopolysaccharides (LPS) extracted from *Bordetella* pertussis, *B. parapertussis* and *B. bronchiseptica* were compared with those of *Escherichia coli* LPS. The LPS preparations from *B. pertussis* showed biological activities comparable to those of *E. coli* LPS in terms of lethal toxicity in galactosamine-sensitized mice, pyrogenicity in rabbits, mitogenicity in C3H/He spleen cell cultures, macrophage activation, and induction of tumour necrosis factor. All the activities of LPS preparations from *B. parapertussis*, except mitogenicity, were lower than those of *E. coli* LPS. LPS from *B. parapertussis* gave the greatest mitogenic action of all those tested. Biological activities stronger than or comparable to those of *E. coli* LPS were observed for LPS from *B. bronchiseptica*.

Introduction

Lipopolysaccharides (LPS) extracted from Gram-negative bacteria are potent molecules which elicit in animals a range of characteristic responses. These are often due to stimulation of host mediators such as interleukin-1 and tumour necrosis factor (TNF) (Haefner-Cavaillon et al., 1984; Nowotny, 1985). Among these responses are the induction of the Shwartzman reaction (Movat & Burrowes, 1983), antitumour effects (Freinman et al., 1987) and adjuvant activity (McGhee et al., 1980).

LPS from *Bordetella pertussis* also exhibits the biological activities described above (Chaby & Caroff, 1988), although lipid A derived from this LPS is non-toxic and does not induce the Shwartzman reaction (Ayme et al., 1980). There is little information on the biochemical, immunological and biological properties of LPS from the other two species of *Bordetella*, namely *B. parapertussis* and *B. bronchiseptica*. In the preceding paper (Amano et al., 1990) we described the biochemical and immunological properties of preparations of LPS from the three species of *Bordetella*, and in the present paper we give some of their biological activities, i.e. lethal toxicity in galactosamine-sensitized mice, pyrogenicity, mitogenicity, macrophage activation and TNF-inducing activity. These activities of *Bordetella* LPS were compared to those of *Escherichia coli* LPS.

Methods

Organisms and extraction of LPS fractions. *B. pertussis* strains Tohama [phase I, Bp(l(Tohama)) and AK-168 [phase I, Bp(lAK-168)], *B. parapertussis* strains 21815 [Bp(21815)] and AK-167 [Bp(21815)], and *B. bronchiseptica* strains L3 [Bbs(L3)] and H-214 [Bbs(H-214)] were obtained from our laboratory stock. Cultivation and purification of *Bordetella* strains, extraction of LPS with hot phenol/water, and purification by ultracentrifugation and by treatment with proteolytic enzymes were carried out as described previously (Amano et al., 1990). LPS from *E. coli* O55:B5 was purchased from Difco.

Determination of lethal toxicity. This was done by the method described by Galanos et al. (1979). Various concentrations of LPS in 0.2 ml water were injected intravenously (i.v.) into male 10-week-old C57Bl/6 mice (Shizuoka Animal Center, Shizuoka, Japan) immediately after intraperitoneal (i.p.) administration in 0.5 ml of pyrogen-free saline containing 10 mg b-galactosamine (Sigma).

Determination of pyrogenicity. Pyrogenicity was determined in Japanese white rabbits (2.1–2.4 kg) (Gokita Breeding Service, Tokyo, Japan) of the same sex in groups of three for each dose to be tested, as described by Kumazawa et al. (1988). The sample was injected i.v. into three rabbits. Rectal temperature was monitored continuously for 3 h, and the maximal increase in temperature was recorded for each rabbit.

Measurement of limulus amoebocyte lysate (LAL) gelation. LAL gelation activity was tested using Pregel/S reagent (Seikagaku Kogyo Co.).
**Measurement of macrophage activation.** This was done by measuring phagocytic, cellular lysosomal enzyme (acid phosphatase), and cytostatic activities of macrophages obtained from the peritoneal cavity of ICR mice (Shizuoka Animal Center) which had been injected i.p. with 1 µg of test sample 4 d earlier, as described by Kumazawa et al. (1988).

Phagocytic activity was assessed by measuring the radioactivity of $^{51}$Cr-labelled, antibody-sensitized sheep erythrocytes which were phagocytosed by in vitro-stimulated peritoneal macrophages. Acid phosphatase activity was measured as follows. Peritoneal macrophages stimulated in vitro were lysed in 96-well microplates with 0.1% Triton X-100, and 20 µl of sodium acetate buffer (pH 5.0) plus 20 µl of 24 mM-p-nitrophenol phosphate were added to the macrophage lysates. After incubation at 37°C for 30 min, 200 µl of 0.2 M sodium carbonate was added to the reaction mixture, and the A$_{405}$ was measured. Enzyme activity was calculated from a standard curve made using known amounts of potato acid phosphatase. Cytostasis-inducing activity was assessed by measuring the growth-inhibitory action against EL-4 lymphoma cells. The activity was calculated as the percentage inhibition of $[^3H]$thymidine incorporation into EL-4 cells.

Assay of TNF-inducing activity. This was done by the method described by Kumazawa et al. (1988). Briefly, 10 µg LPS was administered i.v. into ICR mice (Charles River Japan, Atsugi, Japan) primed with Propionibacterium acnes. Sera were separated from blood obtained 1.5 h after administration of LPS and treated at 56°C for 30 min before use. The TNF activity in the sera was determined by measuring the percentage inhibition of $[^3H]$thymidine uptake into L929 cells.

**Results**

Toxicity, pyrogenicity and LAL gelation activity

Toxicity in galactosamine-sensitized mice is shown in Table 1. Among the preparations of LPS tested, Bbs(L3) was the most toxic, while Bbs(H-214) LPS and two samples of B. pertussis LPS possessed toxicity similar to that of E. coli LPS. Weak toxic activity was shown for two preparations from B. parapertussis.

The smallest dose of LPS that consistently evoked a rise in body temperature (over 0.6°C per rabbit) was 1 ng kg$^{-1}$ for Bbs(L3), and 10 ng kg$^{-1}$ for Bpl(TOHAMA), Bbs(H-214) and E. coli LPS; for Bpl(AK-168), Bpp(21815) and Bpp(AK-167) a dose of 100 ng kg$^{-1}$ was required to induce a fever response (Table 1).

In the LAL gelation test, all the LPS preparations from Bordetella species showed activity the same as [Bpl(AK-168), Bpp(21815), Bpp(AK-167) and Bbs(H-214)] or stronger than [Bpl(TOHAMA) and Bbs(L3)] the LPS from E. coli (Table 1).

**Macrophage activation**

This was determined by measuring phagocytosis of $^{51}$Cr-labelled antibody-sensitized sheep erythrocytes (Fig. 1a), increased level of cellular lysosomal enzyme (acid phosphatase) (Fig. 1b), and induction of cytostasis (Fig. 1c). All the LPS preparations from Bordetella species induced significant macrophage activation at a dose of 1 µg per mouse. At this dose level, the most active LPS was Bbs(L3), which had an activity comparable to that of E. coli LPS. The two LPS preparations from B. pertussis and that from Bbs(H-214) were weaker than the LPS from E. coli, and the activities of the two preparations from B. parapertussis were low.

**TNF-inducing activity**

The two preparations of LPS from B. pertussis and the two from B. bronchiseptica exhibited TNF-inducing activity comparable to that of LPS from E. coli (Fig. 2). However, the two preparations from B. parapertussis were less active than LPS from E. coli.

**Mitogenic activity**

The LPS preparations from Bpl(TOHAMA), two strains of B. parapertussis and Bbs(H-214) showed much higher mitogenic activity than E. coli LPS in C3H/He spleen cell cultures at a concentration of 10 ng per 0.1 ml per well (Fig. 3). The mitogenic activity of LPS from Bpl(AK-168) and Bbs(L3) was comparable to that of LPS from E. coli. None of the LPS preparations from Bordetella species showed significant activity in C3H/HeJ mice at the same concentration as used in C3H/He mice (data not shown).

**Discussion**

LPS from B. pertussis is known to have activities similar to those of the LPS from Gram-negative enterobacteria. The endotoxic activities of LPS, such as fever, Schwartzman reactivity and depression of blood pressure leading to shock and death, are produced by the endotoxin from B. pertussis (Finger et al., 1976; Ayme et al., 1980). Our data demonstrate the toxicity of LPS from other Bordetella species. The LPS preparations from B. bronchiseptica were similar in toxicity to LPS from B. pertussis and E. coli, whereas LPS from B. parapertussis had lower toxicity.

Amano et al. (1990) showed molecular mass differences of LPS from three species of Bordetella by SDS-PAGE: LPS from B. pertussis contained two low molecular mass bands, LPS from B. parapertussis...
Biological properties of Bordetella LPS

Fig. 1. Potency of peritoneal macrophages activated in vivo with LPS from Bordetella species or E. coli. Peritoneal macrophages were obtained from ICR mice injected i.p. with 1 μg LPS 4 d before. Control experiments were done with macrophages from unstimulated mice. The results are means ± standard deviation of three separate experiments. (a) Phagocytic activity measured using 51Cr-labelled, antibody-sensitized sheep erythrocytes. (b) Acid phosphatase activity of lysates of 1 × 10^5 cells determined as A_{405} using p-nitrophenyl phosphate as substrate. (c) Cytostatic activity measured using macrophages co-cultivated for 40 h with target EL-4 lymphoma cells in an effector:target cell ratio of 10:1. The activity was assessed by the degree of inhibition of [3H]thymidine uptake into the target cells.

Fig. 2. TNF-inducing activity of LPS from Bordetella species or E. coli. LPS was administered i.v. at a dose of 1 μg into ICR mice primed with Propionibacterium acnes. Sera were obtained i.v. 1-2 h after administration of LPS. TNF activity was expressed as the percentage inhibition of [3H]thymidine uptake into L929 cells. (a) O, BpI(TOHAMA) LPS; △, Bpp(21815) LPS; □, Bbs(L3) LPS; ●, E. coli LPS. (b) O, BpI(AK-168) LPS; △, Bpp(AK-167) LPS; □, Bbs(H-214) LPS; ●, E. coli LPS. The results are from a single experiment.

controlled ladder structural bands in the high molecular mass area and a single low molecular mass band, and LPS from B. bronchiseptica contained ladder structural high molecular mass bands and two low molecular mass bands. Peppler (1984) and Li et al. (1988) also demonstrated the presence of two different LPS components in B. pertussis, by SDS-PAGE and immunoblotting. These physical differences could be reflected in the difference of the toxicity among Bordetella LPS.

Lehmann et al. (1987) reported that TNF may be a mediator of toxicity of LPS from Gram-negative bacteria in galactosamine-sensitized mice. Our data, showing that the TNF-inducing activity of Bordetella LPS correlates with toxicity, support this idea. The macrophage activation ability of the various preparations of Bordetella LPS also correlated with their toxicity. Lasfargues et al. (1986) also reported that B. pertussis endotoxin is able to induce in mouse peritoneal macrophages cytostatic activity against tumour cells, and that this activity was due to the lipid region of the endotoxin.

Endotoxins from B. pertussis are known to be potent mitogens and polyclonal activators for murine B lymphocytes (Girard et al., 1981; Haeffner-Cavaillon et al., 1982). Girard et al. (1981) reported that B. pertussis LPS induces a mitogenic response in C3H/HeJ B lymphocytes (which are unresponsive to enterbacterial LPS) as
Fig. 3. Mitogenic activity of LPS from Bordetella species or E. coli. C3H/He spleen cells were stimulated for 48 h with the indicated dose of LPS. Mitogenic activity is expressed as mean [3H]thymidine uptake of triplicate cultures. (a) ●, BpI(TOHAMA) LPS; □, BpI(AK-168) LPS; ○, E. coli LPS. (b) ○, Bpp(21815) LPS; □, Bpp(AK-167) LPS; ●, E. coli LPS. (c) ○, Bbs(H-214) LPS; □, Bbs(L3) LPS; ●, E. coli LPS.

Table 1. Endotoxic activities of LPS preparations from Bordetella species and E. coli

<table>
<thead>
<tr>
<th>LPS</th>
<th>Lethal toxicity</th>
<th>LAL gelation activity</th>
<th>Pyrogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP~(TOHAMA)</td>
<td>1</td>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>BpI(AK-168)</td>
<td>1</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Bpp(21815)</td>
<td>0.1</td>
<td>0.01</td>
<td>100</td>
</tr>
<tr>
<td>Bpp(AK-167)</td>
<td>0.1</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Bbs(L3)</td>
<td>0.1</td>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>Bbs(H-214)</td>
<td>1</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

* The minimal lethal dose (MLD) was determined by using six galactosamine-sensitized mice for each dose of LPS preparation.

† The minimal effective dose (MED) causing pyrogenicity was determined by using three Japanese white rabbits for each dose of LPS preparation.

well as in C3H/He B lymphocytes. We also demonstrated mitogenic activity in LPS preparations of B. parapertussis, B. bronchiseptica and B. pertussis, although at the LPS concentrations tested, only cells from C3H/He mice, and not C3H/HeJ mice, responded. The mitogenicity of two LPS preparations from B. parapertussis was much stronger than that of E. coli, while the LPS from Bbs(L3) showed comparable mitogenicity to LPS from E. coli. The results on mitogenic activity do not correlate with the other activities of LPS tested, which may indicate that a different mechanism is involved in the induction of mitogenicity and the other biological activities of LPS tested in this work.

The authors wish to thank Dr John J. Munoz for reviewing the manuscript.

References


Biological properties of Bordetella LPS


