

Anaphylactic reactions in mice induced by *Bordetella pertussis* lipopolysaccharide

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Ten days after the parenteral administration of *Bordetella pertussis* vaccine and a heterologous protein, anaphylaxis can be demonstrated in the normally resistant mouse.¹ A heightened sensitivity to the lethal effects of histamine² and serotonin³ which reaches its maximum four days posttreatment concomitantly results. Subsequent studies showed that the sensitizing properties were associated with a soluble extractive, for they were inherent in the cell-free culture supernatant⁴ and in the aqueous extract of acetone-treated organisms.⁵ The present report presents data on sensitization utilizing the isolated (lipo) polysaccharide endotoxic moiety from *B. pertussis*. In addition, studies have been carried out with endotoxins from unrelated gram-negative organisms, natural and synthetic polysaccharides, etc.

MATERIALS AND METHODS

Female white mice of the Swiss-Webster strain, weighing about 18 grams at the start of each experiment, were used exclusively. For sensitization, *B. pertussis*, 6×10^9 organisms, was injected intraperitoneally (i.p.) in 0.5 ml. volume, either alone or with one of these added antigens—0.03 ml. horse serum (H.S.) or 1 mg. recrystallized ovalbumin (Ea). Various concentrations of bacterial endotoxins, lipopolysaccharides, soluble extractives, polyglucoses, dextrans, levans, blood group substances, etc.*, were substituted for the *B. pertussis* in subsequent experiments. In order to equate better the results of the experiments

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Table I. *Effect of lipopolysaccharides on sensitivity to histamine*

<i>Sensitizing agent</i>	<i>Amount (i.p.)[†]</i>	<i>Histamine mortality*</i>
Control	—	1/42
<i>B. pertussis</i> organisms	6×10^9	42/49†
<i>B. pertussis</i> lipopolysaccharide	100γ	16/50†
<i>B. pertussis</i> lipopolysaccharide	200γ	5/20†
<i>B. bronchisepticus</i> lipopolysaccharide	50γ	1/13
<i>B. bronchisepticus</i> lipopolysaccharide	200γ	1/8
<i>H. influenzae</i> organisms	8.75×10^9	0/22 (Ref. 7)
<i>B. abortus</i> organisms	2×10^9	42/83 (Ref. 9)†
<i>B. abortus</i> lipopolysaccharide	200γ	8/34 (Ref. 9)†
<i>S. marcescens</i> lipopolysaccharide	200γ	7/27†
<i>S. typhosa</i> lipopolysaccharide	200γ	17/40†
<i>E. coli</i> 08 endotoxin	100γ	2/9
<i>E. coli</i> 08 endotoxin	200γ	8/18†
Pyrexal	100γ	1/8
Pyrexal	200γ	1/13
Piromen	200γ	3/15
Pn I polysaccharide	200γ	2/20
2-Deoxy-d-glucose	200γ	0/15
d (+) Glucosamine	200γ	0/15
Sulfopolyglucin	200γ	0/10
"A" blood group substance	0.11 ml.	5/10†
"B" blood group substance	0.15 ml.	0/10
"A" and "B" blood group substance	0.10 ml.	4/10†
"A" and "B" blood group substance	1.0 ml.	1/10

*Challenge, 36γ histamine phosphate per gram mouse given intraperitoneally on the fourth day subsequent to the sensitizing inoculation. Mortality, number of animals dead per number challenged.

†These values differ from the control with statistical significance, $p = < 0.01$.

‡ $p = < 0.05$.

on sensitization, toxicity studies were carried out by injecting mice either intra-peritoneally or intravenously with a given endotoxin at several dosage levels.

RESULTS

Enhancement of histamine sensitivity

Mice were challenged with 36 γ histamine phosphate per gram given intra-peritoneally on the fourth day subsequent to the sensitizing inoculation. This dose is approximately the LD₅₀ for pertussis-sensitized mice and is not lethal to untreated controls. A 32 per cent mortality was obtained for mice pre-treated with 100 γ *B. pertussis* lipopolysaccharide (BPL). The results of pre-treatment with other agents and their statistical significance as determined by the χ^2 test can be seen in Table I.

*We are indebted for these materials to the following: *B. pertussis* endotoxin, Mr. A. Perrault, National Cancer Institute; *Serratia marcescens* polysaccharide, *Brucella abortus* lipopolysaccharide, and *Salmonella typhosa* endotoxin, Difco Laboratories; Vi antigen (*Paracolobactrum ballerup*), Dr. E. E. Baker, Boston University School of Medicine; Pneumococcus type I polysaccharide, E. R. Squibb and Sons; Piromen (*Pseudomonas*), Travenol Laboratories, Inc.; *Escherichia coli* 08 endotoxin and Pyrexal (*Salmonella abortus equi*), Dr. O. Westphal, Dr. A. Wander Forschungsinstitut; *Bordetella bronchisepticus* lipopolysaccharide, Dr. A. P. MacLennan, Microbiological Research Establishment; dextrans and levans, Dr. A. Jeanes, United States Department of Agriculture; polyglucoses, Merck Sharp & Dohme; sulfopolyglucin, Riker Laboratories, Inc. The following were purchased on the open market: 2-deoxy-d-glucose and d(+) glucosamine, Nutritional Biochemicals Corp.; "A" and "B" blood group substances, Knickerbocker Biologicals, Inc., and Merck Sharp & Dohme.

Enhancement of anaphylactic sensitivity

On the tenth (or fifteenth) day subsequent to sensitization with the pertussis-antigen mixture, mice were challenged by the following amounts of homologous antigen, given intravenously: 0.1 ml. H.S. or 1 mg. Ea in 0.1 ml. saline. With either antigen a mortality of about 92 per cent was obtained. Table II gives the mortality rates obtained by using other "adjuvants."

The substitution of BPL or one of the other bacterial endotoxins for *B. pertussis* did not materially alter the sensitizing capacity of the mixture. However, substitution by the highly purified Vi antigen or Pn I polysaccharide was ineffective in enhancing anaphylactic reactivity.

Challenge of pertussis-treated mice with heterologous polysaccharides

On the tenth (or fifteenth) day subsequent to the initial inoculation of *B. pertussis*, mice were given an intravenous injection of either BPL or one of the other bacterial endotoxins, lipopolysaccharides, polyglucoses, etc. All the bacterial extractives, to some degree, induced an anaphylaxis-like fatal reaction except for the Vi antigen and Pn I polysaccharide. None of the other products tested produced deaths. The data are presented in Table III.

Table II. *Enhancement of anaphylactic sensitivity by lipopolysaccharides*

Adjuvant	Amount (i.p.)	Mortality*	
		H.S.	Ea
Control (no adjuvant)	—	3/66 (Ref. 7)	0/20 (Ref. 9)
<i>B. pertussis</i> organisms	6×10^9	59/64	22/24
<i>B. pertussis</i> lipopolysaccharide	100 γ	7/10	27/58
<i>B. pertussis</i> lipopolysaccharide	200 γ	5/15	
<i>B. bronchisepticus</i> lipopolysaccharide	50 γ	6/20	
<i>B. bronchisepticus</i> lipopolysaccharide	100 γ	3/19	
<i>B. bronchisepticus</i> lipopolysaccharide	200 γ	0/14	
<i>H. influenzae</i> organisms	8.75×10^9	1/12 (Ref. 7)	
<i>B. abortus</i> organisms	2×10^9	11/55 (Ref. 9)	5/10 (Ref. 9)
<i>B. abortus</i> lipopolysaccharide	100 γ		14/15 (Ref. 9)
<i>B. abortus</i> lipopolysaccharide	200 γ		20/33 (Ref. 9)
<i>S. marcescens</i> lipopolysaccharide	200 γ		13/20
<i>S. typhosa</i> lipopolysaccharide	200 γ		39/60
<i>S. typhosa</i> lipopolysaccharide	250 γ	5/15	
<i>S. typhosa</i> lipopolysaccharide	500 γ	3/7	
<i>E. coli</i> 08 endotoxin	200 γ	4/10	
Pyrexal	200 γ	6/12	
Piromen	200 γ	14/15	
Pn I polysaccharide	200 γ	1/18	
Vi antigen (<i>P. ballerup</i>)	100 γ		0/11
Vi antigen (<i>P. ballerup</i>)	500 γ		2/30
2-Deoxy-d-glucose	200 γ	1/15	
2-Deoxy-d-glucose	5 mg.	3/15	
d (+) Glucosamine	200 γ	1/15	
Sulfopolyglucin	200 γ	0/12	
"A" blood group substance	0.11 ml.		0/14
"B" blood group substance	0.15 ml.		3/19
"A and B" blood group substance	0.10 ml.	4/15	
"A and B" blood group substance	1.0 ml.		1/19

*Challenge dose of homologous antigen (0.1 ml. H.S. or 1 mg. Ea) given 10 days after sensitization with adjuvant and antigen. Mortality, number of animals dead per number challenged.

Table III. *Mortality in mice induced by polysaccharides 10 days after treatment with B. pertussis*

Challenge agent	Amount of i.v. challenge	Mortality*
<i>B. pertussis</i> lipopolysaccharide	100γ	7/11
<i>B. pertussis</i> lipopolysaccharide	200γ	7/10
<i>B. bronchisepticus</i> lipopolysaccharide	50γ	10/10
<i>B. abortus</i> lipopolysaccharide	200γ	10/10
<i>S. marcescens</i> lipopolysaccharide	200γ	7/10
<i>S. typhosa</i> lipopolysaccharide	200γ	9/10
<i>E. coli</i> 08 endotoxin	100γ	9/10
Pyrexal	100γ	7/9
Piromen	200γ	4/11
Pn I polysaccharide	200γ	0/10
Vi antigen (<i>P. ballerup</i>)	500γ	0/10
2-Deoxy-d-glucose	5 mg.	0/6
2-Deoxy-d-glucose	200γ	0/11
d (+) Glucosamine	200γ	0/11
Sulfopolyglucin	200γ	0/12
Polyglucose	200γ	1/10
“A and B” blood group substance	0.1 ml.	0/9
Dextran, Strept. sp.	200γ	0/9
Dextran, Strept. sp. (deproteinized)	200γ	0/12
Dextran, <i>Streptobacterium dextraniceum</i> (L)	200γ	0/9
Dextran, <i>Streptobacterium dextraniceum</i> (S)	200γ	0/9
Dextran, <i>Streptobacterium dextraniceum</i> (B-2-b)	200γ	0/9
Dextran, <i>L. mesenteroides</i> (B-1396)	200γ	1/9
Dextran, <i>L. mesenteroides</i> (B-1399)	200γ	0/10
Dextran, <i>L. mesenteroides</i> (B-1424)	200γ	0/10
Dextran, <i>L. mesenteroides</i> (B-1355)	200γ	0/10
Dextran, <i>L. mesenteroides</i> (B-742 S)	200γ	0/9
Dextran, <i>L. mesenteroides</i> (B-742 B-2b)	200γ	0/10
Dextran, <i>L. mesenteroides</i> (B-742 L-R)	200γ	0/8
Dextran, <i>L. mesenteroides</i> (B-512 B-2-b)	200γ	0/9
Dextran, <i>L. mesenteroides</i> (B-512 S-R)	200γ	0/9
Levan, Bacillus sp.	200γ	0/10
Levan, <i>Erwinia ananas</i>	200γ	0/10
Levan, <i>L. mesenteroides</i>	200γ	0/9

*Mortality, number of animals dead per number challenged.

In like manner, pretreatment of mice with BPL, the polysaccharide from *S. marcescens* or *S. typhosa* endotoxin, did not sensitize; intravenous challenge with the homologous antigen failed to evoke deaths on the tenth day subsequent to the initial inoculation.

Toxicity

The mortality induced by inoculation of the polysaccharide under discussion was considered as a measure of the toxicity. The values obtained by both the intraperitoneal and intravenous administration are shown in Table IV.

DISCUSSION

Not only is the mechanism by which *B. pertussis* increases the susceptibility of the white mouse to the lethal effects of histamine and serotonin obscure,⁶ but also the relationship between anaphylaxis and these mediators remains conjectural.⁷⁻⁹ Since the capacity to sensitize is unique to *B. pertussis* phase I organisms, the isolation of a homogeneous entity possessing these character-

Table IV. Toxicity of lipopolysaccharides in mice

Substance	Amount	Mortality* from i. p. injection	Mortality* from i. v. injection
<i>B. pertussis</i> lipopolysaccharide	100γ	5/109	0/9
<i>B. pertussis</i> lipopolysaccharide	200γ	0/20	1/10
<i>B. bronchisepticus</i> lipopolysaccharide	50γ	2/25	0/5
<i>B. bronchisepticus</i> lipopolysaccharide	100γ	11/30	
<i>B. bronchisepticus</i> lipopolysaccharide	200γ	25/40	
<i>B. abortus</i> lipopolysaccharide	100γ	1/16	
<i>B. abortus</i> lipopolysaccharide	200γ	22/64	0/14
<i>S. mercrescens</i> lipopolysaccharide	200γ	13/40	0/13
<i>S. typhosa</i> lipopolysaccharide	200γ	2/80	2/14
<i>S. typhosa</i> lipopolysaccharide	250γ	5/10	
<i>E. coli</i> 08 endotoxin	100γ	2/20	1/10
<i>E. coli</i> 08 endotoxin	200γ	12/30	
Pyrexal	100γ	12/26	1/10
Pyrexal	200γ	17/30	
Piromen	200γ	0/26	1/10
Pn I polysaccharide	200γ	0/20	
Vi antigen (<i>P. ballerup</i>)	100γ	0/11	
Vi antigen (<i>P. ballerup</i>)	500γ	0/11	

*Mortality, number of animals dead per number challenged.

istics could be useful as an investigative tool. In this regard, Dolby¹⁰ was able to distinguish the histamine-sensitizing factor (HSF) from the two types of protective antigen. On the other hand, Munoz¹¹ subsequently reported that the mouse protective activity was found in the fractions containing HSF activity.

The endotoxins of certain gram-negative organisms have been shown to exert an adjuvant action in rabbits, enhancing antibody production.¹² Although preliminary studies in mice indicated that *S. typhosa* endotoxin neither engendered a heightened anaphylactic response nor enhanced histamine sensitivity,¹³ it was subsequently determined that antibody titers were elevated by the use of the lipopolysaccharide.¹⁴

Although antibody is probably necessary for gross anaphylaxis, treatment with pertussis vaccine produces an increased reactivity or sensitivity to antigen-antibody complexes separate from any action the vaccine may have on antibody formation.¹⁵ Both a cell-free culture supernatant⁴ and an aqueous extract of *B. pertussis*⁵ were found effective in enhancing anaphylactic sensitivity as well as decreasing the resistance of the mouse to histamine. Lipopolysaccharide is commonly found in both fractions above. Studies with *B. pertussis* endotoxin showed that this lipopolysaccharide was capable of engendering these sensitivities, although neither effect was produced to the same degree as by the vaccine.

The present studies indicate that the endotoxins of several gram-negative organisms also can induce sensitization. However, Pyrexal (from *S. abortus equi*) and Piromen (from *Pseudomonas*) failed to decrease histamine resistance. Since these fractions are apparently devoid of HSF, they might conceivably be used to probe into the mechanism of the development of anaphylactic sensitivity. Unfortunately, Pyrexal is relatively toxic in the required dose and Piromen is not a single lipopolysaccharidic entity.¹⁶ All the endotoxins except Piromen and the lipopolysaccharide of *B. pertussis* were toxic when given intra-

peritoneally. The relationship between toxicity and ability to sensitize has not been established, but it exaggerates the difference between *B. pertussis* and the other organisms studied. However, toxicity is less apparent when the endotoxins are given intravenously.

The death which follows intravenous challenge of pertussis-treated mice resembles the anaphylactic reaction. It has been suggested that gram-negative bacteria contain a common antigenic substance^{17, 18} indicating a true cross-reactivity reaction. However, *H. influenzae*, which is antigenically related to *B. pertussis*, has been found incapable of sensitization.¹⁹ The lipopolysaccharide of *S. marcescens* fails to evoke passive cutaneous anaphylaxis with an antiserum to *B. pertussis*.²⁰ The lipopolysaccharide of *B. pertussis* gives a single line when tested against this antiserum by the Ouchterlony agar plate technique, although the endotoxins of *S. marcescens*, *B. abortus*, and *S. typhosa* fail to precipitate.²⁰

The endotoxins are apparently species specific with the polysaccharide entity as the determinant hapten. Westphal and Lüderitz²¹ have indicated that the lipid moiety is common to the endotoxins of the different types of gram-negative organisms. None of the other polysaccharides studied, including the synthetics and those obtained from native sources, showed any cross-reactivity. In addition, none of the latter are toxic, and none contain lipid. The integrity of the lipid may well be a determinant in identifying the capacity to sensitize, even as it is a prerequisite for the toxic effects of endotoxin. The superiority of lipopolysaccharides in stimulating immunity (and sensitivity) has been considered as being determined by the lipid A content.^{22, 23} The lipid, however, is not strongly haptenic and fails to contribute to serologic cross-reactions in vitro.

The data in Tables I and II indicate that the larger doses of lipopolysaccharide from both *B. pertussis* and *B. bronchisepticus* seem to engender a lesser sensitivity reaction. This is not an unusual paradox in biologic systems. Comparable findings were described by Barnes and Holt,²⁴ who studied the adjuvant effect of *B. pertussis* on diphtheria toxoid and concluded that there was an optimum ratio of pertussis to toxoid above which the adjuvant effect was decreased. Farthing²⁵ described similar experiences with *B. pertussis* and its lipopolysaccharide. The relationship between adjuvance and interference seems to be a quantitative one. This concept is supported by the studies of Biozzi and co-workers²⁶ and Jenkin and Rowley²⁷ on the RES. An optimum amount of bacteria or endotoxin is probably necessary for adjuvance with a greater quantity impairing antibody production.

SUMMARY

The lipopolysaccharide of *Bordetella pertussis* is capable of engendering a heightened sensitivity to histamine in the white mouse, although not to the same degree as the intact bacteria. A somewhat parallel anaphylactic sensitivity results concomitantly from the administration of a mixture of the endotoxin and a heterologous protein. The lipopolysaccharides of certain gram-negative organisms similarly possess this capacity to sensitize. Mice pretreated with *B. pertussis* will show fatal anaphylaxis-like reactions when challenged by the intravenous injection of these lipopolysaccharides.

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