

Properties of the immunogenic and sensitizing activities of *Bordetella pertussis* in mice

II. Enhancement of susceptibility to bacterial endotoxins

Robert E. Pieroni and Leo Levine, Boston, Mass., with the technical assistance of Edward J. Broderick and Anthony Pellegrino

*The component of *Bordetella pertussis* that enhances the susceptibility of mice to challenge with homologous and heterologous endotoxin parallels the protective antigen and histamine-sensitizing factor (HSF) during chemical fractionation of pertussis vaccine. This finding, together with evidence of the lability of all three of the above activities to heat and trypsin, adds another to the lengthening list of sensitizing activities of pertussis vaccine in mice that share these same properties. This is consistent with our thesis that all of the diverse sensitizing activities of pertussis vaccine are functions of the immunogen itself. The induction by endotoxin of a state of partial tolerance to a subsequent injection of a lethal dose of endotoxin was confirmed. This, in addition to the findings of others, would appear to eliminate the lipopolysaccharide component of pertussis vaccine as the agent that elicits hyper-reactivity to bacterial endotoxins. It is postulated that the combined effects on the autonomic nervous system of the sensitizing component in pertussis vaccine and the challenge endotoxin itself are responsible for this hypersensitivity state in mice.*

One of the many well-known sensitizing effects of pertussis vaccine for the mouse is the enhancement of its susceptibility to the endotoxins of gram-negative organisms, including that of the pertussis bacillus itself.^{1, 2} The component of pertussis vaccine responsible for this effect and its mechanism of action are unknown. We have shown that some of the sensitizing activities of pertussis

From the Division of Biologic Laboratories, Institute of Laboratories, Massachusetts Department of Public Health, and the Department of Microbiology, Harvard School of Public Health, Boston, Mass.

Supported by United States Public Health Service Research Grant AI-04195 from the National Institute of Allergy and Infectious Diseases through the Massachusetts Health Research Institute.

Received for publication July 20, 1966.

vaccine have certain physicochemical properties in common, namely, heat lability, susceptibility to tryptic degradation, and parallel fractionation.^{3, 4} The latter refers to the fact that these activities distribute similarly among fractions when the fractionation of pertussis vaccine is carried out.

Kind¹ has already shown that the ability of pertussis vaccine to enhance the susceptibility of mice to endotoxins can be abolished by heating the vaccine at 80° C. for 30 minutes. We have found a reduction of this activity of the vaccine following treatment with trypsin.⁵ The following experiments demonstrate parallel fractionation of three activities: immunogenicity, histamine sensitization, and enhancement of endotoxin lethality. The latter activity thus takes its place with those that have been shown to share some or all of the same properties, including immunogenicity,^{4, 6} sensitization to histamine,³ enhancement of peptone shock,⁴ induction of hyperacute experimental allergic encephalomyelitis,⁷ and adjuvance for unrelated antigens.⁸ These findings are consistent with our working hypothesis that all of the diverse sensitizing activities of pertussis vaccine are functions of the immunogen itself.

EXPERIMENTAL METHODS

These experiments utilize the final precipitate and supernatant produced by adding ammonium sulfate (to 35 per cent saturation) to clarified vaccine lysate.⁶ We have shown that this precipitate contains most of the immunogen and the histamine-sensitizing factor (HSF), whereas the supernatant, although containing the bulk of the organic bacterial substance, has minor amounts of these activities. The object of the experiment was to compare these two fractions, using intraperitoneal injection in mice, with respect to all three activities: immunogenicity, histamine-sensitization, and increased susceptibility to endotoxin challenge. A graded 3 dose design based on nondialyzable nitrogen, identical for each activity, was adopted for the biometric estimation. The dosage for precipitate was selected to span the previously determined median sensitizing dose of HSF. These doses were 2.3, 6.9, and 20.7 meg. nitrogen. The highest feasible dose of supernatant, constrained by volume and toxicity considerations, was about 10 times the highest precipitate dose. Groups of mice were injected with doses of supernatant which varied by twofold increments (56, 112, and 224 meg. nitrogen). The mouse intracerebral immunogenicity test and the HSF test have been previously described.⁶ Susceptibility to endotoxin was determined by sensitizing mice intraperitoneally with pertussis vaccine fractions, followed in 5 days by intraperitoneal challenge with either pertussis vaccine (19×10^9 cells) or *S. typhosa* 0901 endotoxin (50 meg.). Toxic deaths were tabulated at 48 hours. Female CFW mice weighing 16 to 21 grams. were used throughout the studies.

RESULTS

The results are given in Table I in terms of mouse-survival rates for the immunogen and death rates for histamine, pertussis vaccine, and typhoid endotoxin. It is apparent that the supernatant showed a very low level for all three of these activities. At the highest dose administered, the survival rate for intracerebrally challenged mice was only 2 in 11, and the death rate from

Table I. *The parallel fractionation of the immunogenic, the histamine-sensitizing, and the endotoxin-enhancing activities of pertussis vaccine*

<i>Pertussis vaccine fraction</i>	<i>Mouse dose (mcg. nitrogen)</i>	<i>Immuno-genicity survival rate</i>	<i>Sensitization to histamine, death rate</i>	<i>Sensitization to endotoxin, death rate</i>	
				<i>Pertussis vaccine (19 × 10⁹ cells)</i>	<i>Typhoid endotoxin (50 mcg.)</i>
35% SAS supernatant	56	1/11	0/16	0/10	0/10
	112	0/13	0/16	0/10	1/10
	224	2/11	3/16	0/10	0/10
35% SAS precipitate	2.3	6/10	3/16	0/10	0/10
	6.9	6/10	11/15	1/10	4/10
	20.7	10/10	14/16	5/10	9/10
Controls	None	0/10	0/15	0/10	0/10

histamine challenge was only 3 in 16; 0 in 20 died after challenge with either pertussis vaccine or typhoid endotoxin. On the other hand, groups receiving the precipitate in nitrogen dosage ranging from 1/100 to 1/10, the highest supernatant dosage, showed dose-response regression for all three activities.

Because bacterial endotoxins have been found capable of enhancing the sensitivity of mice to histamine,^{2, 3} we carried out a supplementary experiment to determine whether the endotoxin of pertussis vaccine, contained in our fractions used for sensitization, might be the agent for lethality-enhancement of subsequently injected endotoxin through an anaphylactic or other mechanism. One of two groups of mice was sensitized with 50 mcg. of typhoid endotoxin and the other served as control. After 5 days each group was challenged with a lethal dose of the same endotoxin, that is, 500 mcg. All the control mice died (8/8), but only 5 out of 10 of the sensitized mice died, showing that, far from inducing anaphylaxis or other lethal effect, previous treatment with endotoxin induced partial tolerance. This, in addition to the findings of others to be discussed, would appear to indicate that the endotoxin that may have been contained in our precipitate fraction could not have been the sensitizing agent responsible for the deaths tabulated in the last two columns of Table I.

DISCUSSION

These results constitute evidence that one component of pertussis vaccine may be responsible for three different activities in mice, namely, protection against an intracerebral challenge of virulent pertussis bacilli and enhancement of their sensitivity to histamine and to endotoxins. All of these activities were precipitated from a pertussis vaccine lysate by 35 per cent saturation with ammonium sulphate. The resulting supernatant, although containing the bulk of the organic bacterial components and injected at a much greater dosage level than the precipitate, was inactive in all of the above respects. It is this association of apparently diverse activities in a relatively minor portion of the bacillus

substance, together with the common lability to heat and trypsin, that forms the basis of our suggestion that these activities are the functions of a single bacterial component.

Although bacterial endotoxins are capable of increasing the sensitivity of mice to histamine challenge, their effect is weak and irregular.^{2, 3} We have shown that pertussis endotoxin cannot be the main source of the typical histamine sensitization of pertussis vaccine.³ Several findings militate against the thesis that it is pertussis endotoxin that enhances the lethal potential of subsequently injected endotoxin through an anaphylactic or other mechanism. Prior exposure to endotoxin has been shown by many workers, and was presently demonstrated using typhoid endotoxin, actually to reduce susceptibility to a later injection of endotoxin. Kind¹ has shown that heated pertussis vaccine is incapable of sensitizing mice to endotoxin challenge. This would appear to rule out heat-stable endotoxin as being the sensitizing factor in vaccine. Finally, Malkiel and Hargis² found that extracted pertussis lipopolysaccharide could not sensitize mice to endotoxin challenge, although it acted as an effective shocking agent in mice pretreated with whole pertussis vaccine.²

Fishel, Szentivanyi, and Talmage⁹ have suggested that blockade of the beta-adrenergic receptors of the autonomic nervous system may be the mechanism by which pertussis vaccine enhances the susceptibility of mice to histamine and serotonin. We have shown that this may also be the case with vaccine enhancement of peptone shock.⁴ Since (1) a heat-labile, proteinaceous component of pertussis vaccine, quite possibly its immunogen, has been shown to possess the histamine-, peptone-, and endotoxin-sensitizing attributes of pertussis vaccine and (2) sensitization to at least histamine and peptone is apparently mediated through this molecule's effect direct or indirect, on adrenergic receptors, it would appear logical to propose that the hyperreactivity to endotoxin engendered by this vaccine is also mediated through autonomic mechanisms. Adrenergic involvement in many of the biological effects of endotoxin, including its lethal property, has been the subject of numerous reports.^{9, 10} Quite possibly the augmented susceptibility of pertussis-sensitized mice to the lethal effects of bacterial endotoxins results from the compounding of the effects on the autonomic nervous system elicited by both the sensitizing molecule of pertussis vaccine, presumably its immunogen, and endotoxin used for challenge. Preliminary experiments performed in this laboratory appear consonant with this thesis and will be the subject of a future report.

REFERENCES

1. Kind, L. S.: Effects of Heat on the Sensitizing and Shocking Properties of *Hemophilus pertussis*, *J. Immunol.* 77: 115, 1956.
2. Malkiel, S., and Hargis, B. J.: Anaphylactic Reactions in Mice Induced by *Bordetella pertussis* Lipopolysaccharide, *J. ALLERGY* 35: 306, 1964.
3. Pieroni, R. E., Broderick E. J., and Levine, L.: Endotoxin-Induced Hypersensitivity to Histamine in Mice. I. Contrasting Effects of Bacterial Lipopolysaccharides and the Classical Histamine-Sensitizing Factor of *Bordetella pertussis*, *J. Bact.* 91: 2169, 1966.
4. Pieroni, R. E., and Levine, L.: Properties of the Immunogenic and Sensitizing Activities of *Bordetella pertussis*. I. Enhancement of Peptone Shock, *J. ALLERGY* 39: 25, 1967.
5. Pieroni, R. E., and Levine, L.: Unpublished observations.
6. Pieroni, R. E., Broderick, E. J., and Levine, L.: The Soluble Protective Antigen and Histamine-Sensitizing Factor of *Bordetella pertussis*, *J. Immunol.* 95: 643, 1965.

7. Levine, S., Wenk, E. J., Devlin, H. B., Pieroni, R. E., and Levine, L.: Hyperacute Allergic Encephalomyelitis: Adjuvant Effect of Pertussis Vaccines and Extracts, *J. Immunol.* **97**: 363, 1966.
8. Pieroni, R. E., and Levine, L.: Adjuvant Principle of Pertussis Vaccine in the Mouse, *Nature* **211**: 1419, 1966.
9. Fishel, C. W., Szentivanyi, A., and Talmage, D. W.: Adrenergic Factors in *Bordetella pertussis*—Induced Histamine and Serotonin Hypersensitivity in Mice, *in*, Landy, M., and Braun, W., editors: *Bacterial Endotoxins*, New Jersey, 1964, Rutgers University Press, pp. 474-481.
10. Gourzis, J. T., Hollenberg, M. W., and Nickerson, M.: Involvement of Adrenergic Factors in the Effects of Bacterial Endotoxin, *J. Exper. Med.* **114**: 593, 1961.