Differing Protective Effects of Acellular Pertussis Vaccines in Neonatal and Young Mice in a Murine Model of Respiratory Infection

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The protective effects on neonatal (3.5 weeks old) and young mice (7 weeks old) of eight pertussis vaccines prepared from various components at various concentrations were investigated in a murine model of respiratory infection (aerosol challenge model). Neonatal mice were more sensitive than young mice to infection by *Bordetella pertussis* after aerosol challenge. In young mice with all vaccines, there were significant differences between immunized mice and control mice. The efficacy of vaccines was increased by the inclusion of additional filamentous hemagglutinin (FHA), pertussis toxin (PT), or pertactin (PRN) in the basic vaccine (FHA : PT : PRN, 7 : 2 : 1, w/w). An elevated level of FHA strongly increased the efficacy of the vaccine in young mice. It was, however, more difficult to induce protection against *B. pertussis* in neonatal mice than in young mice, irrespective of the levels of the various components in the vaccines. Our data suggest that pertussis vaccines are less effective in neonatal mice than in young mice, as assessed by the aerosol challenge model.

Key words —— DPT vaccine, aerosol challenge model, *Bordetella pertussis*, filamentous hemagglutinin, pertussis toxin, pertactin

INTRODUCTION

Whooping cough caused by *Bordetella pertussis* is a serious disease in children, and the causative agent infects the upper respiratory tract and induces paroxysmal coughing.[1)] Acellular pertussis vaccines have been shown to be effective and associated with low frequencies of adverse reactions.[2–7)] Filamentous hemagglutinin (FHA), pertussis toxin (PT), pertactin (PRN), and fimbriae are all effective components of pertussis vaccines.[2)] A murine model of respiratory infection (aerosol challenge model) was reported to be useful for tests of the potency of various types of acellular pertussis vaccine.[8)] In general, mice for assays of pertussis vaccines are 7 to 12 weeks old (immunized at 3.5 to 9 weeks old) when they are infected with *B. pertussis*.[8–11)] However, it is possible that differences among efficacies of vaccines might be more easily detected in neonatal mice since the symptoms of whooping cough are serious in young infants in particular. In the present study, we examined an aerosol challenge model for its potential utility in tests of the efficacy of various combinations of components of acellular pertussis vaccines using neonatal and young mice. In addition, we also examined the roles of various components of vaccines in the protection of mice against *B. pertussis*.

MATERIALS AND METHODS

Animals —— Specific-pathogen-free female dd-Y mice, either suckling (2 to 3 days old, referred to as neonatal) or 3.5 weeks old (referred to as young), were obtained from Japan SLC (Hamamatsu, Japan). The immunization and aerosol challenge schedule is shown in Table 1.

Preparation of Vaccines —— Components of pertussis vaccine (FHA, PT, and PRN) were purified from *B. pertussis* strain Tohama as described previously.[11–13)] Solutions of the purified components were inactivated by treatment with formalin as described previously.[14,15)] Aluminum phosphate (final concentration, 0.1 mg/ml) was added as an adjuvant to each solution, and then the solutions were stored at 4°C for 1 week. Solutions of each component were mixed at the relative levels indicated in Table 2 to give the indicated concentrations of protein (as protein nitrogen, PN) in each mixture. The resultant preparations were used as vaccines (nos. 2 through 8).

We also tested a commercial diphtheria, tetanus, and acellular pertussis combined vaccine (DTaP; lot

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Table 1. Immunization and Aerosol Challenge Schedule

Mice	Immunized at	Aerosol challenged at
Neonatal	0.5 weeks	3.5 weeks
Young	4 weeks	7 weeks

33), which was a co-purified type of vaccine manufactured by the Kitasato Institute, Tokyo, Japan.[14,15]

Evaluation of the Efficacy of Pertussis Vaccines in a Murine Model of Respiratory Infection —— We examined the efficacy of the pertussis vaccines in a murine model of respiratory infection.[11–13,15] Vaccines were diluted to 1 : 8 (v/v) with saline before immunization [1/4 single human dose (SHD) per milliliter]. Young mice (4 weeks old) were immunized by intraperitoneal injection with diluted vaccine (0.5 ml/mouse, *i.e.*, 1/8 SHD/mouse) or with saline as a control. Neonatal mice (0.5 weeks old) were immunized by intraperitoneal injection with undiluted vaccines (0.1 ml/mouse, *i.e.*, 1/5 SHD/mouse) or with saline as a control. Three weeks later, immunized mice were allowed to inhale a suspension (1×10^{10} cells/ml) of *B. pertussis* strain 18-323 (obtained from the National Institute of Infectious Disease, Tokyo, Japan) for 30 min in a sealed aerosol chamber within a biosafety cabinet. The number of viable *B. pertussis* cells in each mouse lung after such treatment was approximately 10^5 colony-forming units (CFUs) per lung. Two weeks or at the indicated times after the aerosol challenge, mice were sacrificed, and the lungs were dissected and homogenized in 10 ml/lung of phosphate-buffered saline in a Teflon homogenizer on ice. After appropriate dilutions, each lung homogenate was spread on Bordet-Gengou (BG) agar plates supplemented with 20% (v/v) defibrinated horse blood and incubated for 4 days at 37°C. The number of viable bacteria was recorded after logarithmic transformation of the number of CFUs. The numbers of viable bacteria in lungs of mice were expressed as a mean ± standard deviation (S.D.). The significance of differences between nonimmunized mice and each group of immunized mice was examined using Student's *t*-test. A *p*-Value of less than 0.05 was considered significant.

RESULTS

The time courses of numbers of bacterial cells in mouse lungs after an aerosol challenge in nonimmunized neonatal (3.5 weeks old at the aerosol challenge) and young (7 weeks old at the aerosol challenge) mice are shown in Fig. 1. The mice were challenged with *B. pertussis* as described in Materials and Methods. Five mice each in the neonatal group and in the older group were sacrificed at time 0 and after 1, 2, 3, and 4 weeks for quantitation of viable bacteria in their lungs. The initial number of viable bacteria in lungs of mice infected with *B. pertussis* was $10^{4.6}$ and $10^{5.1}$ CFU/lung in neonatal mice and young mice, respectively. The number of bacteria in the lungs of neonatal mice and young mice increased approximately 490-fold and 34-fold, respectively, during the first week after the challenge and then declined slowly. In young mice, the numbers of bacteria in the lungs after aerosol challenge were fewer after 3 and 4 weeks than in the neonatal mice. There were significant of differences between the two groups of mice at 1, 3, and 4 weeks ($p < 0.05$; Fig. 1).

Acellular pertussis vaccines containing various

Table 2. Composition of Vaccines

Vaccine no.	Relative levels of vaccine components (FHA : PT : PRN, w/w)	Relative levels of components compared to those in vaccine no. 2	Protein (PN μg/ml)*
1	Lot 33 (DTaP)	—	25
2	7 : 2 : 1	—	10
3	14 : 2 : 1	FHA × 2	17
4	28 : 2 : 1	FHA × 4	31
5	7 : 4 : 1	PT × 2	12
6	7 : 8 : 1	PT × 4	16
7	7 : 2 : 2	PRN × 2	11
8	7 : 2 : 4	PRN × 4	13

*: PN, protein nitrogen. See text for other abbreviations.

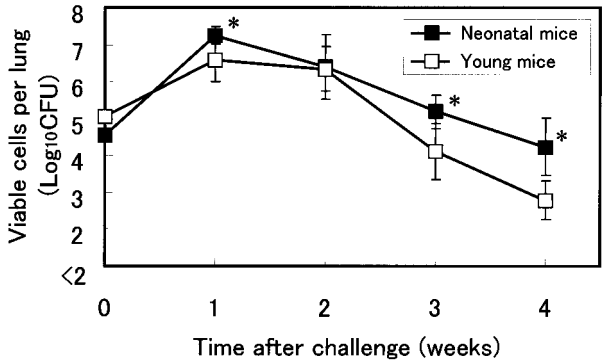

Fig. 1. Time Course of Changes in Numbers of CFU in Lungs of Neonatal Mice (3.5 Weeks Old) and Young Mice (7 Weeks Old) After Infection with *B. Pertussis*

Mice were infected by exposure to an aerosol of *B. pertussis* strain 18-323, as described in Materials and Methods. Mouse lungs were removed 0, 1, 2, 3, and 4 weeks after the challenge, and then bacteria in mouse lungs were counted. Results are presented in terms of $\log_{10}$CFU and are mean values per lung, as estimated from individual lungs of 5 mice for each group at each time point. Each symbol with a vertical line represents a mean ± S.D. *$p < 0.05$ *versus* the group of older mice.

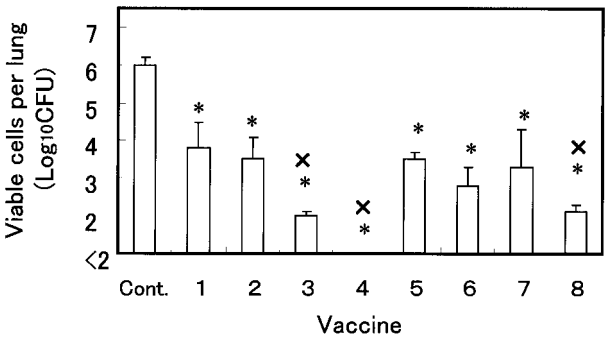

Fig. 2. Protective Effects on 7-Week-Old Mice of Pertussis Vaccines Prepared with Various Combinations of Components

The components of each acellular pertussis vaccine are described in Table 2. Mice were challenged 3 weeks after immunization by exposure to an aerosol of *B. pertussis* strain 18-323. Mouse lungs were removed 14 days after the challenge, and bacteria in the lungs were counted. Each column with a vertical line represents the mean and S.D. of results from 5 mice. Cont, control mice. *$p < 0.05$ *versus* the control group. ×$p < 0.05$ *versus* the group immunized with vaccine no. 2.

combinations of components were prepared as shown in Table 2. These vaccines were examined for their protective effects on young mice exposed to an aerosol challenge with *B. pertussis* at 7 weeks, *i.e.*, 3 weeks after immunization (Fig. 2). There were significant differences ($p < 0.05$) between the immunized mice and the control mice for all the vaccines (Fig. 2). The efficacy of vaccines was increased by the addition of FHA (nos. 3 and 4) or pertactin PRN (no. 8) to the basic vaccine (no. 2).

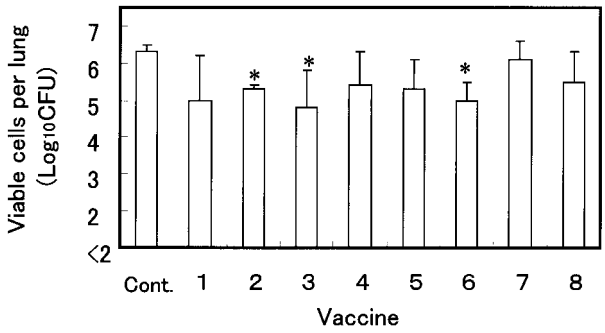

Fig. 3. Protective Effects on 3.5-Week-Old Mice of Pertussis Vaccines Prepared with Various Combinations of Components

See legend to Fig. 2 for details. *$p < 0.05$ *versus* the control group.

None of the tested vaccines provided much protection in neonatal mice when they were tested 3 weeks after immunization (Fig. 3). The efficacy of vaccines was not increased by the addition of FHA (nos. 3 and 4), PT (nos. 5 and 6), or PRN (nos. 7 and 8) to the basic vaccine (no. 2).

DISCUSSION

Whooping cough is a serious disease in infants. In the case of nonimmunized mice, the numbers of bacterial cells in mouse lungs increased for 1 week after the aerosol challenge in both neonatal (3.5 weeks old) and young mice (7 weeks old). The maximum number of bacterial cells was detected 1 week after the aerosol challenge in both groups. The numbers of bacteria in the lungs 1 week after aerosol challenge had increased 490-fold and 34-fold in the neonatal mice and young mice, respectively. Subsequently, the numbers of bacteria in the lungs of both groups of mice declined slowly. However, the number of bacteria remained significantly higher in neonatal as compared with young mice. These data indicate that neonatal mice are more sensitive to aerosol challenge with *B. pertussis* and might explain why symptoms of whooping cough are especially serious in young infants.

In the present study, we investigated the protective effects of acellular pertussis vaccines when neonatal and young mice were exposed to an aerosol challenge 3 weeks after immunization. In the case of young mice, the efficacy of vaccine no. 2 (FHA : PT : PRN, 7 : 2 : 1) in protecting mice against the aerosol challenge was similar to that of the commercial DTaP vaccine. Vaccine no. 2 supple-

mented with an amount equal to (no. 3) or three times greater than (no. 4) the original amount of FHA was more effective than vaccine no. 2 in the aerosol challenge model in young mice. Thus there was a relationship between the amount of FHA and efficacy. Vaccine no. 2 supplemented with three times the original amount of PRN (no. 8) was also more effective than vaccine no. 3 alone in the aerosol challenge model. However, the difference between vaccine no. 7 and vaccine no. 2 was not significant.

The addition of FHA to the basic vaccine (no. 2) increased its efficacy in young mice (nos. 3 and 4). However, the total protein content of vaccine no. 4 was PN 31 μg/ml. The protein content of Japanese acellular pertussis vaccines is maintained below PN 20 μg/ml, as specified by the Japanese minimum requirements for biological products.[16] Previously, we reported about well ratio of FHA and PT on the vaccine efficacy.[15] A strong protective efficacy was obtained with vaccines that contained a small amount of FHA and a large amount of PT (ratios of FHA : PT of 1 : 11 or 2 : 10).[15] However, it has been postulate that adverse reactions to pertussis vaccines might be related to residual activity of PT.[17,18] Therefore vaccines should not been supplemented with an excess of PT. The formulations of vaccines no. 3 and no. 8 might represent appropriately effective relative levels of FHA, PT, and PRN.

In neonatal mice challenged 3.5 weeks after immunization at 0.5 weeks, none of the vaccines was very effective. The immune systems of mice at 0.5 weeks might still be poorly developed. Our results suggest that we should use 7-week-old mice, immunized at 3 to 4 weeks, in assays of the protective efficacy of vaccines in the aerosol challenge model. Further analysis is necessary to clarify the role of immune systems, such as antibody responses and cell-mediated immunity, in the determination of the protective effects of vaccines in neonatal mice and young mice.

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