Effect of Acellular Pertussis Vaccine Against Various Strains of *Bordetella Pertussis* in a Murine Model of Respiratory Infection

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(Received June 24, 2002; Accepted August 8, 2002; Published online August 9, 2002)

The protective effects of an acellular pertussis vaccine were investigated in a murine model of respiratory infection (aerosol challenge model) with various strains of *Bordetella pertussis* (*B. pertussis*) as challengers. There were no significant differences in terms of the time course of increases in numbers of bacterial cells in mouse lungs after aerosol challenge among the tested phase I strains. The vaccine had a strong protective effect against of *B. pertussis* strain Tohama the phase I strain used for production of the vaccine, however was less effective against other clinical isolates of pertussis. Our data suggest that a novel vaccine(s) should be developed from the strains derived from current clinical isolates to eliminate the incidence of pertussis.

Key words —— acellular pertussis vaccine, DPT vaccine, aerosol challenge model, *Bordetella pertussis*

INTRODUCTION

The introduction of whole-cell pertussis vaccine (wP) in the late 1940's resulted in a dramatic reduction in the annual incidence of cases of pertussis in Japan.[1] In 1955, the vaccine was improved by replacing the original strain of *Bordetella pertussis* (*B. pertussis*) with a K-agglutinogen-rich phase I strain (Tohama and Maeno).[1] The new vaccine reduced the incidence of pertussis still further. In the 1960's, the introduction of pertussis vaccine in combination with diphtheria toxoid (DwP) and then with tetanus toxoid (DTwP) resulted in higher rates of acceptance of pertussis vaccine.[2] As a result, the annual number of reported cases of pertussis stabilized at levels of less than 0.5 cases per 100000 population until the early 1970's.[2,3] However, because of adverse reactions of DTwP in 1974 and 1975, mass vaccinations were temporarily suspended in 1975,[2] and the incidence of pertussis rose to 11.3 cases per 100000 population in 1979.[2,3] In 1981, an acellular pertussis vaccine (aP), composed mainly of pertussis toxin (PT) and filamentous hemagglutinin (FHA) from *B. pertussis* strain Tohama, was introduced and widely distributed.[4] As a result, the incidence of pertussis fell again, reaching about 0.4 cases per 100000 population in 1988.[2,3] The incidence of pertussis fell still further, to close to zero cases per 100000 population, in 1998.[3] Since 1981, acellular pertussis vaccines have been produced from *B. pertussis* strain Tohama, a phase I strain, by six manufacturers in Japan.[4,5] *B. pertussis* strain 18-323, the type strain of this species,[6] is used for potency tests (Kendrick test)[7] of whole-cell pertussis vaccines worldwide and of acellular pertussis vaccines in Japan.[8] A murine model of respiratory infection (aerosol challenge model) was reported recently to be useful for tests of the potency of various types of pertussis vaccine.[9,10] In such thesis, various strains of *B. pertussis* were used as challengers, as indicated in Table 1.[9,11–15] In this study, we examined the efficacy of an acellular pertussis vaccine using various strains of *B. pertussis* in the aerosol challenge test.

MATERIALS AND METHODS

Animals —— Specific-pathogen-free female dd-Y mice were obtained from Japan SLC (Hamamatsu, Japan). All mice were 3.5 weeks old at the start of experiments.

Commercial DPT Vaccine —— We used a commercial diphtheria, tetanus and acellular pertussis combined vaccine (DTaP), which was a co-purified vaccine and had been manufactured by the Kitasato Institute, Tokyo, Japan.[16] The vaccine contained formalin-treated pertussis components (pertussis toxin, filamentous hemagglutinin, pertactin and fimbriae, all purified from *B. pertussis* strain Tohama), diphtheria toxoid and tetanus toxoid.

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Table 1. Strain of *B. pertussis* Used for Respiratory Challenge

Strain	Infection method	Reference
18-323	Aerosol challenge	Watanabe *et al.*[11]
18-323	Intranasal challenge	Guiso *et al.*[12]
18-323	Aerosol challenge	Bruss and Siber[13]
Wellcome 28	Aerosol challenge	Xing *et al.*[9]
Wellcome 28	Aerosol challenge	Guiso *et al.*[12]
Wellcome 28	Aerosol challenge	Millis *et al.*[14]
BP536 (Tohama derivative)	Intranasal challenge	van den Berg *et al.*[15]

Bacterial Strains and Culture Conditions —— Three phase I strains (virulent strains) of *B. pertussis*, namely, 18-323,[6] 3779B[17] and Tohama,[18] and one phase III strain (avirulent strain), namely, Sakairi,[18] were used to challenge mice in this study. Cells were grown on Bordet-Gengou (BG) agar supplemented with 20% (v/v) defibrinated horse blood at 37°C. They were harvested in phosphate-buffered saline (PBS) on ice, and each suspension of cells was adjusted to 1×10^{10} cells/ml after measurement of its optical density at 660 nm.

Evaluation of the Efficacy of the Pertussis Vaccine in a Murine Model of Respiratory Infection —— The efficacy of the commercial pertussis vaccine was examined in a murine model of respiratory infection.[1,19–21] The vaccine was diluted 1 : 8 (v/v) with saline before immunization of mice [1/4 single human dose (SHD) per milliliter]. Mice were immunized by intraperitoneal injection with the diluted vaccine (0.5 ml/mouse, *i.e.*, 1/8 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* for 30 min in a sealed aerosol chamber within a biosafety cabinet. Two weeks or at indicated times after the aerosol challenge, mice were sacrificed and lungs were dissected out and homogenized in 10 ml of PBS in a Teflon homogenizer on ice. After appropriate dilutions, each lung homogenate was spread on BG plates and incubated for four days at 37°C. The number of viable bacteria was recorded after logarithmic transformation of the number of colony-forming units (CFU). The significance of differences between non-immunized mice and each group of immunized mice was examined by Student's *t*-test. A *p*-value of less than 0.05 was considered to indicate a significant difference.

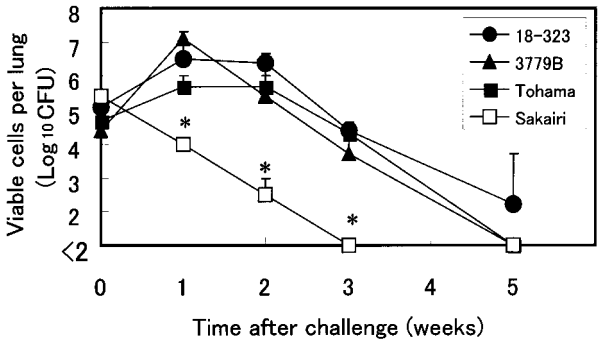

Fig. 1. Time Course of Changes in Numbers of CFU in Lungs of Mice after Infection with Four Strains of *B. pertussis*

Mice were infected by exposure to an aerosol of *B. pertussis* strain 18-323, 3779B, Tohama or Sakairi, as described in Materials and Methods. Mouse lungs were removed 0, 1, 2, 3 and 5 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 five mice for each group at each time point. Each symbol with a vertical line represents a mean ± standard deviation. *: $p < 0.05$ versus the results for strain 18-323.

RESULTS AND DISCUSSION

The time courses of increases in numbers of bacterial cells in mouse lungs after the aerosol challenge are shown in Fig. 1. The mice were challenged with the various strains of *B. pertussis* as described in Materials and Methods. Five mice in each group were sacrificed at time 0 and after 1, 2, 3 and 5 weeks for quantitation of viable bacteria in their lungs. The initial number of viable bacteria in lungs of mice infected with *B. pertussis* strains 18-323, 3779B, Tohama and Sakairi were $10^{5.1}$, $10^{4.2}$, $10^{4.8}$ and $10^{5.3}$ CFU per lung, respectively. The numbers of bacteria in lungs of mice infected with phase I strains 18-323, 3779B and Tohama increased approximately 100-fold, 30-fold and 300-fold, respectively, during the first week after the challenge and then declined slowly (Fig. 1). There were no significant differences in terms of CFU in lungs at each time point between mice infected with strain 18-323 and those infected

with other phase I strains ($p < 0.05$). In the case of the phase III strain, we detected an approximately 50-fold reduction in the number of bacteria in the lungs one week after the aerosol challenge, and the bacteria were rapidly cleared from the lungs thereafter. There were no detectable bacteria in the lungs three weeks after the challenge. There was a significant difference ($p < 0.05$) in terms of CFU in lungs between strain 18-323 and strain Sakairi from one to three weeks after the challenge (Fig. 1). The number of bacterial cells in mouse lungs increased for one week after the aerosol challenge with each of the phase I strains. Maximum numbers of bacterial cells were detected one to two weeks after the aerosol challenge with each phase I strain. However, in the case of the phase III strain, the number of bacterial cells had already decreased one week after the challenge. These results suggest that phase III strains are unable to replicate in mouse lungs.

We investigated the efficacy of a commercial acellular pertussis vaccine using phase I and phase III strains of *B. pertussis* in the aerosol challenge model. Mice immunized with DTaP vaccine were challenged with an aerosol of a phase I or phase III strain. Two weeks after the aerosol challenge, the number of CFU in the lungs of each mouse was determined as described in Materials and Methods. The number of CFU in the lungs of non-immunized mice ranged from approximately $10^{6.3}$ to $10^{5.3}$ two weeks after the aerosol challenge with the phase I strains (Fig. 2). However, the number of CFU in the lungs of non-immunized mice was approximately $10^{2.3}$ two weeks after aerosol challenge with the phase III strain (Fig. 2). The numbers of CFU in the lungs of mice immunized with the DTaP vaccine were lower than those in lungs of non-immunized mice (Fig. 2). There were significant differences ($p < 0.05$) between the immunized mice and the non-immunized mice (Fig. 2). In mice challenged with the Tohama strain, the number of CFU in the lungs of immunized mice was significantly lower than that in the immunized mice challenged with strain 18-323 ($p < 0.05$), which might nor be surprising since the pertussis components in the DTaP vaccine were prepared from the Tohama strain.[4]

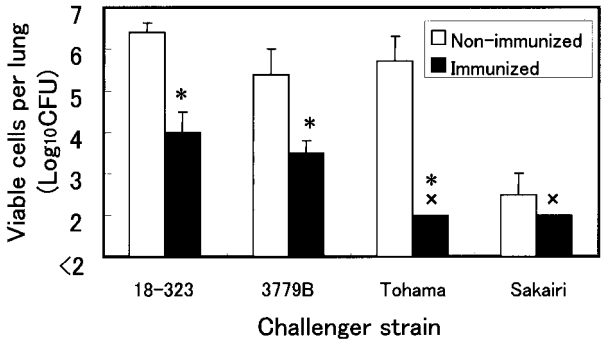

Fig. 2. Protective Effects of a Commercial Pertussis Vaccine in the Respiratory Challenge Model with Various Strains of *B. pertussis*

Mice were challenged, 3 weeks after immunization, by exposure to an aerosol of *B. pertussis* strain 18-323, 3779B, Tohama or Sakairi. Mouse lungs were removed 14 days after the challenge and bacteria in the lungs were counted. Each column with a vertical line represents a mean and standard deviation (5 mice in each group). *: $p < 0.05$ versus the value for the respective non-immunized group. ×: $p < 0.05$ versus the value for immunized mice challenged with strain 18-323.

Thus, when the challenger strain and the strain used for preparation of the vaccine were the same, the efficacy of the vaccine was greater than when the strains were different. Guiso *et al.*[22] noted that, in the past few years, concerns have been raised about the possibility that antigenic variability might exist in isolates of *B. pertussis* that might affect the efficacy of pertussis vaccines in The Netherlands, Finland and Italy. They also reported variations in the *ptxs1* and *prn* genes from various strains of *B. pertussis*.[22–24] As shown in Table 2, strains 18-323 and Tohama have different respective *ptxs1* and *prn* genes. These differences might explain why the efficacy of the vaccine against the Tohama strain was greater than against strain 18-323. Our results suggest that more effective acellular pertussis vaccines might be manufactured from components of current prevalent strains or from strains in recent clinical isolates. It is now necessary to prepare pertussis vaccines from such strains and to investigate their protective effects.

Table 2. Characteristics of the *B. pertussis* Strains 18-323 and Tohama[22–24]

Strain	Geographic location	Year of isolation	Fim type	PT S1 type	PRN type
18-323	U.S.A.	1947	2,3	E	6
Tohama	Japan	1952	2	B	1

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