

CHARACTERIZATION OF THE PROTECTIVE CAPACITY AND IMMUNOGENICITY OF THE 69-kD OUTER MEMBRANE PROTEIN OF *BORDETELLA PERTUSSIS*

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Bordetella pertussis is a gram-negative coccobacillus that is the etiologic agent of pertussis (whooping cough). Pertussis may be characterized by episodes of paroxysmal coughing and can be fatal, especially in children less than 1 year old. The World Health Organization estimates that 600,000 deaths per year are due to pertussis, most of them in unimmunized infants (1). In the United States, widespread immunization of children with an inactivated whole cell pertussis vaccine has been successful in controlling disease (2). However, certain local and systemic reactions, such as pain and swelling at the injection site and fever, have been associated with the whole cell pertussis vaccine (3, 4). To formulate a safe and effective acellular pertussis vaccine, current research efforts have been directed towards characterizing purified antigens of *B. pertussis* and analyzing their potential as protective antigens.

The 69-kD outer membrane protein (OMP)¹ of *B. pertussis* is a nonfimbrial membrane-associated protein that induces agglutinating antibodies in mice (5). The 69-kD OMP is detectable on all virulent strains of *B. pertussis* (6) and an antigenically similar protein is found on the closely related *Bordetella* species *B. parapertussis* and *B. bronchiseptica* (5).

We have analyzed the ability of the 69-kD OMP to protect neonatal mice against lethal *B. pertussis* respiratory challenge. Aerosol infection of mice is a suitable model for the analysis of protection in that certain parameters of the disease process in humans, including adherence of *B. pertussis* to the ciliated epithelium of the respiratory tract, leukocytosis, and an age-related severity of symptoms, are also observed in mice (7-10). In addition, we demonstrate the human immunogenicity of this protein by examining the antibody response in children receiving a single dose of an acellular vaccine that, as we report here, contains significant amounts of the 69-kD OMP.

Materials and Methods

Mice. BALB/cAnNcR mice were obtained on day 3 post partum (pp), with mothers, from the Animal Production Program, Division of Cancer Treatment, National Cancer Institute, Frederick, MD.

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¹ Abbreviations used in this paper: OMP, outer membrane protein; pp, postpartum; WBC, white blood cell.

Antigens. The 69-kD OMP was affinity purified from *B. pertussis* BP353 as previously described (5). Briefly, heat extracts of *B. pertussis* BP353, passed through a fetuin-Sepharose column to remove pertussis toxin, were adsorbed to a column of agarose-linked mAb BPE3 (IgM anti-69-kD OMP), washed, and the bound antigen was eluted with 6 M urea. The preparations of 69-kD OMP used were pure as monitored by SDS-PAGE followed by silver staining (11). Purified 69-kD OMP is frequently observed as a doublet band by SDS-PAGE (5). These preparations contained <0.25% endotoxin as determined by a *Limulus* amoebocyte assay (12), and were free of contaminating pertussis toxin, as determined by the Chinese Hamster Ovary cell agglutination assay (13).

Pertussis toxin was purchased from the Michigan Department of Public Health and was demonstrated to be pure by SDS-PAGE. Pertussis holotoxin was inactivated with 0.15% glutaraldehyde as previously described (14). A portion of the 69-kD OMP was also treated with 0.15% glutaraldehyde by the same method.

Pertussis toxoid, 69-kD OMP, and tetanus toxoid (Connaught Laboratories Inc., Swiftwater, PA) were diluted in PBS containing 0.2% gelatin and adsorbed at 4°C overnight to aluminum hydroxide gel (Superfos a/s; Vedbaek, Denmark). Mice were immunized on days 5 and 12 pp with 0.1 ml antigen administered intraperitoneally.

Samples of unadsorbed Takeda acellular pertussis vaccine, which had not been chemically inactivated, were provided for analysis by Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY. Samples of human sera were provided for analysis by Dr. P. Wright, Department of Pediatrics, Vanderbilt University, Nashville, TN.

Monoclonal Antibodies. The IgG1 anti-69-kD OMP mAbs BPD8 and BPE8 were purified from ascites fluid by ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE sepharose (5). BPE3, an IgM anti-69-kD OMP mAb, was purified by ammonium sulfate precipitation followed by gel filtration on Sepharose 4B. The purity of each mAb preparation was verified by SDS-PAGE. BPD8, BPE8, and BPE3 have previously been determined to react specifically with the 69-kD OMP (5).

Protein A-Sepharose-purified 18.1.7, an IgG1 anti-tetanus toxin mAb (15), was a generous gift of Dr. Jane Halpern, Laboratory of Bacterial Toxins, FDA. The anti-FHA mAb MO8X3E was provided by Dr. James Kenimer, Laboratory of Cellular Physiology, FDA.

In passive protection experiments, 17-d-old mice were injected intravenously with 250 µg of purified mAb 24 h before aerosol challenge.

Aerosol Challenge. Lyophilized *B. pertussis* 18323 was plated on Bordet-Gengou agar containing 15% defibrinated sheep blood. A 21-h culture of bacteria was harvested from slants and resuspended in sterile saline to a concentration of ~10⁹ bacteria/ml. An aliquot of the final suspension was diluted and plated in order to determine the viability of the challenge inoculum.

An aerosol chamber (constructed by the Biomedical Engineering and Instrumentation Branch, National Institutes of Health, Bethesda, MD) contained in a Biosafety Level 3 glove box (Blickman Co., Weehawken, NJ) was used to administer the challenge inoculum to the mice for a 30-min period of aerosol generation as previously described (7). 1 h after termination of the aerosol challenge, at which point no viable *B. pertussis* can be cultured from the surface of the mice or the chamber (7), the mice were removed and caged with filtered cage covers. At this time, two animals were killed in order to determine the number of viable *B. pertussis* in the lungs. All mice tested had ~10⁴ CFU in their lungs 1 h after aerosol infection.

Mice were weighed and bled periodically after infection, and any deaths were noted. Leukocyte counts were determined in a model ZM Coulter counter (Coulter Electronics, Hialeah, FL).

Analysis of Serum and Respiratory Ig. Mice anesthetized with 2,2,2-tribromoethanol (Aldrich Chemical Co., Milwaukee, WI) were exsanguinated from the brachial artery. After the diaphragm was cut, the trachea was cut below the pharynx, and a piece of PE-50 polyethylene tubing (Clay Adams, Parsippany, NJ) was inserted into the trachea, held in place by tightening a loop of suture (6-0 silk; Ethicon, Inc., Somerville, NJ). PBS (0.3 ml) was gently instilled into the lungs and withdrawn three times. The bronchoalveolar lavage fluid was centrifuged to pellet leukocytes, and the supernatant was removed and frozen at -20°C until analyzed.

Murine serum and bronchoalveolar lavage fluid were analyzed for specific antibody to the 69-kD OMP by an ELISA (15). Microtiter plates (Immunolon I; Dynatech Laboratories, Chantilly, VA) were coated with 5 $\mu\text{g}/\text{ml}$ of the 69-kD OMP in 0.1 M carbonate buffer, pH 9, and incubated overnight. The plates were washed and incubated with dilutions of mouse serum or bronchoalveolar lavage fluid in PBS containing 0.05% Tween and 0.2% sodium azide for 3 h. After washing, the plates were incubated for 2 h with alkaline-phosphatase-conjugated goat anti-mouse total Ig, or with alkaline phosphatase-conjugated goat anti-mouse IgM, IgG, or IgA (Southern Biotechnology, Inc., Birmingham, AL). The sensitivity and isotype specificity of the alkaline phosphatase conjugates were established using a panel of mAbs generously provided by Dr. John Cebra, University of Pennsylvania, Philadelphia, PA. The plates were read 30 min after addition of Sigma 104 phosphatase substrate (Sigma Chemical Co., St. Louis, MO), using a Bio-Tek EL 312 reader (Biotek Instruments, Winooski, VT). Total anti-69-kD OMP titers are expressed as the reciprocal of the endpoint dilution, calculated by extrapolation to zero from the linear part of the titration curve. The relative amounts of IgM, IgG, or IgA anti-69-kD OMP are reported as the OD_{405} of a 1:100 dilution of serum or a 1:4 dilution of bronchoalveolar lavage fluid.

Human sera were analyzed for specific antibody to the 69-kD OMP by ELISA as described above with the following modifications. Plates coated with 2 $\mu\text{g}/\text{ml}$ 69-kD OMP were incubated with dilutions of human sera, washed, and were incubated overnight with alkaline phosphatase-conjugated goat anti-human IgG (Cappel Laboratories, Malvern, PA) before addition of substrate. Antibody unitage was calculated by a parallel line method (16).

Electrophoretic Analysis. SDS-gel electrophoresis was performed by the method of Laemmli (17). Electrophoresis of 69-kD OMP or Takeda pertussis vaccine and transfer to nitrocellulose (18) was done as previously described (5). For immunoblotting, dilutions of sera were incubated with individual strips of nitrocellulose for 2 h at room temperature before incubation with alkaline phosphatase-conjugated goat anti-human Ig for 2 hours (Southern Biotechnology, Inc., Birmingham, AL), and were developed with the Protoblot substrate system (Promega, Inc., Madison, WI).

Results

Protection Mediated by Active Immunization with the 69-kD OMP. Immunization of adult mice with our preparation of 69-kD OMP elicited polyclonal antisera that reacted only with a single band of 69-kD when immunoblotted to tricine-urea-solubilized antigens of *B. pertussis* separated by SDS-electrophoresis (data not shown).

Immunization with the 69-kD OMP protected young mice from lethal respiratory challenge with *B. pertussis* 18323. All mice immunized on days 5 and 12 pp with 16 μg 69-kD OMP survived a respiratory challenge with *B. pertussis* 18323 on day 19 pp (Fig. 1); these mice exhibited a mild leukocytosis that subsequently returned to baseline after day 21 (data not shown) and gained weight at a rate similar to uninfected controls (Fig. 1). Mice immunized with pertussis toxoid, as a positive control, exhibited no leukocytosis and were protected from death (Fig. 1). In contrast, mice immunized with tetanus toxoid and then challenged with an aerosol of *B. pertussis* failed to gain weight and exhibited a pronounced leukocytosis; all of these mice were dead within 19 d after challenge (Fig. 1). As the dose of 69-kD OMP per immunization decreased from 16 to 1 μg , the proportion of mice with severe leukocytosis ($>70 \times 10^3$ WBC/ μl) increased and the percentage of survivors declined (Table I). However, $\sim 50\%$ of the mice injected with 1 μg 69-kD OMP survived *B. pertussis* respiratory challenge.

Since vaccine components are frequently chemically inactivated, a preparation of the 69-kD OMP was treated with glutaraldehyde in a manner similar to that used to inactivate pertussis toxin. This glutaraldehyde treated preparation was also pro-

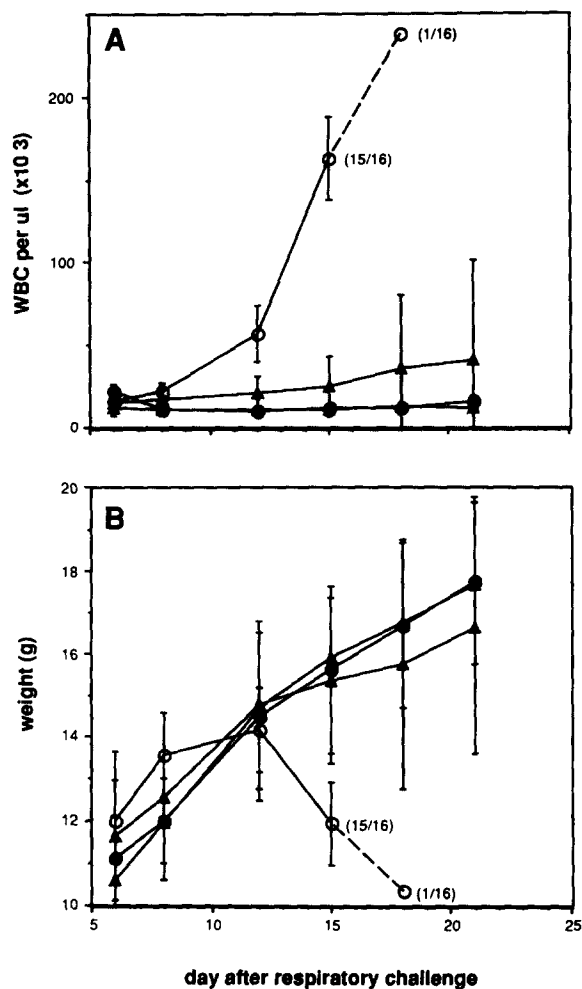


FIGURE 1. Protection against respiratory *B. pertussis* infection by immunization with the 69-kD OMP. (A) Change in peripheral WBC after *B. pertussis* aerosol challenge. Each point represents the mean WBC/ μl and SD for an entire group, except in cases where the (number scored/number infected) is indicated on the graph due to deaths in the group. (B) Weight gain. The mean weight and SD for each group is indicated. (N) indicates the number of mice infected: (●) 8 μg pertussis toxoid (13); (○) 8 μg tetanus toxoid (16); (▲) 16 μg 69-kD OMP (15); (Δ) uninfected controls (10).

TABLE I
Dose-dependent Protection by 69-kD OMP

Dose of 69-kD OMP per injection	n	Severe leukocytosis*	Survivors
μg		%	%
1	16	38	50
4	17	47	47
8	15	27	87
16	9	0	100
16 [†]	12	17	92

* Severe leukocytosis is defined as a count $>70 \times 10^3$ WBC/ μl anytime during the 21-d period after challenge. The mean leukocyte count of uninfected mice was 12×10^3 WBC/ μl .

[†] 69-kD OMP treated with 0.15% glutaraldehyde.

tective. Of the mice immunized with 16 μg of glutaraldehyde-fixed 69-kD OMP, 92% survived respiratory challenge with *B. pertussis* (Table I).

Detection of Specific Antibody. Serum and bronchoalveolar lavage titers of anti-69-kD antibody were measured in mice that had been immunized days 5 and 12 pp with 8 μg 69-kD OMP and challenged on day 19 with an aerosol of *B. pertussis* 18323 (Table II). A low serum IgM as well as an IgG anti-69-kD antibody response was detected in immunized mice that were not challenged. Between 1 and 3 wk after aerosol challenge, the reciprocal endpoint titer of anti-69-kD antibody in serum pooled from a group of five immunized mice rose from 1,200 to 3,200; all of this antibody was of the IgG isotype. Low levels of IgG anti-69-kD antibodies were detected in the bronchoalveolar lavage fluid of immunized mice before challenge, and increasing amounts were detected after challenge.

Passive Protection. The mAb BPE8, a mouse IgG1 anti-69-kD OMP, passively protected animals against respiratory *B. pertussis* infection when administered intravenously to 17-d-old mice 24 h before aerosol challenge (Fig. 2). Of the mice injected with 250 μg purified BPE8, 90% survived *B. pertussis* respiratory challenge, and had peak WBC counts $<70 \times 10^3$ WBC/ μl on day 16 after challenge (Fig. 2). BPE8 was detected by ELISA in the bronchoalveolar lavage fluid of mice within 1 h after intravenous injection and at increased levels 24 h after injection (Table III), demonstrating that the antibody had reached the site of infection in the lungs. In contrast to the protection observed with BPE8, 80% of the mice injected with a second IgG1 anti-69-kD OMP mAb, BPD8, died within 23 d after challenge, with all but 3 of 10 mice displaying leukocyte counts of $>70 \times 10^3$ WBC/ μl (Fig. 2). BPD8 was also detected by ELISA in the bronchoalveolar lavage fluids of mice 24 h after intravenous injection (footnote to Table III). Thus, the inability of BPD8 to protect cannot be attributed to its failure to transude to the lungs from the circulation. Passive administration of a mAb to an unrelated toxin, 18.1.7, an IgG1 anti-tetanus toxin (14), also failed to protect against *B. pertussis* aerosol challenge.

Detection of the 69-kD OMP in an Acellular Pertussis Vaccine. Immunoblot analysis, using the anti-69-kD OMP mAb BPE3 (5), revealed the presence of 69-kD OMP

TABLE II
Detection of Anti-69-kD OMP in Sera and Bronchoalveolar Lavage Fluid
from Immunized Mice

Days after challenge	Serum				Lavage			
	Total anti-69-kD*	A ₄₀₅ †			Total anti-69-kD*	A ₄₀₅		
		IgM	IgG	IgA		IgM	IgG	IgA
0	200	0.44	0.28	0.04	<4	0.03	0.02	0.01
7	1,200	0	0.69	0.01	<4	0.04	0.10	0
14	1,600	0	0.65	0.01	16	0	0.44	0.02
21	3,200	0	0.96	0.02	40	0.01	0.73	0.05

Mice were immunized with 8 μg 69-kD OMP on day 5 and 12 pp and challenged at 19 d of age.

* Endpoint dilution, as described in Materials and Methods.

† Absorbance at 405 nm, determined at a 1:100 dilution of serum and a 1:4 dilution of lavage fluid. Absorbance values from unimmunized mice were 0.06 for serum IgG and IgA, 0.28 for serum IgM and 0.05 for all lavage fluids. These values were subtracted from raw data from immunized mice.

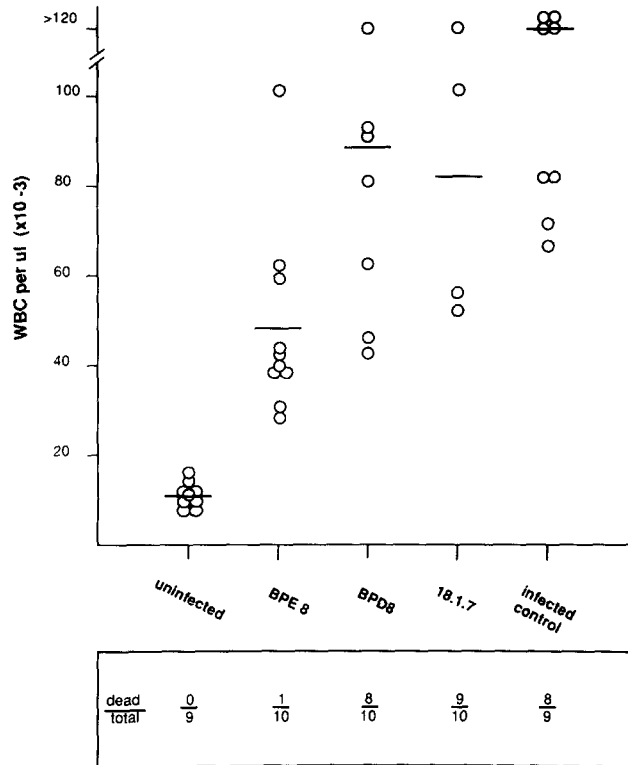


FIGURE 2. Passive protection against respiratory *B. pertussis* infection by the mAb BPE8. Each point represents the number of WBC/ μ l of peripheral blood after aerosol challenge with *B. pertussis* 18323. Bars indicate the arithmetic mean of each group. The leukocyte count for mice injected with BPE8 differs significantly from that of mice receiving BPD8 ($p < 0.05$, Student's t test). The cumulative number of deaths at day 23 after challenge over the total number infected is also shown for each group.

TABLE III
Appearance of BPE8 in Bronchoalveolar Lavage Fluid
of Intravenously Injected Mice

Time after inoculation	A ₄₀₅ *
<i>h</i>	
Lavage	
1	0.47 (0.27-0.64)
6	0.56 (0.48-0.77)
25	0.69 (0.50-0.94)
Serum	
1	0.78 (0.75-0.88)
6	0.62 (0.55-0.75)
25	0.59 (0.54-0.63)

* Mean absorbance and range of five animals per timepoint, determined at a 1:100 dilution of serum and a 1:4 dilution of lavage fluid by ELISA, using microtiter plates coated with purified 69-kD OMP. Background absorbances (405 nm) of sera and lavage fluid from uninjected mice were 0.15 and 0.14, respectively. The absorbance of pooled serum and lavage fluid from mice 24 h after intravenous injection with BPD8 was 0.77 and 1.4, respectively.

in a preparation of a Takeda acellular pertussis vaccine (Fig. 3 A). Densitometry analysis of silver-stained gels reveals that the 69-kD OMP comprises as much as 5%, by weight, of the total protein in this preparation.

The fact that this vaccine contained the 69-kD OMP offered the opportunity to examine the human serum antibody response to this protein. Immunoblot analysis was done on paired sera from 10 children who had received whole cell pertussis vaccine at 2, 4, and 6 mo of age and then were boosted at 18 mo with the above preparation of acellular pertussis vaccine. These children were bled immediately before receiving acellular pertussis vaccine, as well as 1 mo after the 18-mo booster dose. 6 of the 10 paired samples had a marked increase in reactivity with purified 69-kD OMP in postimmunization serum taken 1 mo after boosting. Immunoblot analysis of two of these paired sera is shown in Fig. 3 B. The IgG anti-69-kD OMP serum antibody response, measured in four of the six paired sera by ELISA, is shown in Table IV. All four individuals demonstrated 10–100-fold rises in IgG anti-69-kD OMP in response to immunization with the Takeda acellular vaccine containing 69-kD OMP.

Discussion

We have demonstrated that the 69-kD OMP is a protective antigen in an animal model of *B. pertussis* respiratory infection. Neonatal mice immunized with the 69-kD OMP or a glutaraldehyde-treated preparation of 69-kD OMP before respiratory challenge were protected from death and did not exhibit the severe leukocytosis and weight loss observed in unprotected controls. Protection was dose dependent, as only 50% of the mice immunized with $\leq 4 \mu\text{g}$ 69-kD OMP survived *B. pertussis* respiratory challenge. That antibody-mediated protection was due to immunological reactivity to the 69-kD OMP and not due to a contaminant in the preparation was established as polyclonal antisera raised to the 69-kD preparation reacted with a single

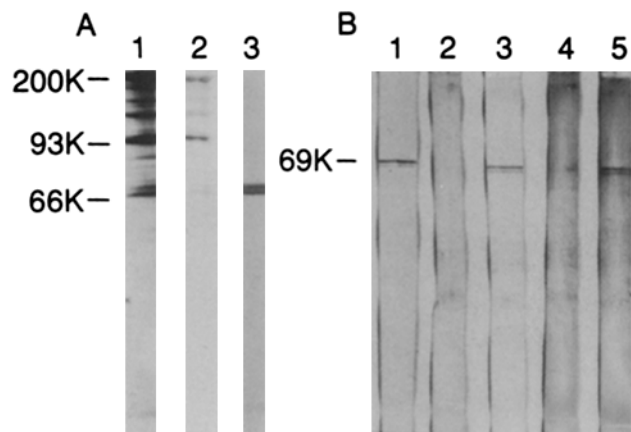


FIGURE 3. Presence of the 69-kD OMP in an acellular vaccine and antibody response to this protein after vaccination. (A) A sample (20 μg) of unadsorbed vaccine that had not been chemically inactivated was separated by SDS-PAGE and stained with silver (lane 1) or transferred to nitrocellulose and probed with a mAb directed against FHA (lane 2) or the anti-69-kD OMP mAb BPE3 (lane 3). (B) Nitrocellulose filters containing purified 69-kD OMP were probed with BPE3 (lane 1) or with human sera (1:100) collected before (lanes 2 and 4) or 30 d after (lanes 3 and 5) administration of the vaccine shown in A. The positions of molecular mass markers and the 69-kD OMP are shown.

TABLE IV
*IgG Antibody Response to 69-kD OMP in 18-Mo-Old Children**
Receiving a Single Immunization with a T-type Acellular Pertussis Vaccine

Patient no.	IgG antibody to 69-kD OMP [†]	
	Preimmunization	Postimmunization
	<i>U/ml</i>	
1	24	3,000
2	9	100
3	42	6,100
4	130	4,700

* Children had previously received whole cell pertussis vaccine at 2, 4, and 6 months of age.

[†] Units of antibody activity as measured by ELISA on microtiter plates coated with purified 69-kD OMP. Reference serum is arbitrarily defined as containing 50 U/ml of IgG anti-69-kD OMP. Antigen-coated wells incubated with PBS instead of serum gave values of <2 U/ml.

band of 69-kD when blotted to solubilized antigens of *B. pertussis* separated by SDS electrophoresis. Active immunization of neonatal mice with 69-kD OMP resulted in detectable levels of IgG anti-69-kD OMP antibody in the serum at the time of aerosol challenge. Although antibody to the 69-kD OMP was not detected in the lungs at the time of aerosol challenge, anti-69-kD antibodies were detected 2–3 wk after challenge. The appearance of anti-69-kD OMP in the lungs of immunized and challenged mice could be due to transudation of antibody from the serum as well as to local production of protective antibody. We have previously shown that intraperitoneal immunization of neonatal mice at days 5 and 12 pp with pertussis toxoid disseminates a primed population of antigen-specific B lymphocytes to the respiratory mucosa (19); this memory population may be stimulated to produce protective antibody in the lung upon respiratory challenge with *B. pertussis*.

The mAb BPE8 passively protected mice challenged with an aerosol of *B. pertussis* against leukocytosis and death, while BPD8 did not. Both BPE8 and BPD8 could be detected in the lungs of mice 24 h after intravenous injection, at the time of challenge. Transudation of IgG1 antibody from the systemic circulation to the lung has previously been demonstrated by Toews et al. (20). Since both BPE8 and BPD8 are IgG1 mAbs that recognize the 69-kD OMP (5) and both were found in the lung at the time of challenge, the ability of BPE8 to protect suggests that this antibody recognizes a protective epitope on the 69-kD OMP distinct from the epitope recognized by BPD8. Alternatively, the observed differential protection could be due to the differences in the affinity of each mAb for the 69-kD OMP. IgG1 antibody to the 69-kD OMP is thus sufficient to prevent leukocytosis and death in neonatal mice challenged with *B. pertussis*, presumably by transudation from the serum to the lung, although active immunization with the 69-kD OMP may elicit mechanisms of protective immunity in addition to serum antibody.

The mAb BB05, which was raised to the 68-kD protein of *B. bronchiseptica*, and which reacts by immunoblot with the 69-kD protein purified from *B. pertussis* (5), has been shown to passively protect mice from atrophic rhinitis and death when challenged with an aerosol of *B. bronchiseptica* (21). Active immunization of pregnant sows with the 68-kD OMP purified from *B. bronchiseptica* has also been shown to

prevent atrophic rhinitis in young offspring challenged intranasally with *B. bronchiseptica* (22). Presumably these piglets were protected by specific antibody in mother's milk, as there is no transplacental transfer of Ig in ungulates (23). Thus both the 69-kD OMP purified from *B. pertussis* and an immunologically related protein isolated from *B. bronchiseptica* have been shown to be protective antigens.

We show here that significant amounts of the 69-kD OMP are detectable in a T-type acellular vaccine prepared by Takeda. T-type vaccines are sucrose gradient-purified preparations of *B. pertussis* extracts that have been demonstrated to contain a mixture of antigens, including pertussis toxin, filamentous hemagglutinin, and fimbriae (24, 25). Since purified FHA is easily degraded and commonly runs as several bands on an acrylamide gel, including some near 69-kD, the presence of 69-kD OMP in a T-type acellular pertussis vaccine has until now gone undetected. Using mAbs specific for these antigens we have demonstrated the presence of the 69-kD OMP as well as FHA in the vaccine by immunoblotting.

In the preliminary studies described here, an antibody response to the 69-kD OMP was detected by immunoblot analysis in 18-mo-old children boosted with the Takeda acellular pertussis vaccine. One to two log increases in the concentration of serum anti-69-kD OMP antibodies were also detected by ELISA. Serum antibody responses to the 69-kD OMP have also been detected in children within 3–9 mo after completing a course of vaccination with whole cell vaccine (26), as well as in convalescent serum and saliva (27). In addition, De Magistris et al. have demonstrated that several human T cell clones, derived from a volunteer convalescent from pertussis, specifically proliferated in response to stimulation with 69-kD OMP purified from *B. pertussis* (28). The 69-kD OMP antigen, therefore, elicits both B cell-mediated and T cell-mediated immunity in humans.

The majority of the doses of acellular vaccine administered in Japan are T-type, containing inactivated pertussis toxin, filamentous hemagglutinin, and surface proteins that induce agglutinating antibodies (4). The ability of a whole cell pertussis vaccine to elicit agglutinating antibody has previously been correlated with clinical protection (29). However, it remains to be established if serum antibodies to the 69-kD OMP will provide a correlate of protective immunity for acellular pertussis vaccines.

We have demonstrated that the 69-kD OMP induces agglutinating antibodies (5), elicits specific antibody detected in the serum and lungs of mice, and is protective in a murine model of *B. pertussis* infection. A passively administered anti-69-kD OMP mAb, detected in the lungs at the time of challenge, was also found to protect mice. We further show that the 69-kD OMP elicits an antibody response in humans, and is present in an acellular pertussis vaccine preparation. The 69-kD OMP has recently been cloned and sequenced (6) and a protocol suitable for the large scale purification of antigen has recently been developed (30). Thus, the availability of sufficient quantities of purified antigen will allow for the analysis of the role and mechanism of the 69-kD OMP in eliciting protective immunity in humans.

Summary

Immunization with the 69-kD outer membrane protein (OMP) of *Bordetella pertussis* protected neonatal mice against lethal respiratory challenge with *B. pertussis* 18323. Active immunization elicited a serum IgG anti-69-kD OMP response at the

time of challenge, with IgG anti-69-kD OMP antibodies detected in bronchoalveolar lavage fluid after challenge. Intravenous administration of BPE8, a monoclonal IgG1 anti-69-kD OMP, also protected young mice against *B. pertussis* challenge. Intravenously injected BPE8 was detected in the lungs of mice at the time of aerosol challenge, suggesting that the presence of specific antibody in the lungs may mediate protection. Thus the 69-kD OMP of *B. pertussis* is a protective antigen in mice that elicits specific serum antibody that can transude to the lung.

The 69-kD OMP was detected in a preparation of a Takeda acellular vaccine by immunoblot analysis and a serum antibody response to the 69-kD OMP was observed in 18-mo-old children boosted with this preparation of Japanese acellular vaccine. Our results demonstrate that the *B. pertussis* 69-kD OMP is a protective antigen in animals, is immunogenic in humans, and is present in a preparation of acellular pertussis vaccine that is widely used in Japan. These findings indicate that the 69-kD OMP should be seriously considered as a candidate for inclusion in new formulations of antigenically defined acellular pertussis vaccines.

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References

1. World Health Organization. 1987. Six killers of children. *World Health*. January/February. 7:344.
2. Centers for Disease Control. 1982. Annual Summary 1981: reported morbidity and mortality in the United States. *Mortality Morbidity Weekly Rev.* 30:54.
3. Cody, C. L., L. J. Baraff, J. D. Cherry, S. M. Marcy, and C. R. Manclark. 1981. Nature and rates of adverse reactions associated with DTP and DT immunizations in infants and children. *Pediatrics*. 68:650.
4. Cherry, J. D., P. A. Brunell, G. S. Golden, and D. T. Karzon. 1988. Report of the task force on pertussis and pertussis immunization. 1988. *Pediatrics*. 81:939.
5. Brennan, M. J., Z. M. Li, J. L. Cowell, M. E. Bisher, A. C. Steven, P. Novotny, and C. R. Manclark. 1988. Identification of a 69 kilodalton nonfimbrial protein as an agglutinin of *Bordetella pertussis*. *Infect. Immun.* 56:3189.
6. Charles, I. G., G. Dougan, D. Pickard, S. Chatfield, M. Smith, P. Novotny, and N. Fairweather. 1989. Molecular cloning and analysis of P.69, a vir-controlled protein from *Bordetella pertussis*. *Proc. Natl. Acad. Sci. USA.* 86:3554.
7. Sato, Y., K. Izumiya, H. Sato, J. L. Cowell, and C. R. Manclark. 1980. Aerosol infection of mice with *Bordetella pertussis*. *Infect. Immun.* 29:261.
8. Sato, Y., K. Izumiya, M. A. Oda, and H. Sato. 1979. Biological significance of *Bordetella pertussis* fimbriae or hemagglutinin: a possible role of the pathogenesis and antibacterial immunity. *In International Symposium on Pertussis*. C. R. Manclark and J. C. Hill, editors. U. S. Department of Health, Education and Welfare, Government Printing Office, Washington, DC. Publication No. (NIH) 79-1830. 51-57.
9. Mallory, F. B., and A. A. Horner. 1912. Pertussis: the histological lesion in the respiratory tract. *J. Med. Res.* 27:115.
10. Linneman, C. C. 1979. Host-parasite interactions in pertussis. *In International Symposium on Pertussis*. C. R. Manclark and J. C. Hill, editors. U. S. Department of Health, Education and Welfare, Government Printing Office, Washington, DC. Publication No.

- (NIH) 79-1830. 3-18.
11. Tsai, C. M., and C. E. Frasch. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal. Biochem.* 119:115.
 12. Code of Federal Regulations. 1989. *Limulus amoebocyte lysate potency test*. Title 21, CFR 660.102. U. S. Government Printing Office, Washington, DC.
 13. Hewlett, E. L., K. T. Sauer, G. A. Myers, J. L. Cowell, and R. L. Guerrant. 1983. Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. *Infect. Immun.* 40:1198.
 14. Arciniega, J. L., D. L. Burns, E. Garcia-Ortigoza, and C. R. Manclark. 1987. Immune response to the B oligomer of pertussis toxin. *Infect. Immun.* 55:1132.
 15. Kenimer, J. G., W. H. Habig, and M. C. Hardegree. 1983. Monoclonal antibodies as probes of tetanus toxin structure and function. *Infect. Immun.* 42:942.
 16. Manclark, C. R., B. D. Meade, and D. G. Burstyn. 1986. Serological Response to *Bordetella pertussis*. In *Manual of Clinical Laboratory Immunology*. 3rd ed. N. R. Rose, H. Friedman, and J. L. Fahey, editors. American Society of Microbiology, Washington, DC. 388-394.
 17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680.
 18. Burnett, W. N. 1981. "Western blotting." Electrophoretic transfer of proteins from SDS polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 122:195.
 19. Shahin, R. D., M. Simmons, and C. R. Manclark. 1989. Analysis of protection against *Bordetella pertussis* respiratory infection in mice by B oligomer and pertussis toxoid. In *Vaccines 89*. R. A. Lerner, H. Ginsberg, R. M. Chanock, and F. Brown, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 249-252.
 20. Toews, G. B., D. Hart, and E. J. Hansen. 1985. Effect of systemic immunization on pulmonary clearance of *Haemophilus influenzae* type B. *Infect. Immun.* 48:343.
 21. Montaraz, J. A., P. Novotny, and J. Ivany. 1984. Identification of a 68-kilodalton protective antigen from *Bordetella bronchiseptica*. *Infect. Immun.* 47:744.
 22. Novotny, P., M. Kobisch, K. Crownley, A. Chubb, and J. A. Montaraz. 1985. Evaluation of *Bordetella bronchiseptica* vaccines in specific-pathogen-free piglets with bacterial cell surface antigens in enzyme-linked immunosorbent assay. *Infect. Immun.* 50:190.
 23. Kraehenbuhl, J. P., C. Bron, and B. Sordat. 1979. Transfer of humoral, secretory and cellular immunity from mother to offspring. *Curr. Top. Pathol.* 66:105.
 24. Aoyama, T., Y. Murase, T. Gonda, and T. Iwata. 1988. Type-specific efficacy of the acellular pertussis vaccine. *Am. J. Dis. Child.* 142:40.
 25. Noble, G., R. Bernier, E. Esber, M. C. Hardegree, A. Hinman, D. Klein, and A. Saah. 1987. Acellular and whole-cell pertussis vaccines in Japan. Report of a visit by U. S. scientists. *J. Am. Med. Assoc.* 257:1351-1356.
 26. Thomas, M. G., K. Redhead, and H. F. Lambert. 1989. Human serum antibody responses to *Bordetella pertussis* infection and pertussis vaccination. *J. Infect. Dis.* 159:211-218.
 27. Seddon, P. C., P. Novotny, C. A. Hart, and C. S. Smith. 1987. Antibody responses to *Bordetella pertussis* antigens. In *Program and Abstracts of the British Pediatric Association Meeting*, April, 1987. 39. (Abstr.)
 28. De Magistris, M. T., M. Romano, S. Nuti, R. Rappuoli, and A. Tagliabue. 1988. Dissecting human T cell responses against *Bordetella* species. *J. Exp. Med.* 168:1351.
 29. Armitage, P., W. C. Cockburn, D. G. Evans, J. O. Irwin, J. Knowelden, and A. F. B. Standfast. 1956. Vaccination against whooping-cough. Relation between protection in children and results of laboratory tests. *Br. Med. J.* 2:454.
 30. Gould-Kostka, J., D. L. Burns, M. J. Brennan, and C. R. Manclark. 1989. Purification and analysis of the antigenicity of a 69,000 Da outer membrane protein from *B. pertussis*. *FEMS (Fed. Eur. Microbiol. Soc.) Lett.* In press.