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Improved Efficacy of a Licensed Acellular Pertussis Vaccine, Reformulated in an Adjuvant Emulsion of Liposomes in Oil, in a Murine Model▽

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The immunogenicities and efficacies of a licensed diphtheria, tetanus, acellular pertussis, and inactivated poliovirus vaccine and the same vaccine formulated in a liposome/oil emulsion adjuvant were compared in a mouse model of pertussis respiratory infection. A single dose of the liposome/oil emulsion-adjuvanted vaccine produced significantly higher antibody levels than one dose of the licensed vaccine and protected mice from *Bordetella pertussis* infection with an efficacy equivalent to that of three doses of the licensed vaccine.

Pertussis is an acute respiratory disease caused by *Bordetella pertussis* that leads to an estimated 200,000 to 400,000 early infant deaths each year (9). Currently available acellular pertussis vaccines must be given as a multiple-dose series over weeks or months, which results in poor compliance with on-time immunization and a prolongation of the period of risk until the series is completed. In developing countries where pertussis is more prevalent, the need to travel to distant health clinics further impedes compliance with timely immunization (8). Pertussis is also resurgent in developed countries, with peak incidence in adolescents and adults as a result of decreased immunity with age and in infants too young to have completed the three-dose primary immunization series (7). A single-dose, long-lasting pertussis vaccine would improve compliance in both developing and developed countries and might reduce the need for frequent booster doses. VacciMax (VM; ImmunoVaccine Technologies, Halifax, Canada) is a potent vaccine delivery platform composed of liposomes in an oil emulsion that induces both long-lasting humoral and cellular immune responses following a single administration (1, 3). Natural infection with *B. pertussis* results in potent induction of both humoral and cell-mediated immunity that provides relatively long-lived (although not lifelong) protection against subsequent infection (6). Current vaccines still leave infants less than 6 months old at high risk for infection, which is thought to be the result of incomplete protection prior to completion of the primary immunization series, failure to induce mucosal immunity, and the presence of a Th2 bias in immune responses (2). A vaccine mimicking natural infection in design and capable of inducing a balanced immune response might provide quicker and longer-lasting immunity. The purpose of this study was to compare the antibody responses generated by a commercially available acellular pertussis vaccine (delivered in three doses) and the same vaccine formulated in VM (delivered as a single dose) in a preclinical animal model. The abilities of the vaccines to prevent *B. pertussis* lung infection were evaluated in a respiratory mouse model of pertussis.

Female 21-day-old BALB/c mice (Charles River Laboratories, Montreal, Quebec, Canada) were housed four per cage with free access to food and water. Immunizations used a combination of diphtheria and tetanus toxoids, an acellular pertussis component, and trivalent inactivated poliovirus (DTaP-IPV; Quadracel, Sanofi Pasteur Inc., Toronto, Ontario, Canada) as supplied by the manufacturer or reformulated in VM (DTaP-IPV/VM) and were initiated on day 0 in groups receiving one injection and days 0, 21, and 31 in mice given three doses. DTaP-IPV (50 μl/mouse, intraperitoneally injected) contained, per 0.5-ml dose, pertussis toxoid (PT; 20 μg), filamentous hemagglutinin (FHA; 20 μg), pertactin (PRN, 3 μg), fimbriae 2 and 3 (FIM; 5 μg), diphtheria toxoid (15 limit-of-flocculation units), tetanus toxoid (5 limit-of-flocculation units), aluminum (0.33 mg), purified inactivated poliomyelitis vaccine (type 1 [Mahoney], 40 D antigen units; type 2 [M.E.F. 1], 8 D antigen units; type 3 [Saukett], 32 D antigen units), 2-phenoxyethanol (0.7%) as a preservative, and Tween

TABLE 1. Geometric mean antibody titers of sera from mice immunized with three doses of DTaP-IPV, a single dose of DTaP-IPV, or a single dose of DTaP-IPV/VM

Vaccine antigen	Geometric mean antibody titer (EU/ml) for indicated vaccine group[a]		
	Triple-dose DTaP-IPV	Single-dose DTaP-IPV	Single-dose DTaP-IPV/VM
PT	3,596	504[b]	2,713[c,d]
FHA	554	32[b]	214[c,d]
PRN	71	7[e]	25[c,f]
FIM	325	107[c]	502[c,g]

[a] There were seven mice in each vaccine group. EU, enzyme-linked immunosorbent assay units.
[b] P was <0.001 in comparison to the triple-dose DTaP-IPV regimen.
[c] P was not significant in comparison to the triple-dose DTaP-IPV regimen.
[d] P was ≤ 0.001 in comparison to the single-dose DTaP-IPV regimen.
[e] P was 0.004 in comparison to the triple-dose DTaP-IPV regimen.
[f] P was not significant in comparison to the single-dose DTaP-IPV regimen.
[g] P was 0.02 in comparison to the single-dose DTaP-IPV regimen.

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TABLE 2. *B. pertussis* bacterial counts from the lungs of mice vaccinated with one or three doses of DTaP-IPV or one dose DTaP-IPV/VM

Vaccine formulation	Mouse no.	Bacterial lung count (log_{10} CFU/lung)[a]
Triple dose of DTaP-IPV	1	0
	2	0
	3	0
	4	0
	5	0
	6	0
	7	0
Single dose of DTaP-IPV	1	3.66
	2	3.26
	3	5.81
	4	5.10
	5	5.08
	6	0
	7	3.38
Single dose of DTaP-IPV/VM	1	0
	2	0
	3	0
	4	0
	5	0
	6	0
	7	0

[a] The mean nonzero log_{10} numbers of CFU/lung were as follows: for the triple-dose DTaP-IPV regimen, 0; for the single-dose DTaP-IPV regimen, 4.38; for the single-dose DTaP-IPV/VM regimen, 0.

80 (20 ppm). DTaP-IPV/VM (equivalent to 50 μl DTaP-IPV/mouse, intraperitoneally injected) contained the commercially supplied DTaP-IPV (Quadracel) encapsulated in liposomes and suspended in phosphate-buffered saline (PBS; 50 μl), which constituted the aqueous phase of a water-in-oil emulsion (100-μl total volume). The liposomes contained lecithin and cholesterol at a ratio of 10:1 (wt/wt) and were formed as described previously (3). Mineral oil-mannide oleate (8.5:1.5, vol/vol; Sigma) formed the oil phase of the emulsion.

For challenge studies, *B. pertussis* strain Tohama was inoculated onto Bordet-Gengou agar (Becton Dickinson, Cockeysville, MD) with 20% defibrinated horse blood (Quelab, Quebec, Canada) and 1% glycerol and then incubated at 36.5°C for 72 h. Bacteria were then transferred to slant tubes containing the above-described medium supplemented with 1% proteose peptone and incubated at 36.5°C for 24 h. A bacterial suspension was prepared in PBS-Casamino Acids (1%) and the volume adjusted to an optical density at 600 nm that corresponds to 5×10^8 CFU/ml. On day 42 postimmunization, mice were transferred to a HEPA-filtered biohazard containment hood in an isolation room under negative pressure. For the aerosolization challenge procedure, mice were placed in a Plexiglas box (32 by 25 by 34 cm) with a centrally located opening for the nebulizer. Aerosols were generated at a rate of 0.3 ml/min for approximately 50 min using a DeVilbiss model 646 nebulizer (Somerset, PA) at an air pressure of 18 lb/in^2 delivered by a Gast pressure pump (Benton Harbor, MI). Two air samples were collected for quantitative determination of the number of *B. pertussis* bacteria in each aerosol, and the mice were returned to their original cages. One mouse from each group was euthanized immediately after the challenge and the left lung placed in 2 ml PBS containing 1% Casamino Acids. Lungs were homogenized, and the homogenate was serially diluted to determine the number of CFU/lung. On days 50/51 postimmunization, mice (seven mice/group) were euthanized and the left lungs harvested to determine the number of *B. pertussis* bacteria in each lung as previously described (4, 5). Blood samples (days 40/41, seven mice/group) were obtained by tail bleeding and antibody titers against all pertussis components of the vaccine (PT, FHA, PRN, and FIM) determined by an enzyme-linked immunosorbent assay. Geometric mean antibody titers, lung bacterial counts, and 95% confidence intervals were calculated, and comparisons between groups were

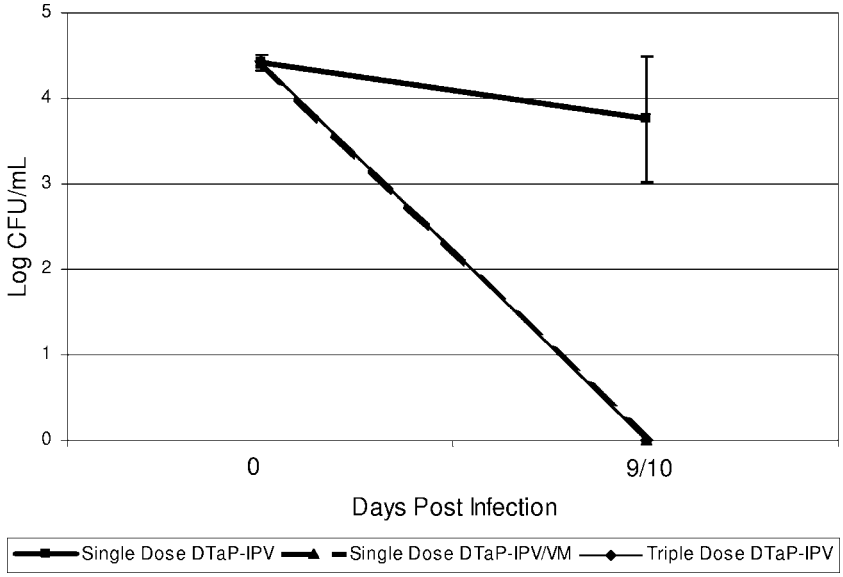

FIG. 1. Log_{10} numbers of CFU/lung (± standard errors of the means) of *B. pertussis* immediately after the challenge (day 0) and 9 to 10 days after the challenge in BALB/c mice immunized with one or three doses of DTaP-IPV or DTaP-IPV/VM.

made using *t* tests; *P* values of <0.05 were considered statistically significant. Prior to analysis, lung bacterial counts of zero were replaced by half the smallest observed nonzero value.

Antibody titers against PT, FHA, PRN, and FIM indicated that a single dose of DTaP-IPV was not very immunogenic compared to the three-dose DTaP-IPV regimen (Table 1). However, a single dose of DTaP-IPV/VM produced antibody titers that were similar to titers obtained after three doses of DTaP-IPV. After the aerosol challenge with *B. pertussis*, the two air samples from the exposure box had bacterial counts of 4,700/ml and 7,500/ml, indicating that mice inhaled similar numbers of *B. pertussis* bacteria during the aerosol challenge. Mice immunized with a single dose of DTaP-IPV had an average bacterial count of 1×10^5 CFU/lung, indicating the failure of this regimen to protect from the pertussis challenge (Table 2). Control mice given three doses of DTaP-IPV were fully protected from the pertussis challenge. A single vaccination with DTaP-IPV/VM also protected all mice from the pertussis challenge, as determined by the absence of bacteria in the lungs (Fig. 1).

In this study, we have demonstrated that a single dose of an existing pertussis vaccine formulated in VM stimulates antibody responses in mice against the pertussis components of DTaP-IPV. The efficacy of the single-dose vaccine was tested in a mouse challenge model of pertussis. Mice immunized once with DTaP-IPV formulated in VM were protected from *B. pertussis* lung infections with an efficacy equivalent to that observed with three doses of the same vaccine without VM. In other studies, we have shown that a single immunization of a VM-based vaccine promotes a rapid, robust, and therapeutic cytotoxic T-lymphocyte response to a human papillomavirus 16 E7 peptide that is protective against a challenge by human papillomavirus 16 E7 protein expressing tumors (3). This study demonstrates that a single vaccination with a commercially available DTaP-IPV formulated in VM also stimulates humoral immune responses and is protective against a bacterial challenge in a mouse aerosol model of pertussis.

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