Dose Response of Attenuated *Bordetella pertussis* BPZE1-Induced Protection in Mice

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Received 7 August 2009/Returned for modification 19 October 2009/Accepted 18 January 2010

Despite the availability of efficacious vaccines, the incidence of whooping cough is still high in many countries and is even increasing in countries with high vaccine coverage. Most severe and life-threatening pertussis cases occur in infants who are too young to be sufficiently protected by current vaccine regimens. As a potential solution to this problem, we have developed an attenuated live *Bordetella pertussis* vaccine strain, named BPZE1. Here, we show that after a single administration, BPZE1 induces dose-dependent protection against challenge with virulent *B. pertussis* in low-dose and in high-dose intranasal mouse lung colonization models. In addition, we observed BPZE1 dose-dependent antibody titers to *B. pertussis* antigens, as well as cell-mediated immunity, evidenced by the amounts of gamma interferon (IFN-γ) released from spleen cells upon stimulation with *B. pertussis* antigens. These two parameters may perhaps be used as readouts in clinical trials in humans that are currently being planned.

Pertussis, or whooping cough, caused by the exclusively human pathogen *Bordetella pertussis*, is a highly contagious acute disease of the respiratory tract and is responsible for approximately 300,000 deaths in children worldwide every year. Despite the availability and intensive use of efficacious vaccines for several decades, pertussis has not been eliminated in any country. In fact, the incidence of the disease is increasing in many countries with high vaccine coverage, and whooping cough remains globally among the top 10 causes of childhood death (4). Although by far most pertussis-linked deaths occur in young infants, adolescent and adult pertussis is also a growing and often underestimated problem in countries with high vaccine coverage (33). In contrast to infant infections, many *B. pertussis* infections in adolescents and adults are mild or subclinical and are usually not life-threatening (13). However, infected adults and adolescents are now considered an important reservoir for the whooping cough agent, able to transmit the infection to infants before they are sufficiently protected by vaccination. This epidemiological change is likely to be due to progressive waning of vaccine-mediated immunity during adolescence. However, other epidemiological features may also potentially contribute to the increasing pertussis burden in areas of high vaccine coverage, such as adaptation of *B. pertussis* strains in response to vaccine-induced immunity (3, 9, 10, 26).

Several strategies to solve this problem can be envisaged. As one of the potential solutions, it has been proposed to provide regular booster doses to adolescents and adults (7). However, repeated administrations of current pertussis vaccines are sometimes associated with local adverse effects, such as large swellings that may involve the entire limb (15). In addition, compliance of adolescents and adults to receiving booster doses is usually low for any vaccine (32). Maternal immunization has also been described as a potential approach to protect newborns (for a recent review, see reference 25). Nevertheless, the vaccination schedule will have to be carefully defined, both safety and efficacy of such a strategy still need to be assessed in clinical trials, and the acceptance among mothers may constitute an important hurdle. As an alternative, infant vaccination occurring as early as possible, preferably at birth, has been proposed in order to protect children during their most vulnerable period (2, 28). However, early protection by vaccination is hampered by the relative immaturity of the neonatal and infant immune system, especially of the cell-mediated immune arm (31), known to be important for protection against *B. pertussis* (23). In addition, optimal protection requires at least three doses of the current vaccines (6), usually given at 1- or 2-months intervals. Therefore, acceptable protection would not be achieved before 3 to 4 months, even if vaccination was started at birth.

In contrast to vaccination, infection with *B. pertussis* is able to quickly induce a strong Th1-type immune response in very young children, characterized by the production of high levels of *B. pertussis* antigen-specific gamma interferon (IFN-γ) (18). Furthermore, studies of nonhuman primates have led to the conclusion that “ultimate protection against whooping cough probably best follows a live *B. pertussis* inoculation” (14). This has prompted us to construct an attenuated *B. pertussis* strain as a live vaccine candidate by genetically altering or removing three *B. pertussis* toxins, pertussis toxin (PTX), tracheal cytotoxin (TCT), and dermonecrotic toxin (DNT). Briefly, this strain, named BPZE1, expresses an enzymatically inactive PTX by altering two key amino acids for the enzymatic activity of the toxin, shows a 100-fold reduction in TCT activity by the replacement of the *B. pertussis ampG* gene with that of *Escherichia coli*, and does not produce DNT by the deletion of its
structural gene. We showed that BPZE1 still colonizes the mouse respiratory tract and is able to provide protection against *B. pertussis* challenge after a single nasal administration in a mouse model (22).

In this study, we investigated the dose response of a single nasal administration of BPZE1 in mice to identify the protective doses needed against challenge infection with virulent *B. pertussis*.

**MATERIALS AND METHODS**

*B. pertussis* strains and growth conditions. Virulent *B. pertussis* BPSM, a streptococcal-resistant Tohama I derivative (20), and the attenuated vaccine strain BPZE1 (22) were grown on Bordet-Gengou (BG) agar (Difco, Detroit, MI) supplemented with 1% glycerol, 20% defibrinated sheep blood, and 100 μg/ml streptomycin (Sigma, St. Louis, MO). The bacteria were then harvested and suspended in sterile phosphate-buffered saline (PBS).

Intranasal infection, vaccination, and challenge. Groups of 8-week-old or 3-week-old (for the weight gain testing) female BALB/c mice (Iffa Credo, L’Arbresle, France) were kept under specific-pathogen-free conditions, and all experiments were carried out under the guidelines of the Institut Pasteur de Lille animal study board. Mice were intranasally infected with the indicated quantities of bacteria in 20 μl PBS, and the lung colonization was evaluated as previously described (21). For challenge infection, mice were intranasally infected 2 months after vaccination with the indicated doses of BPSM in 20 μl PBS. Lung colonization was determined 3, 7, and 30 days later. For intraperitoneal vaccination with acellular pertussis vaccine, one-fifth of a human dose of Tetravac (Aventis Pasteur, France) vaccine containing filamentous hemagglutinin (FHA) and pertussis toxoid was used as described previously (22). The limit of detection for bacterial colonization was 10 viable bacteria per individual lung.

Cytotoxicity assay. Human pulmonary epithelial (A549; ATCC CCL-185) and monocytic (THP-1; ATCC TIB 202) cell lines were cultured at 37°C in 5% CO2 in K-12 and RPMI 1640 media, respectively, supplemented with sodium penicillin G (1,000 U/ml) and streptomycin (50 μg/ml) (Gibco), 2 mM L-glutamine (Gibco), and 10% heat-inactivated fetal calf serum (FCS; Gibco). Before infection, cells were scrapped, washed with PBS to remove antibiotics, and plated at the required concentration in 1% FCS medium in 24-well plates. Cells were infected with 100 μl of bacterial suspension containing BPZE1 or *Escherichia coli* K-12 (multiplicity of infection [MOI] 0, 1, 10, or 100) for 5 h at 37°C in 5% CO2 and then extensively washed with PBS to remove extracellular bacteria. Infected cells were reincubated with fresh complete medium supplemented with 200 μg/ml of amikacin (Merck, France) to kill remaining extracellular bacteria for different time periods. The percentage of viable cells was evaluated by trypan blue exclusion.

Antibody determination. Mice were sacrificed, and sera and bronchoalveolar lavage fluids were collected for assessment of antibody responses by enzyme-linked immunosorbent assays (ELISAs) as previously described (21). Briefly, 96-well plates (Maxisorb; Nunc) were coated overnight at 4°C with 50 μl of FHA (10 μg/ml) purified from PTX-deficient *B. pertussis* BPRA (1), as previously described (19), or with 10 μg/ml PTX purified from FHA-deficient *B. pertussis* BPR4 (17), as previously described (29). Samples were then added in 2-fold serial dilutions and incubated overnight at 4°C. Goat anti-mouse IgG(H+L) or IgA horseradish peroxidase-conjugated antibodies (Southern Biotechnologies Associates, Inc., Birmingham, AL) were then added for 2 h at 37°C. The ELISAs were developed using tetramethylbenzidine and hydrogen peroxide (Interchim, Montluçon, France), according to the manufacturer’s specifications. The results are expressed in titers, defined as the reciprocal of the dilution giving an optical density at 492 nm of three times that of the conjugate control.

IFN-γ assay. Spleen cells from individual mice were harvested 6 weeks after BPZE1 administration and stimulated at 105 cells/ml with heat-killed *B. pertussis* BPSM (105 cells/ml) and 5.0 μg/ml of heat-inactivated PTX or FHA. Supernatants were harvested from triplicate cultures after 72 h of incubation at 37°C in the presence of 5% CO2, and IFN-γ concentrations were determined by immunoassays (BD OptEIA set; Pharmingen, San Diego, CA).

Statistical analysis. The results were analyzed using the unpaired Student t test and the Kruskal-Wallis test, followed by Dunn’s posttest (GraphPad Prism program) when appropriate. Differences were considered significant at P values of ≤0.05.

**RESULTS**

BPZE1 safety assessment. In vivo and in vitro tests were used to assess the safety of BPZE1. Infant (3-week-old) mice were inoculated with 1 × 106 BPZE1, and weight changes were monitored over a period of 2 weeks. No clinical symptoms were observed following BPZE1 administration in infant mice, and their weight gain was similar to that of nonimmunized mice (Fig. 1a). Histopathological analysis of the lungs of mice at 5 days after intranasal administration of BPZE1 showed minimal cell infiltration, similar to that observed in lungs from mice inoculated with sterile PBS (Fig. 1b). In contrast, nasal infection with the virulent *B. pertussis* BPSM strain induced strong peribronchovascular infiltrates and recruitment of inflammatory cells in the lungs of infected mice.

In vitro assays were used to measure the potential cell toxicity of BPZE1. The kinetics of the viability of human pulmonary epithelial A549 cells and monocytic THP-1 cells were measured following infection with different MOIs of live *B. pertussis* BPZE1 or live *E. coli* K-12 as a control. As shown in Fig. 1c, BPZE1 expresses no toxic effect on pulmonary epithelial A549 cells at any MOI tested. A slight reduction of the monocyte THP-1 viability was observed 6 days after interaction with BPZE1 after incubation at the highest bacterial load (MOI = 10). However, this reduction was similar to that observed after infection with nonpathogenic *E. coli*, indicating that BPZE1 is not more toxic than nonpathogenic *E. coli*.

Mouse lung colonization after administration of different doses of BPZE1. We have previously shown that BPZE1 is able to colonize and to persist in the lungs of mice as long as virulent *B. pertussis* (22). Bacterial persistence may be important for prolonged immune stimulation and thus for induction of efficient long-term immunity. Therefore, we first determined the colonization profile of different doses of BPZE1 administered nasally. Groups of BALB/c mice were intranasally inoculated with 10-fold increasing doses of BPZE1. Initial inoculum sizes reaching mouse lungs were determined 3 h after nasal administration of BPZE1 and were shown to rank from 5 × 102 to 1 × 109 CFU. Bacterial loads in the lungs were subsequently assessed every week for each group of mice. As shown in Fig. 2 and consistent with our previous data (22), inoculation with the highest dose of BPZE1 (109 CFU) resulted in persistent colonization of up to 3 to 4 weeks. Persistence was not strongly affected by decreasing the inoculum size down to 107 CFU, although the bacterial loads at each analyzed time point depended on the initial inoculum size, showing a good dose-dependent colonization profile. However, when the inoculum size was further decreased down to 5 × 105 CFU, the bacteria did not persist, and no evidence for colonization was observed. These results indicate that the minimal nasal dose of BPZE1 to establish lung colonization in BALB/c mice was 105 CFU.

Antibody and IFN-γ responses induced by BPZE1. Both antibody and T-cell responses to *B. pertussis* antigens, such as PTX and FHA, contribute to immunity against pertussis (23). The kinetics of serum antibody responses were first measured after immunization of mice with the highest dose (1 × 106 CFU) of BPZE1. As shown in Fig. 3a, the serum IgG response to heat-killed *B. pertussis* extracts was already detectable 14 days after nasal immunization and increased during the first
Sera were then collected from the different mice inoculated with the various doses of BPZE1 at 2 months after BPZE1 administration, and antibody titers were estimated by ELISA. The results showed that administration of BPZE1 induced serum IgG responses against total heat-killed *B. pertussis* extract and against both PTX and FHA and that the antibody titers increased proportionally to the dose of bacteria administered (Fig. 3b to d). No serum IgA response against whole-cell antigens was detected even after administration of the highest dose (10^6 CFU) of BPZE1 (data not shown). Since BPZE1 is administered nasally, we analyzed local antibody responses to *B. pertussis* extracts after administration of 10^6 CFU of BPZE1 or to *E. coli* K-12 at the indicated bacterium/cell ratio (MOI). Significant levels of IgA and IgG responses against *B. pertussis* whole-cell antigens were measured in bronchoalveolar lavage fluid samples from BPZE1-vaccinated mice (Fig. 4). In comparison, acellular pertussis vaccine, which is administered systemically, induced IgG, but there were no IgA responses in the respiratory tract.

In parallel, the IFN-γ production in response to stimulation with the *B. pertussis* antigens was assessed as a readout to measure T-cell responses. Spleen cells from individual mice were therefore harvested 6 weeks after BPZE1 administration and stimulated with heat-killed *B. pertussis* BPSM, PTX, or

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**FIG. 1.** *In vivo* and *in vitro* safety assessments following BPZE1 inoculation. (a) Weight gain monitoring of 3-week-old BALB/c mice intranasally inoculated with 1 × 10^6 CFU of *B. pertussis* BPZE1 (open circles) compared to noninfected mice (closed circles). (b) Histological analysis of lungs from BPZE1- or BPSM-infected mice compared to controls given PBS. Five days after infection, the lungs were aseptically removed and fixed in formaldehyde. Sections were stained with toluidine blue and examined by light microscopy. Original magnification, ×10. (c) Cytotoxicity assessment of BPZE1. Human pulmonary epithelial A549 and monocytic THP-1 cells were exposed for 5 h to live *B. pertussis* BPZE1 or to *E. coli* K-12 at the indicated bacterium/cell ratio (MOI). After extracellular bacteria were removed, infected cells were reincubated in fresh medium for 1 (white bars), 3 (gray bars), and 6 (black bars) days before determination of cell viability by trypan blue exclusion.

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**FIG. 2.** Lung colonization by decreasing the doses of BPZE1. Eight-week-old BALB/c mice were intranasally inoculated with attenuated *B. pertussis* BPZE1, with doses ranging from 1 × 10^6 (filled square), 1 × 10^5 (open diamond), 1 × 10^4 (filled triangle), 1 × 10^3 (open circle), or 5 × 10^2 (filled diamond) bacteria/mouse. The results are expressed as the number of mean CFU (± the number of standard errors of the mean CFU) from three to four mice per group and are representative of two similar experiments. The horizontal dotted line represents the limit for bacterial counts.

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month. Sera were then collected from the different mice inoculated with the various doses of BPZE1 at 2 months after BPZE1 administration, and antibody titers were estimated by ELISA. The results showed that administration of BPZE1 induced serum IgG responses against total heat-killed *B. pertussis* extract and against both PTX and FHA and that the antibody titers increased proportionally to the dose of bacteria administered (Fig. 3b to d). No serum IgA response against whole-cell antigens was detected even after administration of the highest dose (10^6 CFU) of BPZE1 (data not shown). Since BPZE1 is administered nasally, we analyzed local antibody responses to *B. pertussis* extracts after administration of 10^6 CFU of BPZE1 or to *E. coli* K-12 at the indicated bacterium/cell ratio (MOI). Significant levels of IgA and IgG responses against *B. pertussis* whole-cell antigens were measured in bronchoalveolar lavage fluid samples from BPZE1-vaccinated mice (Fig. 4). In comparison, acellular pertussis vaccine, which is administered systemically, induced IgG, but there were no IgA responses in the respiratory tract.

In parallel, the IFN-γ production in response to stimulation with the *B. pertussis* antigens was assessed as a readout to measure T-cell responses. Spleen cells from individual mice were therefore harvested 6 weeks after BPZE1 administration and stimulated with heat-killed *B. pertussis* BPSM, PTX, or
FHA, and the IFN-γ concentrations in the culture supernatants were measured. Consistent with a previous study (22), we found that by using the highest bacterial dose ($1 \times 10^6$ CFU), BPZE1 immunization induced a Th1-type immune response, with high levels of IFN-γ produced by stimulated spleen cells. This IFN-γ response was significantly higher than that induced by stimulated spleen cells from mice immunized with acellular pertussis vaccine (Fig. 5a). Next, we showed that increasing BPZE1 inoculum sizes resulted in increasing IFN-γ responses to all three antigenic stimuli (Fig. 5b and c). A linear dose response was observed for IFN-γ production upon stimulation with PTX and with FHA, starting with significant IFN-γ production after administration of $10^4$ CFU (Fig. 5b), whereas the threshold of $10^4$ CFU resulted in a nearly maximal IFN-γ response to total heat-killed B. pertussis cell extract. Increasing the BPZE1 doses above $10^5$ CFU did not result in a further increase in the level of total heat-killed B. pertussis cell extract-induced IFN-γ (Fig. 5c).

FIG. 3. Serum antibody responses of BPZE1-vaccinated mice. (a) Mice were first intranasally immunized with the highest dose ($10^6$ CFU) of BPZE1, and the kinetics of serum antibody responses against total B. pertussis extract was measured during 1 month. (b to d) Mice were then intranasally immunized with the indicated doses of BPZE1, and serum antibody titers against total B. pertussis extract (b), FHA (c), and PTX (d) were measured 2 months later by ELISA. The results are expressed as mean titers (± standard errors of the mean titers) from three to four mice per group and are representative of two similar experiments. P values of <0.05 (*) compared to naïve mice were considered significant.

FIG. 4. Antibody responses in bronchoalveolar lavage fluids of BPZE1-vaccinated mice to B. pertussis antigens. Mice were immunized intranasally with the highest dose ($10^6$ CFU) of BPZE1 or intraperitoneally with one-fifth of acellular pertussis vaccine (aPv) (Tetravac; Aventis-Pasteur, France). Bronchoalveolar lavage fluids were obtained 2 months later, and antibody responses to a total B. pertussis extract were measured by ELISA. Results are expressed as mean titers (± standard errors of the mean titers) from three to four mice per group. P values of <0.05 (*) compared to naïve mice were considered significant.
Protection induced by the administration of BPZE1. To evaluate the protective immunity induced by nasal vaccination with the different doses of BPZE1, the mice were challenged 2 months after administration of BPZE1 by intranasal infection with $5 \times 10^5$ CFU of virulent *B. pertussis* BPSM, and the colony counts in the lungs were determined 3 h and 7 days later. In the control unvaccinated mice, the colony counts increased by approximately 10-fold between 3 h and 7 days postchallenge. In contrast, mice immunized with a single nasal dose of BPZE1 showed strong protection, as evidenced by the low to undetectable CFU counts 7 days after challenge infection (Fig. 6a). With a vaccine dose as low as $10^4$ CFU of BPZE1, full protection was observed, as evidenced by the total bacterial clearance at day 7 after challenge, compared to that of the nonvaccinated mice.

When the mice were challenged with a 100-fold-higher dose of virulent *B. pertussis* BPSM, a correlation was found between the vaccine dose and the protective effect ($r = 0.99$). Although total bacterial clearance was not observed at day 7 after challenge for any vaccine group, vaccination with $10^6$ CFU of BPZE1 resulted in an approximately 1,000,000-fold reduction in bacterial counts at day 7 postchallenge, compared to that of the nonvaccinated mice, and vaccination with $10^3$ CFU of BPZE1 resulted in an approximately 1,000-fold reduction (Fig. 6b).

To investigate whether nasal vaccination with BPZE1 induces a B-cell memory response, antibody titers to FHA and PTX were determined 7 days after challenge and compared to the titers measured before challenge. At all vaccine doses,
anamnestic responses to either of the antigens were detected after challenge, with maximal antibody titers to FHA and PTX reached at the threshold of 10^5 CFU of BPZE1 (Fig. 7).

**DISCUSSION**

Attenuated live vaccines against bordetellosis have been developed to protect dogs and pigs against *Bordetella bronchiseptica* infections (5, 30). Furthermore, experiments on nonhuman primates carried out more than 40 years ago (14) have suggested that infection by *B. pertussis* may be the best way to protect against subsequent infection. Although for vaccination against whooping cough this strategy has so far received little attention to the benefit of the development of acellular vaccines composed of defined *B. pertussis* protein antigens, attempts to genetically attenuate *B. pertussis* for that purpose have been described several years ago (27). They consisted of deleting the *aroA* gene, which indeed resulted in strong attenuation but rather poor immunogenicity, probably due to the failure of *B. pertussis aroA* mutants to colonize the respiratory tract, and several administrations of very high doses were required to protect mice against subsequent challenge with virulent *B. pertussis*. More recently, we have developed the attenuated BPZE1 vaccine strain, able to persist in the mouse respiratory tract and to induce strong protection after a single nasal administration in a murine respiratory infection model (22). In addition, BPZE1 was shown to protect against *Bordetella parapertussis*, the second most frequent cause of whooping cough-like illness in humans, whereas the acellular vaccine did not, suggesting that BPZE1 may protect against a wide variety of *B. pertussis* isolates. This is particularly interesting in light of the potential vaccine escape by the relatively recent strain adaptation observed for *B. pertussis*, especially in areas of intensive vaccination (3, 9, 10, 26).

Although humans are the only natural reservoir for *B. pertussis* and mice are comparatively more difficult to infect than humans, as substantially more bacteria are needed to establish infection in mice than in humans, mouse models are nevertheless widely accepted models to preclinically test pertussis vaccines. In particular, the intranasal murine lung infection model has been validated as a good assay to discriminate between the efficacies of different pertussis vaccines (8, 24), whereas the classical intracerebral challenge model developed by Kendrick et al. (16) and used in the European pharmacopeia is much less discriminatory. We therefore used this model in the dose-response studies with the BPZE1 vaccine strain and found a strong correlation between the vaccine dose of BPZE1 and protection against subsequent challenge with virulent *B. pertussis*. Both high-dose and low-dose challenge models were used, and in all cases, a single BPZE1 administration significantly protected against challenge infection. In the low-dose challenge model, total bacterial clearance was not observed at any vaccine dose, but a strong correlation of protection with the vaccine dose was observed. In addition, a strong correlation of the vaccine dose with antibody titers against the major *B. pertussis* antigens FHA and PTX, as well as with IFN-γ production by spleen cells upon stimu-
lation with these antigens, was observed, and a vaccine dose-dependent anamnestic response was observed upon challenge infection with virulent B. pertussis. Since both antibodies and cell-mediated immunity appear to contribute to protection against whooping cough, these parameters may be valid assays to use for the monitoring of immune responses induced by BPZE1 in future clinical trials. In addition, administration of BPZE1 resulted in the appearance of anti- B. pertussis antibodies in bronchoalveolar lavage fluids, both of the IgG and of the IgA isotypes. Although local anti-B. pertussis IgG was likely to be excreted from sera, it is possible that the IgA had been locally induced, since no anti-B. pertussis was found in the sera. In contrast to vaccination with BPZE1, after systemic vaccination with a commercial acellular vaccine, no anti-B. pertussis IgA production was detected in the bronchoalveolar lavage fluids. Since B. pertussis causes a strictly mucosal respiratory infection, antigen-specific IgA responses might represent important effectors for immunity against pertussis (12).

Importantly, even after administration of the highest vaccine dose (10^6 CFU), no significant lung pathology was observed, although infection of mice with 10^6 CFU of the virulent strain resulted in strong lung infiltration of inflammatory cells, attesting to the safety of BPZE1, even at the highest dose tested here. Likewise, infection of infant mice with 10^6 CFU of BPZE1 did not affect their weight gain, at least over 15 days. In vitro, infection of pneumocyte cultures with MOIs of up to 100 did not cause any cytotoxicity, and the cytotoxicity of BPZE1 infection on a monocyte cell line was minimal, even at an MOI of 100, similar to that of infection with a nonpathogenic E. coli K-12 laboratory strain. These results have contributed to downgrading BPZE1 from a biosafety level 2 organism to a biosafety level 1 organism in France.

Although the human dose of acellular pertussis vaccines has been validated in this intranasal mouse challenge model, it is difficult to extrapolate the optimal BPZE1 vaccine dose in this model to a human dose. It is likely that colonization of the respiratory tract is an important factor for the induction of infection-induced immune protection. B. pertussis is a strictly human pathogen, and it is highly contagious to humans. Only a few bacteria are necessary to establish an infection in humans (11). B. pertussis is much less infectious to mice. Therefore, the optimal human doses of BPZE1 may actually be lower than the optimal doses for mice. A minimum of 10^3 CFU of BPZE1 is required to establish a persistent colonization in mice, but it is possible that for humans that much less bacteria are needed for colonization to achieve protective immunity. This is an important consideration for the development of BPZE1-based vaccines to protect humans against whooping cough and will be the major issue of the first clinical trials in humans that are currently being planned.

ACKNOWLEDGMENTS

We are grateful to M. Loyens for technical assistance during this study and to C. Creuzi for her expertise in histological analysis. This work was partly supported by the Agence Nationale de la Recherche (project ANR-05-MIME-BPVAC, project no. A05137TES) and by the European Commission (FP7, grant agreement 201502; Child-Innovac).

REFERENCES

1. Antoine, R., and C. Locht. 1990. Roles of the disulfide bond and the carboxy-terminal region of the S1 subunit in the assembly and biosynthesis of pertussis toxin. Infect. Immun. 58:1518–1526.
2. Belloni, C., A. DeSilvestri, C. Tinelli, M. A. Avanzini, M. Marconi, F. Strano, G. Rondini, and G. Chirico. 2003. Immunogenicity of a three-component acellular pertussis vaccine administered at birth. Pediatrics 111:1042–1045.
3. Bouche, V.-V., Caro, E. Levillain, Ga. Guigo, and N. Guigo. 2008. Genomic Content of Bordetella pertussis clinical isolates circulating in areas of intensive children vaccination. PLoS One 3:e2437.
4. Crowcroft, N. S., C. Stein, P. Duclos, and M. Birmingham. 2003. How best to estimate the global burden of pertussis? Lancet Infect. Dis. 3:413–418.
5. de Jong, M. F. 1987. Prevention of atrophic rhinitis in piglets by means of intranasal administration of a live non-AR-pathogenic Bordetella bronchiseptica vaccine. Vet. Q. 9:123–133.
6. Edwards, K. M., and M. D. Dekker. 1996. Acellular pertussis vaccines for infants. N. Engl. J. Med. 334:391–392.
7. Forsyth, K., T. Tan, C. H. von König, J. J. Caro, and S. Plotkin. 2005. Potential strategies to reduce the burden of pertussis. Pediatr. Infect. Dis. J. 24:S69–S74.
8. Guiso, N., C. Capiau, G. Carletti, J. Poolman, and P. Hauser. 1999. Intranasal murine model of Bordetella pertussis infection. I. Prediction of protection in human infants by acellular vaccines. Vaccine 17:2366–2376.
9. He, Q., and J. Mertsola. 2008. Factors contributing to pertussis resurgence. Future Microbiol. 3:329–339.
10. Heikkinen, E., T. Kallonen, L. Saarinen, R. Sara, A. J. King, F. R. Mooi, J. T. Sointi, J. Mertsola, and Q. He. 2007. Comparative genomics of Bordetella pertussis reveals progressive gene loss in Finnish strains. PLoS One 2:e904.
11. Heisinger, U. 2001. Pertussis: an old disease that is still with us. Curr. Opin. Infect. Dis. 14:329–335.
12. Hellwig, S. M., A. B. van Spriel, J. F. Schellekens, F. R. Mooi, and J. G. van de Winkel. 2001. Immunoglobulin A-mediated protection against Bordetella pertussis infection. Infect. Immun. 69:4846–4850.
13. Hewlett, E. L., and K. M. Edwards. 2005. Clinical practice: pertussis—not just for kids. N. Engl. J. Med. 352:1215–1222.
14. Huang, C. C., P. M. Chen, J. K. Kuo, W. H. Chu, S. T. Lin, H. S. Lin, and Y. C. Lin. 1962. Experimental whooping cough. N. Engl. J. Med. 266:105–111.
15. Jackson, L. A., B. A. Carstie, D. Malais, and J. Froeschle. 2002. Retrospective population-based assessment of medically attended injection site reactions, severe, allergic responses and febrile episodes after acellular pertussis vaccine combined with diphtheria and tetanus toxoids. Pediatr. Infect. Dis. J. 21:781–786.
16. Kendrick, P. L., G. Elderling, M. K. Dixon, and J. Misner. 1947. Mouse protection tests in the study of pertussis vaccine: a comparative series using the intracerebral route for challenge. Am. J. Public Health 37:803–810.
17. Locht, C., M. C. Geoffroy, and G. Renaud. 1992. Common accessory genes for the Bordetella pertussis filamentous hemagglutinin and fibrinase sequence similarities with the papC and papD gene families. EMBO J. 11:3175–3183.
18. Mascart, F., V. Verscheure, A. Malfroot, M. Hainaut, D. Pierard, S. Temerianu, A. Pelletier, A.-S. Debrie, J. Levy, G. Del Giudice, and C. Locht. 2003. Bordetella pertussis infection in 2-months-old infants promotes type I T cell responses. J. Immunol. 170:1504–1509.
19. Menozzi, F. D., C. Gantiez, and C. Locht. 1991. Interaction of the Bordetella pertussis filamentous haemagglutinin with heparin. FEMS Microbiol. Lett. 52:59–64.
20. Menozzi, F. D., R. Mutombo, G. Renaud, C. Gantiez, J. H. Hannah, E. Leininger, M. J. Brennan, and C. Locht. 1994. Heparin-inhibitable lectin activity of the filamentous hemagglutinin adhesin of Bordetella pertussis. Infect. Immun. 62:769–778.
21. Miecare, N., J. Cornette, A. M. Schacht, R. J. Pierce, C. Locht, A. Capron, and G. Riveau. 1997. Intranasal priming with recombinant Bordetella pertus- sis for the induction of a systemic immune response against a heterologous antigen. Infect. Immun. 65:544–550.
22. Miecare, N., A. S. Debrie, D. Rase, J. Bertout, C. Rouanet, A. Ben Younes, C. Creuzi, J. Engle, W. E. Goldman, and C. Locht. 2006. Live attenuated B. pertussis as a single-dose nasal vaccine against whooping-cough. PLoS Pathog. 2:e62–670.
23. Mills, K. H. G. 2001. Immunity to Bordetella pertussis. Microbes Infect. 3:331–3346.
24. Mills, K. H., M. Brady, E. Ryan, and B. P. Mahon. 1998. A respiratory challenge model for infection with Bordetella pertussis: application in the assessment of pertussis vaccine potency and in defining the mechanism of protective immunity. Dev. Biol. Stand. 95:51–61.
25. Mooi, F. R., and S. C. De Greeff. 2007. The case for maternal vaccination against pertussis. Lancet Infect. Dis. 7:514–624.

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26. Mooi, F. R., I. H. M. van Loo, M. van Gent, Q. He, M. J. Bart, C. J. Heuvelman, S. C. de Greeff, D. Diavatopoulos, P. Teunis, N. Nagelkerke, and J. Mertsola. 2009. *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. Emerg. Infect. Dis. 15: 1206–1213.

27. Roberts, M., D. Maskell, P. Novotny, and G. Dougan. 1990. Construction and characterization in vivo of *Bordetella pertussis* *aroA* mutants. Infect. Immun. 58: 732–739.

28. Roduit, C., P. Bozzotti, N. Mielcarek, P. H. Lambert, G. del Giudice, C. Locht, and C. A. Siegrist. 2002. Immunogenicity and protective efficacy of neonatal vaccination against *Bordetella pertussis* in a murine model: evidence for early control of pertussis. Infect. Immun. 70: 3521–3528.

29. Sekura, R. D., F. Fish, C. R. Manclark, B. Meade, and Y. L. Zhang. 1983. Pertussis toxin. Affinity purification of a new ADP-ribosyltransferase. J. Biol. Chem. 258: 14647–14651.

30. Shade, F. J., and R. A. Goodnow. 1979. Intranasal immunization of dogs against *Bordetella bronchiseptica*-induced tracheobronchitis (kennel cough) with modified live-*Bordetella bronchiseptica* vaccine. Am. J. Vet. Res. 40: 1241–1243.

31. Siegrist, C. A. 2001. Neonatal and early life vaccinology. Vaccine 19: 3331–3346.

32. Storsaeter, J., J. Wolter, and C. Locht. 2007. Pertussis vaccines, p. 245–288. In C. Locht (ed.), *Bordetella* molecular microbiology. Horizon Bioscience, Norfolk, United Kingdom.

33. Wirsing von König, C. H., S. Halperin, M. Riffelmann, and N. Guiso. 2002. Pertussis of adults and infants. Lancet Infect. Dis. 2: 744–750.