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Heterologous prime-boost immunization with live attenuated *B. pertussis* BPZE1 followed by acellular pertussis vaccine in mice

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ABSTRACT

Pertussis is a severe and life-threatening infectious disease. Two successive generations of vaccines have strongly reduced its incidence over the last 70 years. However, despite excellent global vaccine coverage, it is still not under control and constitutes today the most frequent vaccine-preventable childhood disease. New vaccination approaches are therefore needed. Here, we provide preclinical proof of concept for a heterologous prime-boost strategy, using the live attenuated *Bordetella pertussis* vaccine candidate BPZE1 to prime infant and neonatal mice intranasally and a currently available acellular pertussis vaccine (aPV) as a booster. Intranasal vaccination with BPZE1 provided strong protection against challenge in neonatal mice, which could be boosted with a single dose of aPV. Furthermore, BPZE1 priming induced a strong Th1/Th17 response, which was maintained after repeated aPV administrations, in contrast to non-primed mice, in which aPV administrations resulted in Th2 skewing. In addition to T cell responses, intranasal administration of BPZE1 to infant or neonatal mice also primed antibody responses to *B. pertussis* antigens, with a strong preference of the IgG2a over the IgG1 isotypes, which was not seen in non-primed animals. Finally, neonatal BPZE1 priming strongly enhanced aPV-induced protection against *B. pertussis* challenge. These results lend support for a heterologous prime-boost strategy to control pertussis by using BPZE1 early in life and considering the current aPV administrations as booster vaccinations, thereby bridging the gap from birth to the first aPV immunizations and avoiding aPV-mediated Th2 skewing. A first-in-man clinical trial on BPZE1 has recently been successfully completed, which provides hope that these findings may be translated into human applications in the future.

1. Introduction

Infection by *Bordetella pertussis*, the causative agent of whooping cough, remains one of the leading causes of neonatal morbidity and mortality worldwide, despite of the wide-spread use of efficacious vaccines. Two types of vaccines are currently available. Since the 1990, acellular pertussis vaccines (aPV) are progressively replacing the first-generation, whole-cell vaccines. They have shown an improved safety profile, with comparable short-term efficacy [1,2]. Vaccination usually starts at two months of age and requires at least three immunizations, given at one-to-two months intervals, for optimal protection. Thus, young infants (<6 months of age) are not fully protected, yet they constitute the most vulnerable age group, with most of the pertussis-linked deaths [3–5].

The recent resurgence of pertussis in countries with high aPV vaccination coverage [6] has raised the concern that aPV-induced immunity wanes faster than immunity induced by whole-cell vaccines or by infection [7]. In addition, aPV administration skews the immune response to a Th2 type, both in children and in mice [8–10]. Th2 skewing in the first months of life has been associated with a genetic risk of atopy [11–16]. It may also reduce vaccine efficacy and longevity of protection [17,18].

The observation that immunity induced by natural *B. pertussis* infection wanes much later than vaccine-induced immunity [19], and that newborns are able to mount a strong Th1 response

Abbreviations: aPV, acellular pertussis vaccine; FHA, filamentous hemagglutnin; i.n., intra-nasal; i.p., intra-peritoneal; prn, pertactin; PBS/Tw, PBS containing 0.01% Tween; PTX, pertussis toxin.

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upon natural infection [20] has prompted the development of a live attenuated pertussis vaccine candidate to be administered by the intra-nasal (i.n.) route [21]. This vaccine candidate, named BPZE1, has now successfully undergone a phase I clinical trial in humans [22]. In preclinical mouse models, a single i.n. administration of BPZE1 induced strong and sustained B cell and Th1T cell responses, leading to full protection against challenge infection [21,23–26]. In infant mice, protection induced by a single i.n. administration of BPZE1 was stronger than that induced by two administrations of aPV [21] and was of significantly longer duration [25,26]. Therefore, BPZE1 is an attractive candidate to efficiently prime immunity in neonates in order to protect infants through their first months of life. Current vaccination schedules may then perhaps effectively boost anti-pertussis immunity.

Here, we investigated the immune responses induced in a neonatal mouse model by heterologous prime-boosting using BPZE1 for priming and aPV as a booster. We compared immune responses and protection in animals primed as neonatal (7 to 10 days old) mice with those primed in infancy (3 weeks old). We found that priming with BPZE1 prior to aPV immunization maintained the Th1 responses to *B. pertussis* antigens both in neonatal and infant mice. In addition, the BPZE1 prime-aPV boost regimen led to a significant increase in the production of anti-*B. pertussis*-specific antibodies compared to either BPZE1 or aPV vaccination alone.

2. Materials and methods

2.1. Bordetella strains and growth conditions

The *B. pertussis* strains used here were streptomycin-resistant BPSM [27] and BPZE1 [21], both derived from *B. pertussis* Tohama I. All strains were grown on Bordet-Gengou agar (Difco Laboratories, Detroit, Michigan, United States) supplemented with 1% glycerol, 20% defibrinated sheep blood and 100 μg/ml streptomycin at 37 °C. After growth, the bacteria were harvested by scraping the plates and resuspended in PBS at the desired density.

2.2. Animals, immunization and B. pertussis infection

Seven to 10 days-old and three weeks-old female BALB/c mice were obtained from Charles River (l'Abresle, France) and maintained under specific pathogen-free conditions in the animal facilities of the Institut Pasteur de Lille. All animal work was carried out according to the institutional guidelines of the Institut Pasteur de Lille. The mice were i.n immunized as previously described [21,23–25]. Briefly, groups of 4–5 mice were slightly sedated with pentobarbital (CEVA Santé Animale—La Ballastière, France) and inoculated by pipetting 10 μl (for neonatal mice) or 20 μl PBS (for infant mice) containing approximately 10^6 CFU of BPZE1 onto the tips of the nares. For challenge with 10^6 CFU were administered in 20 μl. For vaccination with four-component (diphtheria-tetanus-pertussis-poliomyelitis) aPV (PENTAVAC®, Sanofi Pasteur, France, composed of purified diphtheria toxoid, purified tetanus toxoid, purified pertussis toxoid, purified filamentous hemagglutinin [FHA], inactivated type 1, type 2 and type 3 poliovirus, and *Haemophilus influenzae* type b polysaccharide), the mice were immunized intra-peritoneally (i.p.) with 20% of the human dose and boosted 3 weeks later using the same dose.

For the heterologous prime-boost experiments, the mice were first immunized with 10^4 or 10^6 CFU BPZE1 at 7–10 days or 3 weeks of age, followed three weeks later by one or two i.p. vaccinations with aPV given at a three-week interval. Three weeks after the last aPV immunization, the mice were challenged i.n. with 10^6 CFU BPSM and sacrificed 7 days later. Their lungs were harvested, homogenized in PBS and plated in serial dilutions onto BG-blood agar for CFU quantification.

2.3. Antibody determination

The levels of antibodies to filamentous hemagglutinin (FHA), pertussis toxin (PTX) and pertactin (Prn) in the sera were determined by ELISA as described [25]. Briefly, F96 Maxisorp Microtiters plates (Nunc A/S, Roskilde, Denmark) were coated overnight at 4 °C with 50 μl per well of 10 μg/ml FHA (purified from PTX-deficient *B. pertussis* BPRA [28]), 5 μg/ml PTX (purified from FHA-deficient *B. pertussis* BPGR4 [29]) or 5 μg/ml recombinant Prn (purified from recombinant *Escherichia coli* [30] and kindly provided by Dr. Anne-Marie Buisman, Bilthoven; The Netherlands). After washing with PBS, the plates were blocked with PBS containing 5% low-fat milk powder and then washed again twice with PBS containing 0.01% Tween (PBS/Tw). Pooled serum samples serially diluted in PBS/Tw containing 0.5% gelatin were then added to the coated plates and incubated for 2 h at 37 °C. The plates were then washed three times in PBS/Tw before the addition of goat anti-mouse IgG-horseradish peroxidase conjugates (Southern Biotechnology Associates, Inc., Birmingham, Ala.) and incubation for 2 h at 37 °C. After four washes with PBS/Tw, 100 μl of horseradish peroxidase Substrate TMB solutions (Interchim, Montluçon, France) was added, and the plates were incubated for 30 min at room temperature. The reaction was stopped by the addition of 50 μl of 1 M H_3PO_4. The optical density was measured with a Biokinetic reader EL/340 microplate (Bio-Tec™ instruments Inc., Paris, France) at 450 nm, and a linear regression curve of log optical density versus the serum dilution was plotted. The results are expressed in titers, corresponding to the reciprocal of the dilution giving an optical density three times that of the conjugate control.

2.4. Cell-mediated immunity measurement

Spleens were removed aseptically from the mice, and splenocytes were prepared as single cell suspensions. 10^6 spleen cells were resuspended in 200 μl of RPMI-supplemented medium and placed in *U*-shaped 96-well cultures plates (Nunc A/S, Roskilde). They were stimulated with FHA and PTX as previously described [24]. After 60 h, the supernatants were harvested from triplicate cultures and analyzed for IFN-γ, IL-5, IL-13 and IL-17 production using ELISA kits (BD Biosciences, San Diego, USA) according to the manufacturer's instructions.

2.5. Statistical analysis

The results were analyzed using the unpaired Student's *t* test and the Kruskal–Wallis test followed by the Dunn post-test (GraphPad Prism program) when appropriate. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Nasal administration of BPZE1 protect neonatal mice from B. pertussis infection

We have previously shown that a single i.n. administration of BPZE1 protects adult (8 weeks old) and infant (3 weeks old) mice against challenge with *B. pertussis* [21]. As the primary targets of the BPZE1 vaccine are the newborns, we first tested here the potential of BPZE1 to protect neonatal mice. Seven- to 10-days-old BALB/c mice were thus immunized i.n. with a single dose of BPZE1 or i.p. with two doses of aPV given three weeks apart. This protocol was chosen as a comparator, as it has been established as a good model for predicting pertussis vaccine efficacy in children [31].

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