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Cytokine and antibody profiles in 1-year-old children vaccinated with either acellular or whole-cell pertussis vaccine during infancy

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ABSTRACT

Two different types of pertussis vaccines are currently available to protect children against whooping cough, the first-generation whole-cell (Pw) vaccines and the more recent acellular (Pa) vaccines. Both types provide good protection, yet induce different types of immune responses in 6-month-old infants, with a strong Th1 response induced by Pw vaccines compared to a mixed Th1/Th2 response and a delay in non-specific IFN-γ secretions after the administration of Pa vaccines. We show here that at 13 months of age, most Pw- or Pa-vaccinated children display *Bordetella pertussis*-specific T-cell responses, in addition to significant antibody levels, although a higher Th2/Th1 cytokine ratio remained in Pa recipients compared to Pw recipients. In contrast, the proportion of children with tetanus toxin-specific T-cell responses was lower in Pa than in Pw vaccine recipients, although most children had protective anti-tetanus toxin IgG levels. In addition, the global Th2 bias observed in 6-month-old infants vaccinated with a Pa vaccine was normalized at 13 months.

1. Introduction

Pertussis is one of the few infectious diseases whose incidence is increasing in many countries despite the existence of effective vaccines. The vaccines have reduced the incidence of pertussis by 90–95% in developed countries, but *Bordetella pertussis* continues to circulate even in populations with high vaccine coverage of infants and children. In the USA the reported incidence has increased approximately 10-fold over the last 20 years [1], and worldwide roughly 40 million whooping cough cases are still recorded each year, mostly in developing countries [2]. Protection against pertussis relies both on humoral immunity and on cellular Th1-type immune responses, as highlighted in several studies during the last 15 years [3–8]. Two different types of pertussis vaccines are currently available, the first-generation whole-cell (Pw) vaccines and the more recent acellular (Pa) vaccines. Both types of vaccines provide good protection, yet induce different types of immune responses. They both induce specific antibodies (Abs) with higher titers obtained after the administration of a Pa compared to a Pw vaccine for the one to five antigens included in Pa vaccines [9,10]. In addition, whereas Pw vaccines induce a strong antigen-specific Th1 response, Pa vaccines induce mixed Th1/Th2 responses in humans [11–13].

One factor involved in the persistence of whooping cough even in countries with high vaccine coverage is probably the lack of sufficiently long-lasting protection induced by the administration of pertussis vaccines during childhood. This is illustrated by the fact that the pertussis incidence is currently increasing in adolescents and young adults [14], who therefore now constitute an important reservoir and source of transmission for the pathogen. Few data are available in the literature on the longevity of *B. pertussis*-specific immune responses in humans [9,15]. The duration and the quality of T-cell memory immune responses appear to be imprinted at the time of the immune priming [16], implying that these memory immune responses may differ according to the type of vaccine administered during infancy. Priming with pertussis vaccines most often occurs between 2 and 6 months of age by means of three vaccine injections, given at 1- or 2 months intervals [17]. We have recently reported that a primary vaccination with a two-component Pa vaccine is associated with a general Th2-skewed profile of cytokine secretion in 6-month-old infants, in comparison to the cytokine profile observed in infants vaccinated with a Pw vaccine [11].

In this paper, we characterized the antigen-specific cytokine profiles in 13-month-old children vaccinated in infancy either with

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a Pa or with a Pw vaccine, to assess whether the *B. pertussis*-specific cytokine profiles detected at 6 months are sustained at 13 months, before the administration of the first vaccine booster. As tetanus toxoid (TT) was co-administered with the pertussis vaccines, antigen-specific immune responses to TT were compared in addition to those to two different *B. pertussis* antigens.

2. Material and methods

2.1. Children included in the study

67 children were enrolled in the study, comprising 56 children already included in our previous study on cellular immune responses induced by pertussis vaccine administrations [11], as well as 11 newly enrolled children who were not yet included at the time of the first study. We obtained their parent's informed consent, and the ethical committee of the Saint-Pierre Hospital (Brussels, Belgium), where the infants were enrolled, had approved the study. Like in our previous study, all the enrolled infants were born from HIV-infected mothers and received during their first 6 weeks of life preventive therapy with zidovudine, although they were themselves HIV-negative. This group of infants was chosen for ethical reasons, as, at the time of the study, blood samples were routinely collected up to 12–13 months of age for the follow-up of these infants, so that no additional blood puncture had to be done for this study. All the infants were vaccinated against *B. pertussis*, Tetanus, Diphtheria, Poliomyelitis, *Haemophilus influenzae* type b and Hepatitis B according to the recommendations in Belgium. These infants received at 2, 3 and 4 months of age the two-component (pertussis toxin (PT)/filamentous haemagglutinin (FHA)) Pa vaccine (Tetravac™, sanofi pasteur, Lyon, France) or the Pw vaccine (Tetracoq™, sanofi pasteur, Lyon, France), mixed just before the administration with the TT-conjugated *H. influenzae* type b polysaccharide vaccine (Act-Hib™, sanofi pasteur, Lyon, France). The recombinant Hepatitis B vaccine (HBVAXPRO, sanofi pasteur msd, Lyon, France) was injected at a separate site at 3 and 4 months of age. 47 infants were vaccinated with the Pa vaccine and 20 received the Pw vaccine. Both vaccines contained a minimum of 30 IU of diphtheria toxoid, 40 IU of TT, and 40, 8, and 32 of D-antigen units of inactivated polioviruses type 1, 2, and 3, respectively. The Pw vaccine contained a minimum of 4 IU of inactivated *B. pertussis*, and the Pa vaccine contained 25 μg of PT and 25 μg of FHA.

For this study, blood samples were collected just before the booster Pa vaccine dose at around 13 months of age. An additional blood sample was obtained after the vaccine booster at around 15 months of age for 15 Pa-vaccinated children. Because of the limited availability of the peripheral blood mononuclear cells (PBMC) isolated from some blood samples, not all the in vitro analyses could be performed for all the samples. Fifteen cord-blood samples were collected to determine the cut-off of positive antigen-specific responses for cytokine secretions and lymphocyte proliferation.

2.2. Antigens and mitogen for cellular immune assays

FHA was purified from *B. pertussis* BPRA, a PT-deficient strain [18], and PT was purified from *B. pertussis* BPDR-RE, a FHA-deficient strain that produced genetically inactivated PT-RE, as previously described [19]. To avoid potential mitogenicity, PT was heat inactivated for 20 min at 80 °C. The antigen concentrations of the purified antigens were estimated by the BCA™ Protein Assay Reagent Kit (Pierce Biotechnology, Rockford, USA), and FHA and PT were used at a final concentration of 1 μg/ml. Tetanus toxoid (TT) (sanofi pasteur, Lyon, France) was used at 5 μg/ml, phytohaemagglutinin (PHA) (Remel, Lenexa, KS, USA) at 2 μg/ml and candidin (Stallergenes, Waterloo, Belgium) was used at 5 UI/ml.

2.3. PBMC isolation and culture, cytokine determinations

PBMC were isolated by density gradient centrifugation of the blood on Lymphoprep (Nycomed Pharma, Oslo, Norway) and resuspended in complete RPMI medium (Biowhittaker, Verviers, Belgium) supplemented with 10% of fetal bovine serum (Hyclone, Logan, Utah), as previously described [20]. The cells were then cultured at 2×10^6 cells/ml in the presence of antigens or mitogen at 37 °C under 5% CO_2 atmosphere. Gamma-interferon (IFN-γ), Interleukin-5 (IL-5) and Interleukin-13 (IL-13) concentrations were measured by classical sandwich ELISA in 72 h cell culture supernatants, using commercially available antibody pairs, as well as commercial standards (IFN-γ, BioSource International, Camarillo, CA, USA; IL-5, Pharmingen, San Diego, CA, USA; IL-13, Endogen, Woburn, MA, USA) as described previously [11]. The sensitivity limits of the three assays were 25 pg/ml. When detectable, cytokine concentrations obtained in non-stimulated conditions were subtracted from those obtained for mitogen- or antigen-stimulated cells. A cytokine secretion was considered as significant when higher than the upper limit of the 95% confidence interval of the results obtained for control cord-blood samples tested in parallel, considering that no antigen-specific cellular immune responses should be detectable among cord-blood lymphocytes. These background cytokine secretions obtained on cord-blood samples were very low, being 25, 110, and 25 pg/ml for IFN-γ, 125, 42, and 25 pg/ml for IL-13, and 54, 25, and 25 pg/ml for IL-5, for FHA, PT and TT, respectively (upper limit of the 95% confidence interval of the results).

2.4. Cell proliferation assay

PBMC proliferation was evaluated after 6 days of culture (1×10^6 cells/ml in 0.2 ml of complete RPMI containing 10% human serum, in triplicate) in flat-bottom 96-wells culture plates in the presence of *B. pertussis* antigens, TT, or candidine, used as positive control. [^{3}H]-thymidine was added during the last 16 h of the culture, and the DNA synthesis was evaluated by measuring [^{3}H]-thymidine incorporation. The results were expressed as a proliferative index calculated as a ratio between the responses obtained in the presence or absence of antigen. These proliferative Indexes were considered as positive when higher than the upper limit of the 95% confidence interval of the indexes obtained for the cord-blood samples tested in parallel (indexes of 4, 3, 2 for FHA, PT and TT, respectively).

2.5. Antibody titer determination

Immunoglobulin G (IgG) titers to *B. pertussis* antigens and to TT were assessed by ELISA at the Global Clinical Immunology platform sanofi pasteur, Swiftwater, PA, USA in the sera from 41 Pa-vaccinated infants and from 15 Pw vaccine recipients. Briefly, purified antigen (1.4 μg/ml for FHA, sanofi pasteur, Lyon, France), 1.8 μg/ml for PT (sanofi pasteur, Lyon, France) and 1.25 μg/ml for TT (sanofi pasteur, Swiftwater, PA, USA) was adsorbed to the wells of a microtiter plate (PT and FHA, Greiner Bio-One North America, NC, USA; TT Nunc, Thermo-Fisher Scientific, Rochester, NY, USA). Diluted serum samples or reference standard or quality controls were incubated in the wells for 20–72 h at 2–8 °C for PT and FHA-or 16–20 h at room temperature for TT and enzyme-conjugated anti-human IgG (Fc) (Kirkegard and Perry Laboratories, Inc., Gaithersburg, MD, USA) was added. An alkaline phosphatase substrate (Sigma–Aldrich, St. Louis, MO, USA) was added and the results were read on a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The results were expressed in ELISA units per ml (EU/ml) for FHA and PT and in International units per ml (IU/ml) for TT. A serologic response to both *B. pertussis* antigens was defined