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Modulation of the infant immune responses by the first pertussis vaccine administrations

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Abstract

Many efforts are currently made to prepare combined vaccines against most infectious pathogens, that may be administered early in life to protect infants against infectious diseases as early as possible. However, little is known about the general immune modulation induced by early vaccination. Here, we have analyzed the cytokine secretion profiles of two groups of 6-month-old infants having received as primary immunization either a whole-cell (Pw) or an acellular (Pa) pertussis vaccine in a tetravalent formulation of pertussis–tetanus–diphtheria-poliomyelitis vaccines. Both groups of infants secreted IFN-γ in response to the *Bordetella pertussis* antigens filamentous haemagglutinin and pertussis toxin, and this response was correlated with antigen-specific IL-12p70 secretion, indicating that both pertussis vaccines induced Th1 cytokines. However, Pa recipients also developed a strong Th2-type cytokine response to the *B. pertussis* antigens, as noted previously. In addition, they induced Th2-type cytokines to the co-administrated antigen tetanus toxoïd, as well as to the food antigen beta-lactoglobulin. Furthermore, the general cytokine profile of the Pa recipients was strongly Th2-skewed at 6 months, as indicated by the cytokines induced by the mitogen phytohaemagglutinin. These data demonstrate that the cytokine profile of 6-month-old infants is influenced by the type of formulation of the pertussis vaccine they received at 2, 3 and 4 months of life. Large prospective studies would be warranted to evaluate the possible long-term consequences of this early modulation of the cytokine responses in infants.

Keywords: Human infants; Pertussis vaccines; Th1/Th2 cells

1. Introduction

The neonatal and infant immune system is intrinsically defective in Th1-type responses [1–4], and virtually all human newborns have Th2-skewed immune responses to common environmental antigens [2]. The kinetics of the post-natal maturation of the Th1 function is highly variable among infants but appears generally to be delayed until 12 months of age [5]. A more persistent Th2-skewed immune response during the first months of life has been associated with a genetic risk for atopy [5–8]. In these infants, the first 6 months of life constitute a critical time window for the selection of the T helper cells that will later in life dominate the T memory population [7,8]. High-risk infants developing atopic disease later in life are characterized by a strong Th2 cytokine profile already at 6 months of age [8].

Several environmental factors are able to modulate the Th1/Th2 cytokine balance in infancy and early childhood. Certain bacterial infections, such as *Bordetella pertussis* infection [9], and some vaccines, such as BCG [10] and whole-cell pertussis vaccines (Pw) [9] are able to induce strong antigen-specific Th1-type responses early in infancy. The rapid increase in the incidence of allergic diseases in developed countries has recently been associated with an obvious decrease in the incidence of many infectious diseases [11]. This association has been suggested to be a consequence of the induction by infectious pathogens of Th1-type T cell

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responses, which then play an inhibitory role on the development of allergen-specific Th2 cells [11]. The capacity to induce a Th1- or a Th2-type immune response seems to be linked to the intrinsic properties of the encountered antigens, and this is probably most important in infants, who do not yet have memory cells for these antigens. Factors amplifying early Th2 imprinting may skew subsequent immune responses toward Th2-polarized immune memory, whereas Th1-type cytokine-inducing antigens may antagonize such a Th2-polarized immune memory. The identification of the environmental factors that may orient the immune responses of infants toward a Th2 or a Th1 pattern may therefore be very important.

In order to protect against infectious diseases as early as possible, some vaccines are administered in early childhood, sometimes even at birth in some countries, as is the case for the BCG and hepatitis B vaccines [12]. In addition, major efforts are being undertaken to combine different vaccines in order to decrease the number of injections needed to protect. Combined vaccination against *B. pertussis*, diphtheria, tetanus, poliomyelitis and sometimes *Haemophilus influenzae* type b and hepatitis B are generally initiated at 2 months of life. Early protection against *B. pertussis* appears mandatory in view of the recent re-emergence of pertussis [13] and of the severity of the infection in very young infants (<6 months of age). The classically administered Pw consisting of heat/formalin inactivated whole bacteria is progressively being replaced, at least in developed countries, by acellular vaccines (Pa), because Pa is less reactogenic than Pw [14]. Pa is composed of a variable number of purified components of the bacteria and induces protection levels up to roughly 85%, comparable to the 90–95% protection typically obtained with the best Pw [15], even though the two vaccine types induce different immune responses both in mice and in children [16]. Whereas Pw induces antigen-specific Th1 cells, similar to *B. pertussis* infection [9], the administration of Pa is associated with the appearance of a mixed Th1/Th2 response [17,18].

In view of these differences and of the importance of the first 6 months of life for the imprinting of the Th2/Th1-type orientation, we investigated here whether the change from Pw to Pa administered to 2-month-old infants, may modulate the general and/or antigen-specific cytokine profile of these infants. We have thus analyzed the Th1/Th2 cytokine profiles of 6-month-old infants, vaccinated with either Pa or Pw, in response to the polyclonal mitogen phytohaemagglutinin (PHA), to three parenterally administrated vaccine antigens, tetanus toxoïd (TT), the *B. pertussis* filamentous haemagglutinin (FHA) and pertussis toxin (PTX), as well as to the oral antigen beta-lactoglobulin (BLG).

2. Materials and methods

2.1. Human subjects

Sixty infants were included in the study after having obtained their parent's informed consent and the ethical committee of the Saint-Pierre Hospital (Brussels), where the infants were enrolled, had approved the study. All infants were vaccinated against pertussis (P), tetanus (T), diphtheria (D), poliomyelitis, *H. influenzae* type b and hepatitis B according to the recommendations in Belgium. These consisted of three doses of intramuscular vaccine administration at 2, 3 and 4 months of age of a four-component vaccine (pertussis–tetanus–diphtheria–poliomyelitis) that was mixed with TT-conjugated *H. influenzae* type b polysaccharide (sanofi pasteur) just before the administration. The hepatitis B vaccine (recombinant hepatitis B vaccine, sanofi pasteur) was injected at a separate site at 3 and 4 months. Whereas 36 infants received a two-component Pa vaccine (Tetravac, sanofi pasteur, Lyon, France), 24 infants received a Pw vaccine (Tetracoq, sanofi pasteur). Tetravac and Tetracoq both contained a minimum of 40 I.U. of TT, 30 I.U. of diphtheria toxoid, 40 U of polio type 1, 8 U of polio type 2 and 32 U of polio type 3 inactivated virus. Tetravac contained 25 μg of glutaraldehyde-inactivated PTX and 25 μg of FHA, whereas Tetracoq contained at least 4 I.U. of inactivated *B. pertussis* (heat, formaldehyde, phenoxy ethanol).

All the enrolled infants were born from HIV-infected mothers and received a 6 weeks preventive therapy with zidovudine, although they were themselves HIV-negative. This group of infants was chosen for ethical reasons, as blood samples are routinely collected from these infants at 2, 3 and 6 months of age so that no additional blood puncture had to be done for this study. All the infants were bottle-fed and their socioeconomic status was excellent as a consequence of the Belgian social security system. The two groups of infants were similar with respect to birth weight (data not shown), and cellular immune responses tested at the time of enrolment (2 months of age) by the analysis of the cytokines secreted in response to mitogenic stimulation of their peripheral lymphocytes (see Section 3). The IgG, IgA and IgM concentrations and the absolute lymphocyte counts of these infants were within the normal ranges for their age both at 2 and at 6 months. The percentages of B, T and NK cells, as well as the proportions of $CD4^+$ and $CD8^+$ T lymphocytes were also within normal ranges and were not different between the two groups of infants. 8.1% of the Pa vaccinated infants had a family history of allergic disease, compared to 6.6% of the Pw vaccinated infants. Blood samples were collected before the first vaccine administration at 2 months of age, and 2 months after the third vaccine injection, at 6 months of age.

2.2. Cell isolation, culture conditions and antigen/mitogen stimulation

Peripheral blood mononuclear cells (PBMC) were isolated and assayed for cytokine secretion after antigenic or polyclonal stimulation as previously described [9]. IFN-γ, IL-12 p70, IL-13 and IL-5 concentrations were measured by ELISA in the culture supernatants after 24 or 72 h of culture for IL-12 p70 or the other cytokines, respectively.