Vaccine 33 (2015) 4117–4123

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Humoral and cell mediated immune responses to a pertussis containing vaccine in pregnant and nonpregnant women

Kris Huygen [a,*], Raïssa Nadège Caboré [a], Kirsten Maertens [b], Pierre Van Damme [b], Elke Leuridan [b]

[a] National Pertussis Reference Laboratory, Service Immunology, Scientific Institute of Public Health (WIV-ISP), 1180 Brussels, Belgium
[b] Centre for the Evaluation of Vaccination, Vaccine & Infectious Diseases Institute, University of Antwerp, 2610 Wilrijk, Belgium

ARTICLE INFO

Article history:
Received 28 October 2014
Received in revised form 24 February 2015
Accepted 10 June 2015
Available online 14 July 2015

Keywords:
Pertussis
Diphtheria
Tetanus
Pregnancy
Vaccination
Cell mediated immunity

ABSTRACT

Vaccination of pregnant women is recommended for some infectious diseases in order to protect both women and offspring through high titres of maternal IgG antibodies. Less is known on the triggering of cellular immune responses by vaccines administered during pregnancy. In an ongoing study on maternal pertussis vaccination (2012–2014) 18 pregnant women were vaccinated with a tetanus-diphtheria-acellular pertussis (Tdap) containing vaccine (Boostrix®) during the third pregnancy trimester. Sixteen age-matched nonpregnant women received the same vaccine in the same time period. A blood sample was taken at the moment of, but before vaccination and one month and one year after vaccination. Anti-Pertussis Toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), tetanus toxin (TT) and diphtheria toxin (DT) antibodies were measured by ELISA. Cellular immune responses were analyzed using a diluted whole blood assay, measuring proliferation, and cytokine release in response to vaccine antigens PT, FHA, TT, and to pokeweed mitogen (PWM) as polyclonal stimulus.

Antibody levels to all five vaccine components increased significantly and to the same extent after vaccination in pregnant and nonpregnant women. One year after vaccination, antibody titres had decreased particularly to PT, but they were still significantly higher to all antigens than before vaccination. In contrast, proliferative and IFN-γ responses were increased to TT, PT, and FHA in nonpregnant women one month after vaccination, whereas in pregnant women only TT specific T cell responses were increased and to a lesser extent than in the control group. One year after vaccination, cellular responses equaled the baseline levels detected prior to vaccination in both groups. In conclusion, a Tdap vaccination can increase vaccine specific IgG antibodies to the same extent in pregnant and in nonpregnant women, whereas the stimulation of vaccine specific Th1 type cellular immune responses with this acellular vaccine is transient and impaired during pregnancy.

1. Introduction

Pertussis, caused by *Bordetella pertussis*, is a contagious, potentially life-threatening respiratory illness and a major cause of childhood morbidity and mortality. Although disease incidence declined steadily since the introduction of whole-cell-based vaccines in the fifties, the number of reported cases across all age groups has increased again during the past two decades in many industrialized countries with high vaccination coverage [1]. The underlying mechanism of this evolution is probably multifactorial: waning immunity in adults, switch from whole cell pertussis wP to less reactogenic acellular pertussis aP vaccines, increased awareness of physicians coupled to an easier and earlier diagnosis by PCR and possibly changes in virulence of the circulating pertussis strains. In adults *B. pertussis* infection can often cause only mild disease, as we confirmed in a recent seroprevalence study of 'healthy' adults aged 20–39 years old [2].

In order to protect the youngest infants, who are most vulnerable to severe disease and even death, various strategies such as cocoon vaccination and pre-pregnancy boosting have been proposed [3–6]. Pertussis vaccination in the second or third trimester of pregnancy has been implemented in 2011 in the USA [7], in 2012

* Corresponding author. Tel.: +32 2 373 33 70; fax: +32 2 373 33 67.
E-mail addresses: kris.huygen@wiv-isp.be (K. Huygen), raissanadeg.cabore@wiv-isp.be (R.N. Caboré), kirsten.maertens@uantwerpen.be (K. Maertens), pierre.vandamme@uantwerpen.be (P. Van Damme), elke.leuridan@uantwerpen.be (E. Leuridan).

http://dx.doi.org/10.1016/j.vaccine.2015.06.108
0264-410X/

in the United Kingdom [8], and in 2013 in Ireland [9] and Belgium [10].

Babies born from mothers who received Tdap during pregnancy have significantly higher tetanus, diphtheria, and pertussis antibody concentrations at birth when compared to newborns from mothers who did not receive Tdap [11,12]. A Tdap booster vaccination at 30–32 weeks' gestation is safe [13] and elevated pertussis antibodies can be measured at delivery, and in the infants at birth and at age 2 months [14]. A vaccine effectiveness of 91% for infants younger than 3 months was demonstrated in an observational study, conducted between January 2008 and September 2013 in England [15].

Some immunological questions related to this maternal vaccination strategy, have not been answered yet. For one, protection against pertussis is dependent both on antibody- and cell-mediated immune (CMI) responses [16]. Vaccination induces these pertussis-specific CMI responses in infants [17], adolescents [18–20], and adults [21,22] but CMI responses to pertussis vaccination during pregnancy have so far not been reported. In a number of papers reported on maternal vaccination (for a more recent review [23]), focus has been so far on the induction of antibodies. The only published data on CMI responses stimulated by maternal vaccination are to our knowledge by Yamaguchi et al. who described that the efficacy of a flu vaccination was similar in all stages of pregnancy [24] and by Kay et al. who reported on enhanced natural killer cell and T cell responses to inactivated influenza vaccination during pregnancy [25].

In this paper, we report on Tdap (Boostrix®) induced specific antibody and CMI responses of women enrolled during the third trimester of pregnancy as compared to the responses of age-matched nonpregnant women who received the same vaccine in the same time period.

2. Materials and methods

2.1. Study design

A total of 18 pregnant women who participated in an ongoing study (Clinicaltrials.gov: NCT01698346) on maternal pertussis vaccination [26], received a combined pertussis vaccine (Boostrix®, GSK Biologicals, Rixensart) during the third trimester of pregnancy (25–32 weeks). Inclusion criteria of the study were: pregnant women aged 18–40 years with a normal pregnancy, consenting to be immunized during pregnancy. The pregnancy was considered to be 'normal' by the responsible physician, i.e., all standard prenatal care tests had to be normal, including tests for gestational diabetes and congenital malformations detectable on ultrasound checkup at 20 weeks of gestation. The chromosomal test was not obligatory to enter the study. But when a woman had an abnormal antenatal test, she was not allowed to participate. Chromosomal testing is performed before the gestational age of 20 weeks and women were only included after the testing was performed (if they chose antenatal testing). A detailed ultrasound is performed in all Belgian women at week 20 and the result was obtained for all participating women. Antenatal steroids were not an exclusion criterium for this study.

A control group was identified during the same time period and consisted of 16 age-matched nonpregnant women receiving the same vaccination.

Five cc of heparinized blood was collected at the moment of but before vaccination (month 0), at 28–31 days (month 1) and at 1 year (month 12) after vaccination. Ethical approval has been obtained by the Ethical Committee of the University Hospital of Antwerp on April 4, 2011.

2.2. Proliferation assays

Proliferation assays were performed as described before [27]. Briefly, heparinized whole blood, collected by venipuncture, was diluted 1:10 in RPMI-1640 medium, supplemented with Hepes, L-glutamine, penicillin/streptomycin, and 5×10^{-5} M 2-mercapto-ethanol. Cells were cultured in round bottom microwell plates (Greiner) in a humidified CO_2 incubator at 37° for 7 days. A volume of 180 μl of diluted blood (in 10% autologous plasma) was added to 20 μl of Tetanus Toxoid (TT) (provided by Dr. Y. Fikri, WIV-ISP), heat-inactivated Pertussis Toxin (PT), Filamentous Haemagglutinin (FHA) (both kindly provided by Dr. M. De Ridder (GlaxoSmithKline) to Dr. R. Vanhoof [28]) or Pokeweed Mitogen (PWM) (Sigma, L'Isle d'Abeau, France). Antigens were used at final concentration of 5 μg/ml and PWM at 4 μg/ml. Tritiated thymidine (Perkin Elmer) was added to the cells during the last 20 hours of culture (0.4 μCi/well). Cells were harvested on a Skatron Cell Harvester and filters were counted in a Beckman LS Betaplate scintillation counter. Mean counts per minute (cpm) were calculated from quintuplicate cultures. Mean cpm values of cultures of unstimulated cells (negative control) ranged between 50 and 100 cpm. Because of these very low negative control values, the analysis of antigen/mitogen stimulated responses was based on total cpm values without subtraction of the negative control values. Responses were considered positive when the stimulation index was >5.

2.3. Cytokine production

To avoid interference with circulating cytokine levels, heparinized blood was centrifuged for 10 min at 1500 rpm and plasma was recovered and stored at −20 °C for antibody assays. Plasma was replaced by a same volume of RPMI-1640 medium, supplemented with Hepes, L-glutamine, penicillin/streptomycin, 5×10^{-5}M 2-mercapto-ethanol, and 10% Fetal Calf Serum. Blood was diluted to a final concentration of 10^6 leukocytes/ml in the same complete medium with 10% FCS and cells were stimulated as described for the proliferation assay. Culture supernatants from three wells were pooled after 7 days of culture and stored at −20 °C until assay.

2.4. Cytokine assays

IFN-γ was detected using Human IFN gamma ELISA Ready-SET Go! (eBioscience, cat.nr. 88-7316). Sensitivity 4 pg/ml, Standard curve range 4–500 pg/ml.

IL-10 and IL-13 levels were quantified using Human IL-10 ELISA Ready-SET-Go! (Affymetrix Ref. 88-7106-88) and Human IL-13 ELISA Ready-SET-Go! (Affymetrix 88-7439-88) respectively. Sensitivity of the assays is 2 pg/ml and 4 pg/ml respectively.

2.5. Detection of anti-pertussis, anti-tetanus and anti-diphtheria antibodies

Anti-PT IgG antibodies were detected in plasma by ELISA, using the Virion/Serion kit (ANL Copenhagen). Anti-FHA and anti-Prn antibodies were detected using EuroImmune ELISA. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui ELISA.

Plasma was tested in duplicate at a dilution of 1:100 (PT, TT, and DT), 1:400 (FHA) and 1:800 (Prn). OD results were converted into international units IU/ml. In order to calculate the Geometric Mean Concentration (GMC), all values>1100 IU/ml were used as 1200 IU/ml.

Vaccine responsiveness for pertussis antigens was defined as a postvaccination antibody concentration ≥20 IU/ml if the prevaccination antibody concentration was <5 IU/ml, a postvaccination rise