ELSEVIER

Vaccine 18 (2000) 1971–1974

Vaccine

www.elsevier.com/locate/vaccine

Short communication

Immune responses to pertussis antigens eight years after booster immunization with acellular vaccines in adults

Nhu Nguyen Tran Minh[a, b, c,*], Qiushui He[a, c], Kati Edelman[a, b], Anne Putto-Laurila[b], Heikki Arvilommi[a, c], Matti K. Viljanen[a, c], Jussi Mertsola[a, b, c]

[a]*National Public Health Institute, Department in Turku, Kiinamyllynkatu 13, Fin-20520, Turku, Finland*
[b]*Department of Pediatrics, Turku University Hospital, Kiinamyllynkatu 4-8, Fin-20520, Turku, Finland*
[c]*Turku Immunology Centre, Kiinamyllynkatu 13, Fin-20520, Turku, Finland*

Received 2 June 1999; received in revised form 1 November 1999; accepted 8 November 1999

Abstract

Pertussis-specific antibody and cell-mediated immune (CMI) responses were studied in adults 8 years after booster immunization with either a bicomponent (pertussis toxin and filamentous hemagglutinin) or a monocomponent (pertactin) acellular vaccine and in age-matched healthy controls. The levels of vaccine-induced antibodies were also compared between the serum samples collected before, 1 month, 4 years, and 8 years after immunization. Over the follow-up period, geometric mean values (GMV) of antibodies to the vaccine antigens decreased in both groups of vaccinees. However, the 8-year postimmunization GMV were 3–20 times higher than preimmunization GMV (all P values <0.01). Moreover, both antibody and CMI responses to the vaccine antigens were significantly higher in the vaccinees than in the controls (all $P < 0.01$ for antibody; all $P < 0.001$ for CMI responses). The results show that antibody and CMI responses induced by acellular pertussis vaccines can persist for up to 8 years after booster immunization of adults primed with whole-cell vaccine.

Keywords: Acellular pertussis vaccine; Booster immunization; Immune response

1. Introduction

Childhood immunization against pertussis has been effective in controlling pertussis disease but has not eliminated the circulation of the causative organism *Bordetella pertussis* [1]. Increasing evidence suggests that infections and disease caused by *B. pertussis* are a significant and growing problem among adults in immunized populations [1–3], indicating the need for booster immunizations in this age group. Modern acellular pertussis vaccines, which are less reactogenic than conventional whole-cell vaccines, could be used for this purpose [4–6]. Little is known, however, about the duration of the immune responses induced by acellular vaccines in adults.

We have recently reported that a new acellular pertussis vaccine, designed for booster immunization in older age groups, is highly immunogenic and well tolerated [6]. The three antigens included in the vaccine: pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin (PRN) of *B. pertussis* were initially tested in adult volunteers as part of phase I trials in October 1990. Eight years later, we investigated antibody and cell-mediated immune (CMI) responses to the vaccine antigens in a cohort of the vaccinees.

* Corresponding author. Tel.: +358-2-2519255; fax: +358-2-2519254.
E-mail address: tranminh@utu.fi (N.N. Tran Minh).

0264-410X/00/$ - see front matter
PII: S0264-410X(99)00534-4

2. Materials and methods

2.1. Subjects, vaccines and samples

In the initial study, 40 student volunteers from Turku University were randomly immunized with either a single dose of a bicomponent (25 μg of PT and 25 μg of FHA) or monocomponent (20 μg of PRN) pertussis vaccine (SmithKline Beecham Biologicals, Rixensart, Belgium). Blood samples were collected before, 1 month and 4 years after immunization. For the present study, all the vaccinees who still resided in the urban area of Turku city at the time of the study initiation were recruited. Eighteen vaccinees including both sexes (32–38 years of age) were available: nine had received the bicomponent vaccine (PT + FHA group) and 9 the monocomponent vaccine (PRN group). In addition, blood samples were collected from 9 age-matched healthy controls. All the vaccinees and controls had received primary immunizations with whole-cell pertussis vaccine in childhood and had no history of pertussis.

2.2. Serum antibody measurement

All serum samples included in the present study were analysed in parallel at the laboratory of SmithKline Beecham Biologicals. Immunoglobulin (Ig) G antibodies to PT, FHA and PRN were measured by a standardized enzyme immunosorbent assay (EIA) as described previously [4]. The results were expressed as EIA units/ml. The cut-off value of the assay was 5 EIA units/ml. Results lower than the assay cut-off were assigned an arbitrary value of one-half the assay cut-off.

2.3. Proliferation assay

Preparations of peripheral blood mononuclear cells (PBMC) and culture conditions for the proliferation assay were as described previously [6]. Briefly, all blood samples were processed within 2 h, and triplicate cultures (0.2 ml) of PBMC suspension (5×10^5 cells/ml) were incubated with 1 μg/ml of heat inactivated PT, 1 μg/ml of FHA or 2.5 μg/ml of PRN (SmithKline Beecham Biologicals, Belgium). Phytohemagglutinin (PHA) M and pokeweed mitogen (PWM) served as positive controls, and cultures in absense of antigen were negative controls. After a 5-day incubation at 37°C in an atmosphere with 5% CO_2, [^{3}H]thymidine (0.5 μCi/well) was added and, 16 h later, incorporated radioactivity was measured by scintillation counting (cpm). The mean cpm of stimulated culture was divided by the mean cpm of non-stimulated culture to obtain a stimulation index (SI).

2.4. Statistical analyses

Calculations of geometric mean values (GMV) of antibodies and CMI responses were performed on $\log_{10}$-transformed data, reporting the antilogarithm. Comparisons within and between groups were performed with the paired and unpaired Student's *t*-test, respectively. The Spearman rank correlation coefficient was used for the analysis of correlations. A two-tailed *P* value of <0.05 was considered statistically significant.

3. Results

3.1. Follow-up of vaccine-induced antibodies

The GMV of IgG antibodies to PT, FHA and PRN monitored in the two groups of vaccinees during the 8-

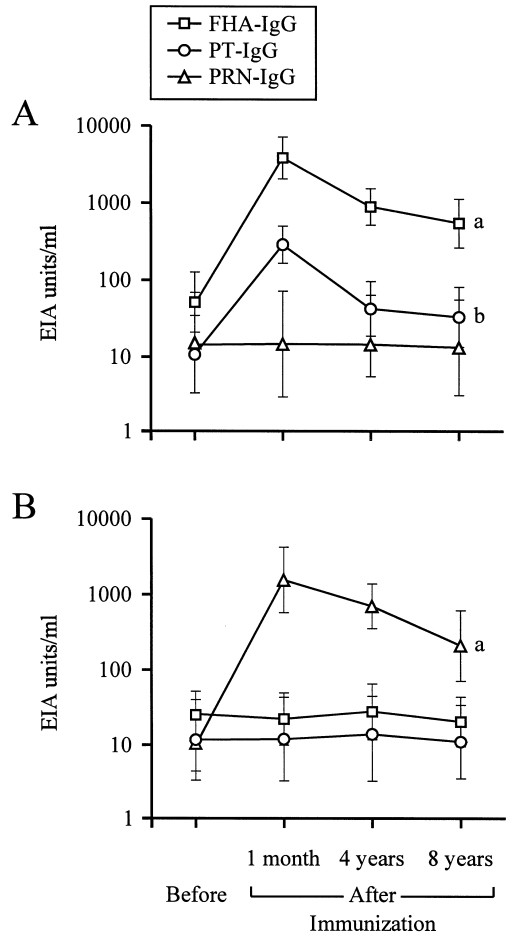

Fig. 1. Geometric mean values of antipertussis IgG antibodies in group (A) immunized with PT + FHA ($n = 9$) and group (B) immunized with PRN ($n = 9$) during the 8-year follow-up period. PT, pertussis toxin; FHA, filamentous hemagglutinin; PRN, pertactin; bars indicate 95% confidence interval; [a]$P < 0.001$ and [b]$P < 0.01$ compared with the corresponding preimmunization value.

year follow-up period are shown in Fig. 1. The GMV of antibodies to the given antigens (either PT + FHA or PRN) rose sharply following immunization, but gradually decreased over the following 8 years. However, the 8-year postimmunization GMV were still significantly, approx. 3–20 times, higher than the preimmunization GMV for the given antigens. By contrast GMV of antibodies to the antigens that were not included in the vaccine (control values) did not show any change over the 8-year follow-up period. Eight years after immunization, GMV of antibodies to the given antigens were significantly higher in the vaccinees than in the controls [Fig. 2(A)].

3.2. CMI responses to the vaccine antigens

CMI responses to PT, FHA and PRN measured in the vaccinees 8 years after immunization and in the controls are presented in Fig. 2(B). The PBMC of the vaccinees showed significantly higher proliferative responses to the given antigens than the cells of the controls. On the other hand, proliferative responses to the antigens that were not included in the vaccine showed no difference to the control groups. Spontaneous proliferation or proliferation induced by PHA or PWM were of the same magnitude in all groups (data not shown).

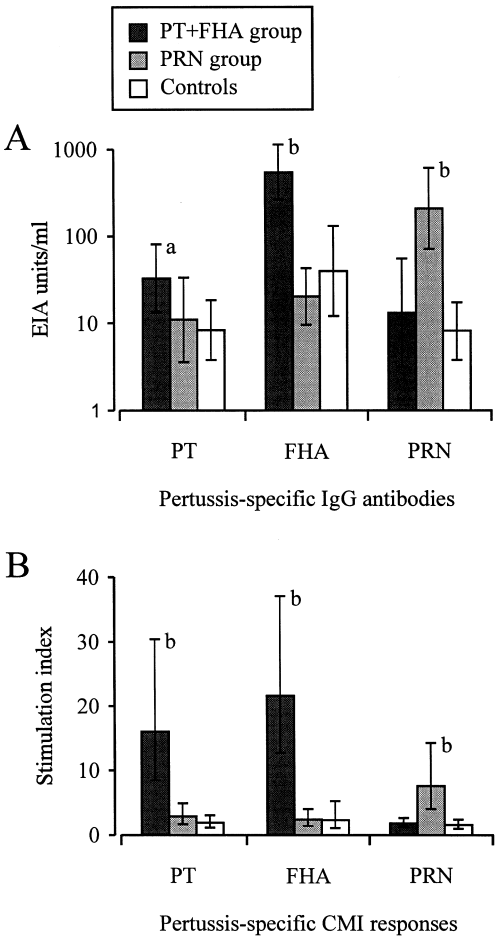

Fig. 2. Pertussis-specific IgG antibody (A) and CMI responses (B) in the two groups of vaccinees ($n = 9$ in each group) 8 years after immunization and in the controls ($n = 9$). Abbreviations are explained in the legend to Fig. 1; bars indicate 95% confidence interval; [a]$P < 0.05$ compared with the control group; [b]$P < 0.001$ compared with the other two groups.

3.3. Relationship between antibodies and CMI responses

A positive correlation was found between the humoral response and corresponding cellular response for each pertussis antigen, as shown in Fig. 2. Spearman correlations coefficients for IgG antibodies to CMI responses were for: PT, $\gamma = 0.6$; FHA, $\gamma = 0.6$; PRN, $\gamma = 0.7$, all P values < 0.001.

4. Discussion

The present study, although based on a limited sample size, represents the first reported evaluation of the long-term duration of humoral and cellular responses following booster immunization of adults with acellular vaccines. The results indicate that antibody and CMI responses induced by acellular pertussis vaccines persist for up to 8 years after booster immunization of adults. These findings are in line with the results of previous studies on the persistence of immune responses induced by acellular vaccines in adults. Edwards et al. [5] showed that levels of antipertussis antibodies in adult vaccinees had declined by 50% one year after booster immunization but remained substantially higher than preimmunization levels. A recent study in Italian adults showed that acellular vaccines induced CMI and antibody responses that remained high during the 2 and 4-year postimmunization follow-up periods, respectively [7].

The persistence of pertussis-specific immune responses may depend not only on the magnitude of responses induced by the vaccine but also on the possible natural boosting effect caused by infection with *B. pertussis*. It was therefore of importance to measure immune responses to antigens that were not included in the vaccine and which could serve as indicators of natural infection. In both groups of vaccinees, these responses showed no alteration and remained at the same level as in the control group throughout the follow-up period. Thus, immunization is likely to be responsible for the sustained high-levels of pertussis-specific responses rather than infection.

Although recent studies [8,9] have suggested that antibody responses may be important in protection against pertussis, CMI may also be an essential host determinant of immunity to *B. pertussis* [10–12]. It is known

that after primary immunization with acellular pertussis vaccines the serum antibodies decline rapidly, whereas measurable CMI stays longer [13,14]. A recent vaccine efficacy trial in Italy demonstrated that two acellular vaccines (one containing the identical antigens tested here) used for primary immunization were able to provide a high degree of protection in the same period when the GMV of antibodies to the vaccine antigens were decreasing to the non-detectable values [15]. In the present study, the CMI responses measured in adults 8 years after immunization were similar in magnitude to those in adolescents and adults recently infected with *B. pertussis* [11,12] or immunized with these same vaccine antigens [6,11]. Thus, pertussis-specific CMI (memory T-cells) may play a role in the development of long-lasting immunity against *B. pertussis*.

Since acellular pertussis vaccines were recently introduced, the duration of immunity remains a notable issue. A recent study found that immunity after priming doses of an acellular vaccine waned more rapidly than after the priming doses of a whole cell vaccine [16]. With this regard, it is important to note that the subjects of this study had been primed with a conventional whole-cell vaccine. One might expect that booster immunization with acellular vaccines following primary immunization with acellular vaccine would not be as immunogenic. Since the degree and duration of protection afforded by acellular vaccines are undetermined particularly in adults, data on the duration of immune responses are of importance. Theoretically, these data could be used as an indicator of the need for and the timing of booster doses. Because postimmunization immunological correlates of protection have not been established, it remains, however, to be shown how well these persisting immune responses correlate with clinical protection. Nevertheless, the persistence of immune responses is a sign of immunological memory. Individuals with such memory can develop more rapid and effective immune responses when they re-encounter the pathogen.

In conclusion, the present study shows that a booster dose of acellular pertussis vaccines can induce long-lasting responses in both arms of the immune system when given in adults previously primed with whole-cell vaccine. These findings suggest that the use of acellular pertussis vaccines in adults is feasible.

Acknowledgements

We thank Birgitta Aittanen, Etta-Liisa Väänänen, Tuula Lehtonen and Erkki Nieminen for exellent technical assistant. This work was supported by grants from the Foundation for Pediatric Research, the Finnish Cultural Foundation and the Academy of Finland.

References

[1] Black S. Epidemiology of pertussis. Pediatr Infect Dis J 1997;16:S85–S89.
[2] Robertson PW, Goldberg H, Jarvie BH, Smith DD, Whybin LR. *Bordetella pertussis* infection: a cause of persistent cough in adults. Med J Aust 1987;146:522–5.
[3] Deville JG, Cherry JD, Christenson PD, et al. Frequency of unrecognized *Bordetella pertussis* infection in adults. Clin Infect Dis 1995;21:639–42.
[4] Ruuskanen O, Noel A, Putto-Laurila A, et al. Development of an acellular pertussis vaccine and its administration as a booster in healthy adults. Vaccine 1991;9:117–21.
[5] Edwards KM, Decker MD, Graham BS, Mezzatesta J, Scott J, Hackell J. Adult immunization with acellular pertussis vaccine. JAMA 1993;269:53–6.
[6] Tran Minh NN, Edelman K, He Q, Viljanen MK, Arvilommi H, Mertsola J. Antibody and cell-mediated immune responses to booster immunization with a new acellular pertussis vaccine in school children. Vaccine 1998;16:1604–10.
[7] Di Tomaso A, Bartalini M, Peppoloni S, Podda A, Rappuoli R, De Magistris MT. Acellular pertussis vaccines containing genetically defoxified pertussis toxin induce long-lasting humoral and cellular responses in adults. Vaccine 1997;15:1218–24.
[8] Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serological correlates of immunity to *Bordetella pertussis* cough illnesses. Vaccine 1998;16:1901–6.
[9] Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of antipertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine 1998;16:1907–16.
[10] Mills KHG, Ryan M, Ryan E, Mahon BP. A murine model in which protection correlates with pertussis vaccine efficacy in children reveals complementary roles for humoral and cell-mediated immunity in protection against *Bordetella pertussis*. Infect Immun 1998;66:594–602.
[11] He Q, Tran Minh NN, Edelman K, Viljanen MK, Arvilommi H, Mertsola J. Cytokine mRNA expression and proliferative responses induced by pertussis toxin, filamentous hemagglutinin and pertactin of *Bordetella pertussis* in the peripheral blood mononuclear cells of infected and immunized persons. Infect Immun 1998;66:3796–801.
[12] Tran Minh NN, He Q, Edelman K, et al. Cell-mediated immune responses to antigens of *Bordetella pertussis* and protection against pertussis in school children. Pediat Infect Dis J 1999;18:366–70.
[13] Zepp F, Knuf M, Habermehl P, et al. Pertussis-specific cell-mediated immunity in infants after immunization with a tricomponent acellular pertussis vaccine. Infect Immun 1996;64:4078–84.
[14] Cassone A, Ausiello CM, Urbani F, et al. Cell-mediated and antibody responses to *Bordetella pertussis* antigens in children vaccinated with acellular or whole-cell pertussis vaccines. Arch Pediatr Adolesc Med 1997;151:283–9.
[15] Giuliano M, Mastrantonio P, Giammanco A, Piscitelli A, Salmaso S, Wassilak SGF. Antibody responses and persistence in the two years after immunization with two acellular vaccines and one whole-cell vaccine against pertussis. J Pediatr 1998;132:983–8.
[16] Simondon F, Preziosi MP, Yam A, Kane CT, Chabirand L, Iteman I, Sanden G, Mboup S, Hoffenbach A, Knudsen K, Guiso N, Wassilak S, Cadoz M. A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal. Vaccine 1997;15:1606–12.