



Differences of humoral and cellular immune response to an acellular pertussis booster in adolescents with a whole cell or acellular primary vaccination

Nikolaus Rieber^{a,*}, Anna Graf^a, Bernd H. Belohradsky^a, Dominik Hartl^a, Simon Urschel^a, Marion Riffelmann^b, Carl-Heinz Wirsing von König^b, Johannes Liese^a

^a University Children's Hospital, Ludwig-Maximilians-University, Munich, Germany

^b Institute for Hygiene and Laboratory Medicine, Helios Klinikum Krefeld, Germany

ARTICLE INFO

Article history:

Received 24 July 2008

Received in revised form

22 September 2008

Accepted 24 September 2008

Available online 11 October 2008

Keywords:

Pertussis vaccination

Adolescents

Cell-mediated immunity

ABSTRACT

To study the pertussis-specific immune response of adolescents with different prevaccination schedules, we measured the humoral and cell-mediated immunity (CMI) to pertussis antigens before and after a five-component Tdap booster vaccination in 78 adolescents, who had previously received either five doses of a two-component acellular pertussis vaccine (aP; last dose age 4–6 years), four doses of aP (last dose age 18–24 months), or four doses of whole cell pertussis vaccine (wCP; last dose age 18–24 months). The proportion of participants with a twofold rise in titre was 79% against pertussis toxin (PT), 94% against filamentous hemagglutinin (FHA), and 99% against pertactin (PRN) without significant differences between the three groups. However, participants with primary wCP vaccination showed higher postvaccination titres to pertussis toxin (geometric mean titre, GMT 50.3 EU/ml) than those with either four (GMT 17.1 EU/ml) or five (GMT 16.4 EU/ml) previous aP doses. CMI indices to PT, FHA, PRN and fimbriae (FIM) increased after vaccination and were similar between groups. The current adolescent Tdap booster immunization induced good humoral and cellular immune response to pertussis. The higher antibody titres to pertussis toxin may indicate a more effective priming of B cell memory after primary whole-cell vaccination.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Despite the high-coverage rates of infants and young children with pertussis vaccines, the number of pertussis cases reported to the Centers for Disease Control and Prevention (CDC) increased from 5158 in 1995 to 25,616 in 2005 [1]. The increase in cases was most pronounced in individuals >10 years of age [2,3]. In Germany, a prospective study between 2001 and 2004 revealed an incidence of pertussis disease in adults of about 165/100,000 per year [4]. Most concerning, young adults are a source for transmission of pertussis to young unvaccinated infants [3,5,6], who are at risk for pertussis-associated life-threatening complications [7,8]. For this reason, pertussis booster vaccinations for all adolescents, for all adults, or for adults with close contact to infants, have recently been recommended in many countries, e.g. the USA, Germany, France and Italy.

For many years, only whole cell pertussis (wCP) vaccines were available. They had, however, a considerable rate of systemic and local adverse effects. Therefore, in a number of countries, wCP vaccines were replaced by acellular (aP) vaccines in the mid-1990s because of their better reactogenicity profile [9]. Most wCP vaccines had performed moderately better than aP vaccines in efficacy studies in children after primary vaccination [9,10]. The immune response induced by wCP and aP vaccines is different: (i) wCP vaccines show a predominant Th1 associated cytokine pattern, whereas aP vaccines lead to a Th2-polarized cytokine pattern in infants and children at 4–6 years of age [11,12]; (ii) wCP vaccines have a stronger, specific T cell proliferative effect [13], whereas aP vaccines lead to higher antibody titres in the peak humoral immune response shortly after immunization in young children [13,14]. Yet, when investigating long-term immune responses, these different effects may be masked by subclinical pertussis infections, so called silent, natural boosters. In aP-vaccinated children, these silent boosters may lead to a switch to a Th1 predominant phenotype and stronger pertussis-specific T cell proliferation [15].

Most immunogenicity studies of pertussis vaccines were carried out shortly after primary or postprimary vaccination in infants or young children. Only a few studies of the long-term immunity in adolescents [16,17], and even fewer in adolescents with exclusive

* Corresponding author at: Department of Antimicrobial Therapy & Infection Immunology, Children's Hospital-Dr. von Haunersches Kinderspital, Ludwig-Maximilians-University Munich, Lindwurmstr. 4, 80337 Munich, Germany. Tel.: +49 89 5160 2811; fax: +49 89 5160 3138.

E-mail address: Nikolaus.Rieber@med.uni-muenchen.de (N. Rieber).

aP vaccination background have been conducted [18,19]. No study up to now has examined the cellular immunity in adolescents with exclusive aP preimmunization.

The majority of our study population was enrolled in their infancy in an effectiveness study of the two-component acellular Biken DTaP vaccine in Germany [10] and had received an immunization schedule with four doses of Biken DTaP at 2, 4, 6 and 15–24 months of age. A part of our study population received a fifth consecutive Biken DTaP dose at 4–6 years of age within a safety and immunogenicity study [20]. They are now among the first cohort of adolescents to have received five consecutive aP vaccinations in childhood before their adolescent aP booster vaccination. This schedule will be followed with increasing frequency in the future. We were therefore interested in differences in long-term humoral and cellular immunity and booster immune response to pertussis antigens between these adolescents, who previously had received five consecutive doses of Biken DTaP (last dose, age 4–6 years), and similar aged adolescents, who received either four doses of Biken DTaP (last dose, age 18–24 months) or four doses of whole cell pertussis vaccine (DTwP, Behringwerke, Marburg, Germany; last dose, age 18–24 months).

2. Materials and methods

This open, nonrandomized, multicenter study was carried out to examine pertussis-specific cellular and humoral immunity before and after aP booster vaccination in adolescents with different childhood pertussis vaccination schedules. Patients were recruited from pediatric practices in Germany and in the outpatient department of the University Children's Hospital, Ludwig-Maximilians-University, Munich.

2.1. Study population

A clinical trial examining reactogenicity of a Tdap vaccination given as the sixth consecutive acellular pertussis dose in 10–14-year-old adolescents in Germany was conducted in parallel with the present study [21]. Study investigators of the reactogenicity trial were asked to participate as investigators in this study. Adolescents 10–14 years of age with no obvious health problems and no immunosuppressive treatment were enrolled, provided they had previously received either five doses of an acellular pertussis vaccine (Biken DTaP, last dose age 4–6 years) [10,20] (*5aP group*), or four doses of an acellular pertussis vaccine (Biken DTaP, last dose age 18–24 months) [10] (*4aP group*), or four doses of a whole-cell pertussis vaccine (DPT-Impfstoff[®], Behringwerke, Marburg, Germany, last dose age 18–24 months) (*4wcp group*). The Biken DTaP was a sterile preparation of a two-component acellular pertussis vaccine with pertussis toxin (PT) and filamentous hemagglutinin (FHA) manufactured by Biken and Tanabe Corp. (Osaka, Japan) combined with adsorbed diphtheria and tetanus toxoids by Aventis Pasteur Inc. (Swiftwater, PA). Each 0.5 ml dose contained 6.7 Lf of diphtheria toxoid, 5.0 Lf of tetanus toxoid, 23.4 µg of detoxified PT and 23.4 µg of detoxified FHA, thimerosal 1/10,000 (25 µg), sodium phosphate and sodium chloride. The whole-cell pertussis vaccine DPT-Impfstoff[®] consisted of 4 IU of *Bordetella pertussis*, 30 IU of diphtheria toxoid, 40 IU of tetanus toxoid and 0.75 mg of aluminum hydroxide/aluminum phosphate and was used as comparative whole-cell vaccine in an acellular pertussis vaccine efficacy study in Germany [10]. At the time of the study, a booster vaccination against pertussis (aP vaccine), diphtheria and tetanus was recommended in Germany for all participants. No participants with a history of clinical pertussis disease were enrolled in the study.

The study protocol was approved by the local ethical review board of the University of Munich, Germany, and by the Paul-Ehrlich-Institute, Langen, Germany. The study was conducted according to Good Clinical Practice and in accordance with the Declaration of Helsinki. Written, informed assent from each participant and written, informed consent from the parent/legal guardian was obtained prior to study entry.

2.2. Study vaccine

Only licensed products provided by Sanofi Pasteur MSD, Leimen, Germany were used. Participants received either REPEVAX[®], COVAXIS[®], or COVAXIS[®] and IPV MERIEUX[®].

COVAXIS[®] contains in each 0.5 ml dose 2.5 µg pertussis toxoid, 5 µg filamentous hemagglutinin, 3 µg pertactin, 5 µg fimbriae types 2 and 3, 2 Lf diphtheria toxoid, 5 Lf tetanus toxoid, 1.5 mg aluminum phosphate and 0.6% 2-phenoxyethanol. COVAXIS[®] and IPV-Merieux[®] are identical to the US-licensed products Adacel[®] and IPOL[®], respectively. REPEVAX[®], the combination of the two, is not marketed in North America.

In addition to the same acellular pertussis antigens and content, each 0.5 ml dose of REPEVAX[®] additionally contains inactivated, Vero cell-derived poliomyelitis vaccine (vero cell origin) (Poliovirus Type 1: 40 D units, Poliovirus Type 2: 8 D units, Poliovirus Type 3: 32 D units).

IPV MERIEUX[®] contains in each 0.5 ml dose inactivated, Vero cell-derived poliomyelitis vaccine (vero cell origin) (Poliovirus Type 1: 40 D units, Poliovirus Type 2: 8 D units, Poliovirus Type 3: 32 D units).

2.3. Study procedures

Participants of the *5aP group* and the *4wcp group* received one dose of REPEVAX vaccine in the left arm, or one dose of COVAXIS vaccine in the left arm plus one optional dose of IPV MERIEUX vaccine in the right arm, as determined by their randomisation in the parallel clinical reactogenicity trial [21].

4aP group participants received one dose of REPEVAX vaccine in the left arm, or one dose of COVAXIS vaccine in the left arm plus one optional dose of IPV MERIEUX vaccine in the right arm as recommended by their physician.

From every participant, a 10 ml heparinized blood sample (20 IU heparin per ml) and a 3 ml serum sample were drawn directly before vaccination and 28–36 days after vaccination. The blood samples were sent at room temperature to the Laboratory of Immunology at the University Children's Hospital, Ludwig-Maximilians-University, Munich, Germany and were processed within 24 h.

2.4. Serum antibody determination

Serum antibodies were measured at the Institute for Hygiene and Laboratory Medicine, Helios Klinikum Krefeld, Germany (the German reference centre for pertussis diagnostics). IgG antibodies against native antigens pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin (PRN) (antigen source: GlaxoSmithKline Biologicals SA; Rixensart, Belgium) were determined using a standardized enzyme-linked immunosorbent assay (ELISA) according to procedures described earlier [22]. Standardized ELISA for IgG antibodies against fimbriae (FIM) 2 or 3 were not then available.

The standard curve was calculated by four-parameters logistics ("softmax" evaluation software, Molecular Devices, Sunnyvale, CA, USA) and results were expressed in ELISA units per milliliter (EU/ml) as referenced to the serum #3 of the CBER/FDA. The minimal level of detection (MLD) for all antibodies was arbitrarily set

Table 1

Seropositivity before and after adolescent Tdap vaccination and percentage of participants with twofold titre rise. The percentages of participants with detectable IgG antibodies (≥ 5 EU/ml) to pertussis antigens PT, FHA and PRN before and 28–36 days after adolescent Tdap vaccination and the percentages of participants with twofold rise in titres against pertussis antigens are shown (5aP group: $N = 36$; 4aP group: $N = 23$; 4wcP group: $N = 18$).

Antibody	Group	Seropositivity prevaccination (%) (95% CI)	Seropositivity postvaccination (%) (95% CI)	Twofold titre rise (%) (95% CI)
IgG-anti-PT	5aP group	36.1 (20.8–53.8)	86.5 (71.2–95.5)	77.8 (60.9–89.9)
	4aP group	30.4 (13.2–52.9)	82.6 (61.2–95.1)	82.6 (61.2–95.1)
	4wcP group	55.6 (30.8–78.5)	94.4 (72.7–99.9)	77.8 (52.4–93.6)
IgG-anti-FHA	5aP group	94.4 (81.3–99.3)	100 (90.5–100)	91.7 (77.5–98.3)
	4aP group	82.6 (61.2–95.1)	100 (85.2–100)	100 (85.2–100)
	4wcP group	61.1 (35.8–82.7)	100 (81.5–100)	88.9 (65.3–98.6)
IgG-anti-PRN	5aP group	88.9 (73.9–96.9)	100 (90.5–100)	100 (90.3–100)
	4aP group	56.5 (34.5–76.8)	100 (85.2–100)	100 (85.2–100)
	4wcP group	72.2 (46.5–90.3)	100 (81.5–100)	94.4 (72.7–99.9)

to 5 EU/ml. Antibody concentrations below the MLD were given a value of one-half the MLD (2.5 EU/ml).

2.5. Preparation of peripheral blood mononuclear cells (PBMC)

Heparinized blood samples were processed within 24 h. PBMC were prepared by Ficoll density gradient sedimentation (Lymphocyte Separation Medium, Biochrom, Berlin, Germany) and washed twice in RPMI 1640 (Biochrom, Berlin, Germany). Trypan blue staining solution at 0.5% differentiated between viable and nonviable cells. Only samples with viability of $>80\%$ were evaluated. PBMCs were resuspended at 1.5×10^6 cells/ml in RPMI 1640 supplemented with 5% heat-inactivated (30 min at 56°C) human serum and 1% penicillin (Biochrom, Berlin, Germany), hereafter referred to as complete medium.

2.6. Lymphocyte proliferation assay

The capacity of PBMC to respond to the pertussis antigens PT, FHA, PRN and FIM 2/3 (all provided by Sanofi Pasteur Inc., Swiftwater, PA, USA) was assessed by antigen-specific proliferation [23]. Lymphocytes (1.5×10^5) were cultured in round-bottom microtitre plates (Greiner bio-one, Frickenhausen, Germany) in 100 μl complete medium/well in the presence of antigen. The following optimal antigen concentrations were added [11]: PT 2 $\mu\text{g}/\text{ml}$ (heat-inactivated at 80°C for 20 min), FHA 10 $\mu\text{g}/\text{ml}$, PRN 10 $\mu\text{g}/\text{ml}$ and FIM 2/3 10 $\mu\text{g}/\text{ml}$.

Cultures with purified phytohemagglutinin (PHA) (Oxoid, London, England) at 1 $\mu\text{g}/\text{ml}$ were positive controls; cultures without antigens were negative controls. Cell cultures were performed in duplicate in a humidified atmosphere at 37°C and 5% CO_2 . After a culture period of 6 days for antigen-specific responses and of 2 days for PHA, 1.5 μCi of [^3H]thymidine (Amersham, UK) was added per well. The cells were harvested after 16 h and incorporated [^3H]thymidine was measured by a scintillation counter (Beckmann, Germany). The results were expressed as mean counts per minute (cpm). Cell-mediated immunity (CMI) was defined to be positive when the antigen-induced proliferation was ≥ 4 -fold higher than spontaneous proliferation (stimulation index [SI] ≥ 4).

2.7. Data analysis and statistical methods

Data were analyzed with REPORT version 6.6 and TESTIMATE version 6.4 (IDV, Gauting, Germany) and GraphPad Prism version 4.01 for Windows (GraphPad Software, San Diego, California, USA). Wilcoxon matched-pair signed-rank test was used for analysis of prevaccination versus postvaccination values for each individual. For the pairwise testing between the three groups, a

Mann–Whitney U -test was performed. Analysis of correlations was performed using the Spearman rank correlation coefficient. For all analyses, a two-tailed p -value ≤ 0.05 was considered to be significant.

3. Results

3.1. Demographic characteristics of participants

The mean age of the 78 study participants (33 female/45 male) was 12.4 years (age range 10–14 years). There were no substantial gender or age differences between the three treatment groups.

3.2. Humoral immunity

We analyzed the titres of IgG antibodies against PT, FHA and PRN (Tables 1 and 2 and Fig. 1). Prior to booster vaccination, IgG antibodies were detected to PT in 39% of all participants, to FHA in 83%, and to PRN in 75%. For the 5aP group, geometric mean titres (GMTs) to PT and FHA after the fifth aP dose at 4–6 years of age were known [20], although these values were measured in a different laboratory. A marked decrease was observed for both antigens since that time point (123.1 EU/ml \rightarrow 4.22 EU/ml for IgG-anti-PT; 241.1 EU/ml \rightarrow 25.2 EU/ml for IgG-anti-FHA). Ninety-four percent of 5aP group participants still had measurable anti-FHA-titres; only 36% had measurable anti-PT titres.

Higher prevaccination PT GMTs were found in the 4wcP group compared with both acellular vaccination groups: i.e. for the 5aP group (9.7 EU/ml versus 4.2 EU/ml, $p = 0.12$) and for the 4aP group (9.7 EU/ml versus 3.9 EU/ml, $p = 0.06$). In the 4wcP group, three participants (17%) had IgG-anti-PT of ≥ 94 EU/ml, which is considered a threshold indicating recent subclinical or clinical infection [24].

In contrast, the 5aP group showed a significantly higher FHA prevaccination GMT than the 4wcP group (25.2 EU/ml versus 10.7 EU/ml, $p = 0.04$). No differences in prevaccination titres against PRN were detected between the three groups.

After vaccination, IgG-titres against PT, FHA and PRN increased significantly ($p < 0.0001$) in all groups. The proportion of participants with twofold increase in titre was 79% against PT, 94% against FHA and 99% against PRN with no significant differences between the three groups.

The postvaccination GMTs to PT were significantly higher in the 4wcP group than in the 5aP group (50.3 EU/ml versus 16.4 EU/ml, $p = 0.001$) or the 4aP group (50.3 EU/ml versus 17.1 EU/ml, $p = 0.006$). No difference between the 5aP group and the 4aP group was detected.

There was a significant ($p < 0.0001$) positive correlation between pre- and postvaccination titres for all 3 antigens.

Table 2
Antibody titres to PT, FHA and PRN before and 28–36 days after adolescent Tdap vaccination. Antibody titres are given in EU/ml. Minimum level of detection (MLD) was 5 EU/ml. Antibody levels below the MLD were given an arbitrary value of half of the MLD (=2.5 EU/ml) (5aP group: N=36; 4aP group: N=23; 4wcP group: N=18).

Antibody	Group	GMT pre vaccination (EU/ml)(95% CI)	GMT post vaccination (EU/ml)(95% CI)
IgG-anti-PT	5aP group	4.2 (3.2-5.5)	16.4 (11.4-23.6)
	4aP group	3.9 (2.8-5.3)	17.1 (9.7-30.1)
	4wcP group	9.7 (4.2-22.5)	50.3 (26.1-97.0)
IgG-anti-FHA	5aP group	25.2 (16.8-37.8)	161.0 (122.3-211.8)
	4aP group	15.0 (9.4-23.9)	127.0 (89.1-181.2)
	4wcP group	10.7 (5.2-21.9)	137.6 (98.9-191.3)
IgG-anti-PRN	5aP group	18.8 (12.5-28.2)	600.7 (449.6-802.6)
	4aP group	13.3 (6.3-28.0)	407.3 (260.6-636.7)
	4wcP group	14.0 (6.3-30.9)	639.0 (370.0-1103.3)

*Statistically significant difference between groups (Mann-Whitney U-test; see text).

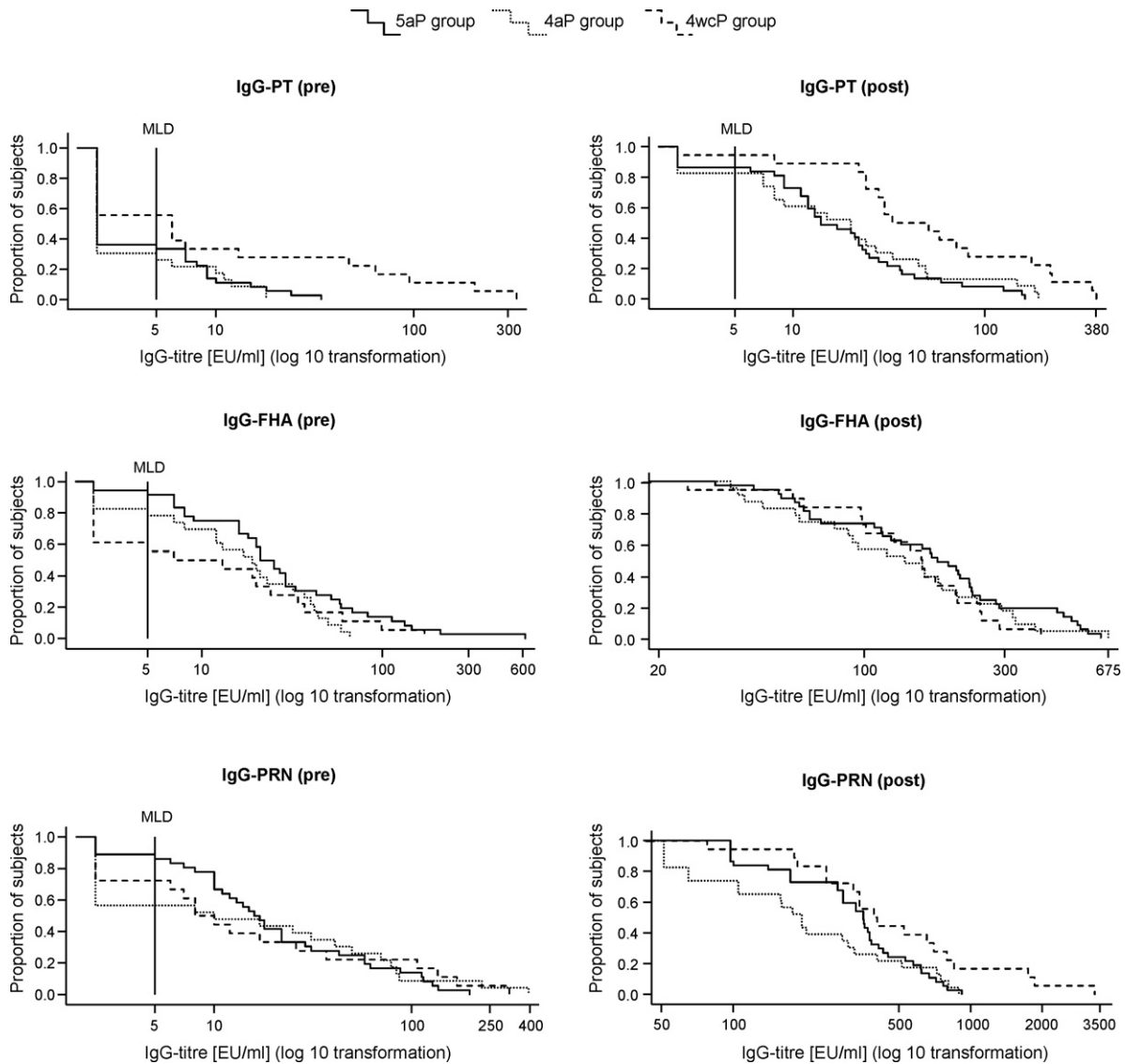


Fig. 1. Reverse cumulative distribution curves of pre- and postvaccination IgG antibodies to PT, FHA and PRN. Antibody titres (EU/ml) to PT, FHA and PRN were measured before and 28–36 days after adolescent Tdap vaccination. Minimum level of detection (MLD) was 5 EU/ml. Antibody levels below the MLD were given an arbitrary value of half of the MLD (=2.5 EU/ml) (continuous line = 5aP group; dotted line = 4aP group; dashed line = 4wcP group).

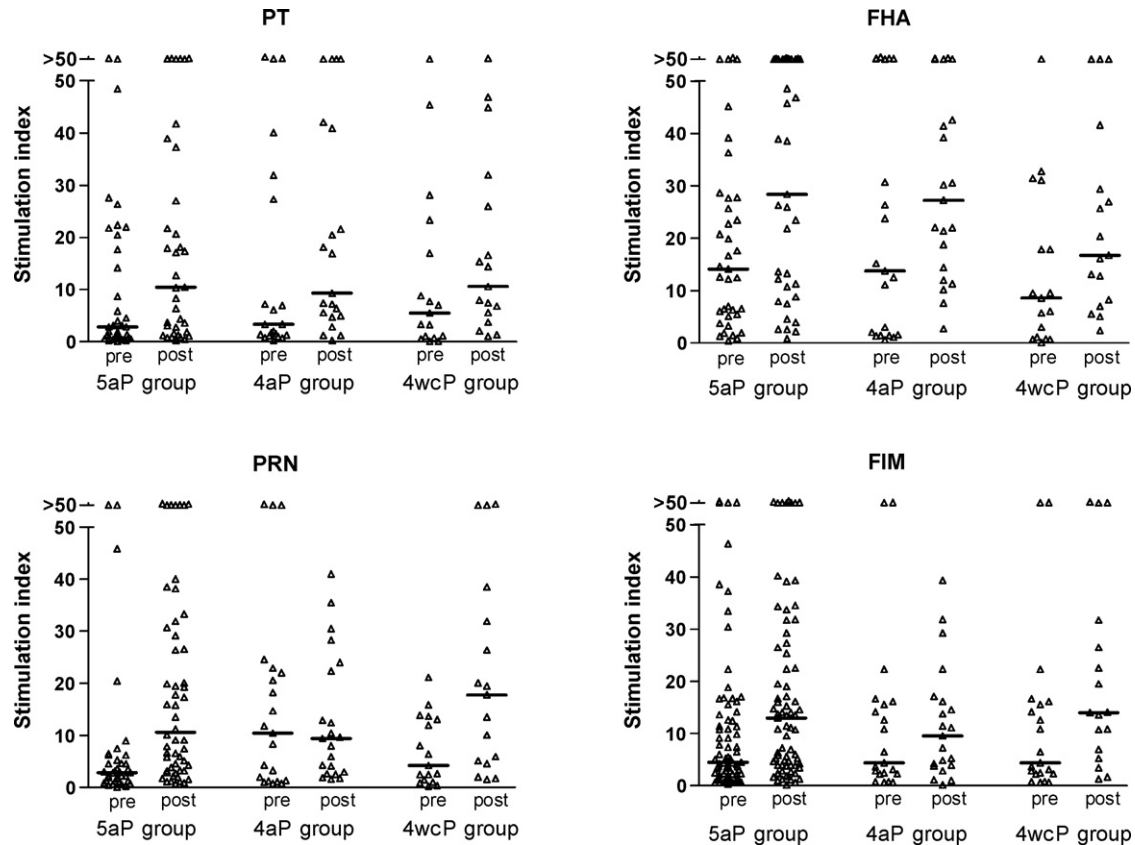


Fig. 2. The stimulation indices (SI) after stimulation with PT, FHA, PRN and FIM before (pre) and 28–36 days after (post) adolescent Tdap vaccination. The horizontal line within the scatter blot indicates the median of the individual stimulation indices. Stimulation indices beyond 50 are merged in this figure for better illustration (5aP group: $N=37$; 4aP group: $N=21$; 4wcP group: $N=17$).

3.3. Cell-mediated immunity (CMI)

Prior to booster vaccination, specific CMI ($SI \geq 4$) was seen in 43% of participants against PT, 71% against FHA, 47% against PRN and 56% against FIM. The lymphocytic proliferation was highest after FHA stimulation. SI to all four tested pertussis antigens (PT, FHA, PRN, FIM) showed a significant increase (Wilcoxon test; $p \leq 0.01$) after vaccination (Fig. 2), as did the percentage of participants with positive CMI ($SI \geq 4$) (Wilcoxon test; $p \leq 0.017$). After adolescent Tdap booster vaccination, specific CMI ($SI \geq 4$) was seen in 71% of participants against PT, 91% against FHA, 72% against PRN and 76% against FIM. With the exception of a significantly higher SI to PRN before vaccination in the 4aP group compared to the 5aP group (Mann–Whitney U -test; $p=0.04$), pre- and postvaccination SI as well as the percentage of participants with positive CMI were similar between groups.

3.4. Correlation between humoral immunity and CMI

Comparing the quantitative values, our data do not indicate a clear correlation between antibody titres and SIs to pertussis antigens before and after booster vaccination. The qualitative comparison (positive/negative immune response) was restricted to PT, because of an overall high-seropositivity rate to FHA and PRN, which made a qualitative analysis problematic. Of those 45 participants without detectable PT antibodies before booster vaccination, 15 (33%) had a positive CMI to PT. On the other hand, 12 of 42 (29%) participants with a negative CMI before booster vaccination had detectable antibody titres. After vaccination, 5 of 10 (50%) participants with negative antibody response had a positive CMI response,

while 17 of 22 (77%) participants with a negative CMI had detectable antibody response. Therefore, even if positive and negative immune responses are compared, the data do not indicate a relationship between humoral and cell-mediated immunity to PT in our adolescents.

4. Discussion

The aim of this study was to elucidate differences in long-term humoral and cellular immunity and booster response to pertussis antigens in adolescents with either a previous exclusive aP schedule of four or five doses or a previous wcP schedule with four doses. This is the first study to evaluate T cell memory and cellular immune response in adolescents who had exclusively received acellular pertussis vaccines in infancy and early childhood. The adolescent Tdap booster immunization induced overall good humoral and cellular immune responses to pertussis. IgG-titres significantly increased against PT, FHA, and PRN in all groups after vaccination. No differences were found between groups. The percentage of participants with a twofold titre increase against PT, FHA, and PRN was similar for all groups. The percentage of participants with positive CMI to pertussis antigens showed a significant increase after vaccination, which was similar for all groups.

The reactogenicity data pertaining to this adolescent acellular pertussis booster immunization will be presented in detail elsewhere. Generally, the immunization was well tolerated with fewer local reactions than seen with the fifth dose of acellular pertussis vaccine in 4–6 years old children [20,25].

Comparing the CMI with antibody titres before vaccination, we found similar percentages of participants with either cellular or humoral long-term immune responses. Therefore, CMI was not

superior to antibody titres as a parameter of immune persistence since the last pertussis vaccination, which is in line with other reports [12,26]. However, the prevaccination immunity may be largely affected by previous natural boosters. This renders it difficult to conclude that long-term effects are solely due to vaccination. The discrepancy with other publications describing a longer persistence of CMI than antibody titre in young children and adolescents 2, 3, and 5 years after pertussis vaccination [16,17,23] may therefore be explained by a high rate of previous natural boosters in our cohort. In our study population, positive prevaccination antibody titres to at least one pertussis antigen have been seen at a rate of 90% 5–12 years after the last pertussis vaccination. Data from Knuf et al. also indicate a high rate of subclinical pertussis infections in Germany, showing a seropositivity rate of 37% against PT and 92% against FHA in an unvaccinated German adolescent population without history of clinical pertussis [27]. In contrast to primary immune responses, we did not observe a correlation between antibody titres and SI to pertussis antigens [12,28]. This is in concordance with several other reports [17,23,29]. The lack of correlation might be explained by reactivation of memory B cells, which is less dependent on T cell help than the primary activation [30]. Thus, long-term T cell reactivity does not necessarily reflect B cell memory response.

From prior studies in mice, one might predict a reduced CMI response in exclusively aP-vaccinated individuals compared with whole cell vaccinated individuals [13,31]. A possible reduced T cell priming in our aP vaccinated individuals may have been overcome by the putative high rate of subclinical natural boosting in our German adolescent population [27].

In contrast to anti-FHA and anti-PRN IgG titres, antibody titres against PT prior to adolescent booster vaccination had declined to undetectable levels in more than half of the participants since their last vaccination at 18–24 months or 4–6 of age. This may be partly explained by the fact, that antibody responses to FHA and PRN also occur after infections with *Bordetella parapertussis* and *Bordetella bronchiseptica* and may additionally be the result of cross-reacting epitopes of nonencapsulated *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and perhaps other bacteria [32]. The antibody titres to PT prior to adolescent booster vaccination showed higher values for the primary wCP immunized adolescents compared with adolescents receiving four or five doses of aP. Similar data was found in a study by Pichichero et al., where antibody titres to PT before an adolescent aP booster immunization showed higher titres in adolescents after five doses of wCP (vaccine by Wyeth Lederle) compared with adolescents previously given five doses aP (vaccine by GlaxoSmithKline or Sanofi Pasteur) during early childhood [19]. A study by Guiso et al. [33] comparing long-term humoral and cell-mediated immunity between 7 and 9 years old children previously immunized with wCP (Sanofi Pasteur) or aP (GlaxoSmithKline) vaccines did not reveal a difference in PT antibody titres approximately 6 years after the last vaccination. The differences that we and others [19] observed in adolescents aged 10–14 years might not yet be detectable in the 7–9 years old children. Differences in the whole cell vaccines used in these studies might also contribute to the unequal results. Remarkably, 3 out of 18 participants in the 4wCP group showed high-IgG-anti-PT titres of over 94 EU/ml indicating recent contact to *B. pertussis* [24]. This highlights the difficulties in relating long-term antibody titres solely to vaccination.

Vaccination of adolescents induced an at least twofold rise in titre to PT in about 80% of our participants in all three groups. When, however, the quantitative antibody titres were compared between the groups the titres to PT were significantly higher in the group with primary wCP immunization than in the two groups with primary aP immunization. As shown by Pichichero et al. [14]

the antibody titres to PT 1 month after the postprimary booster at 18–24 months with the acellular Biken pertussis vaccine were higher than after wCP vaccination with a Connaught vaccine at the same time points. Looking now at the antibody titres before and 4 weeks after the adolescent booster vaccination, the results seem to be reversed. In principle, there are two explanations for this difference: more recent natural pertussis boosters in the 4wCP group or a more effective priming of B cell memory to PT through whole cell vaccination. Since IgG-titres to FHA and PRN prior to booster vaccination, which are less specific, but may also indicate recent exposure, were higher in the 5aP group than in the 4wCP group, the first explanation seems somewhat less likely. The data may therefore indicate a better priming of memory PT-specific B cells and long-lived plasma cells [34] by wCP vaccination, at least with the wCP vaccine used in our population.

LPS or other TLR-ligands of the bacterial cell wall, so-called PAMPs (pathogen associated molecular patterns) have been shown to support the generation of memory B cells and long-lived plasma cells [34,35]. The stronger memory response to PT in our 4wCP group might be attributed to an initially more-pronounced generation of memory B cells and long-lived plasma cells through a specific combination of co-stimulatory signals [36] exhibited by the whole-cell pertussis vaccine compared with the alum-containing acellular vaccine. The specific combination of co-stimulatory factors might also cause the variable efficacies of different whole-cell vaccines [9]. The cell-mediated immune response to PT was quite similar in the three groups indicating that the difference in the anti-PT antibody response is not due to a selectively more efficient induction of PT-specific memory T cells by primary wCP immunization compared to aP vaccination.

Several studies have shown that in populations with high-vaccine coverage of children, *B. pertussis* tends to shift to adolescents and adults [2–4]. This may be due to the absence of a primary vaccination in parts of these age groups and to the limited long-term protection by the available pertussis vaccines. Thus, one major goal in future improvement of pertussis vaccines is better long-term protection through a more effective induction of memory B cells and long-lived plasma cells. This study encourages efforts to identify the essential combinations of adjuvant signals provided by the most efficacious of the wCP vaccines. Irrespective of that, adolescent Tdap booster induced reliable humoral and cellular immune responses in adolescents who had been primarily vaccinated with different acellular and cellular vaccination schedules. Therefore, enforcing adolescent and adult booster vaccination programs will prevent suffering and contracting pertussis disease in these individuals and simultaneously minimize the risk of transmission of *B. pertussis* to young infants, who are at high risk of pertussis-associated life-threatening complications.

Acknowledgements

This work was supported by grants from Sanofi Pasteur Inc., Swiftwater, PA, USA. The authors wish to thank Thomas Malzer, CRO Munich, for organization and monitoring of study procedures, the statistician Marion Ocak for professional help with statistical analyses, Sabine Dönhoff for expert technical assistance and Drs. Michael Decker, David Johnson and Robert Lersch for critical review of the manuscript. This report represents a part of the doctoral thesis by Anna Graf.

References

- [1] Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2005. Published March 30, 2007, for MMWR 2005;54(53):77–9.

- [2] Yih WK, Lett SM, des Vignes FN, Garrison KM, Sipe PL, Marchant CD. The increasing incidence of pertussis in Massachusetts adolescents and adults 1989–1998. *J Infect Dis* 2000;182(5):1409–16.
- [3] Wirsing von König CH, Halperin S, Riffelmann M, Guiso N. Pertussis of adults and infants. *Lancet Infect Dis* 2002;2(12):744–50.
- [4] Riffelmann M, Littmann M, Hulsse C, O'Brien J, Wirsing von König CH. Pertussis: incidence, symptoms and costs. *Dtsch Med Wochenschr* 2006;131(50):2829–34.
- [5] Deen JL, Mink CA, Cherry JD, Christenson PD, Pineda EF, Lewis K, et al. Household contact study of *Bordetella pertussis* infections. *Clin Infect Dis* 1995;21(5):1211–9.
- [6] Wendelboe AM, Njamkepo E, Bourillon A, Floret DD, Gaudelus J, Gerber M, et al. Transmission of *Bordetella pertussis* to young infants. *Pediatr Infect Dis J* 2007;26(4):293–9.
- [7] Tanaka M, Vitek CR, Pascual FB, Bisgard KM, Tate JE, Murphy TV. Trends in pertussis among infants in the United States 1980–1999. *JAMA* 2003;290(22):2968–75.
- [8] Vitek CR, Pascual FB, Baughman AL, Murphy TV. Increase in deaths from pertussis among young infants in the United States in the 1990s. *Pediatr Infect Dis J* 2003;22(7):628–34.
- [9] Edwards KM, Decker MD. Pertussis vaccine. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders; 2008. p. 467–517.
- [10] Liese JG, Meschievitz CK, Harzer E, Froeschle J, Hosbach P, Hoppe JE, et al. Efficacy of a two-component acellular pertussis vaccine in infants. *Pediatr Infect Dis J* 1997;16(11):1038–44.
- [11] Ryan M, Murphy G, Ryan E, Nilsson L, Shackley F, Gothefors L, et al. Distinct T-cell subtypes induced with whole cell and acellular pertussis vaccines in children. *Immunology* 1998;93(1):1–10.
- [12] Esposito S, Agliardi T, Giammanco A, Faldella G, Cascio A, Bosis S, et al. Long-term pertussis-specific immunity after primary vaccination with a combined diphtheria, tetanus, tricomponent acellular pertussis, and hepatitis B vaccine in comparison with that after natural infection. *Infect Immun* 2001;69(7):4516–20.
- [13] Redhead K, Watkins J, Barnard A, Mills KH. Effective immunization against *Bordetella pertussis* respiratory infection in mice is dependent on induction of cell-mediated immunity. *Infect Immun* 1993;61(8):3190–8.
- [14] Pichichero ME, Francis AB, Marsocci SM, Green JL, Disney FA, Meschievitz C. Safety and immunogenicity of an acellular pertussis vaccine booster in 15- to 20-month-old children previously immunized with acellular or whole-cell pertussis vaccine as infants. *Pediatrics* 1993;91(4):756–60.
- [15] Ausiello CM, Lande R, Urbani F, la Sala A, Stefanelli P, Salmasso S, et al. Cell-mediated immune responses in four-year-old children after primary immunization with acellular pertussis vaccines. *Infect Immun* 1999;67(8):4064–71.
- [16] Edelman KJ, He Q, Makinen JP, Haanpera MS, Nguyen Tran Minh N, Schuerman L, et al. Pertussis-specific cell-mediated and humoral immunity in adolescents 3 years after booster immunization with acellular pertussis vaccine. *Clin Infect Dis* 2004;39(2):179–85.
- [17] Edelman K, He Q, Makinen J, Sahlberg A, Haanpera M, Schuerman L, et al. Immunity to pertussis 5 years after booster immunization during adolescence. *Clin Infect Dis* 2007;44(10):1271–7.
- [18] Meyer CU, Zepp F, Decker M, Lee M, Chang SJ, Ward J, et al. Cellular immunity in adolescents and adults following acellular pertussis vaccine administration. *Clin Vac Immunol* 2007;14(3):288–92.
- [19] Pichichero ME, Casey JR, Francis AB, Marsocci SM, Murphy M, Hoeger W, et al. Acellular pertussis vaccine boosters combined with diphtheria and tetanus toxoid boosters for adolescents: safety and immunogenicity assessment when preceded by different 5-dose DTaP/DTwP schedules. *Clin Pediatr (Phila)* 2006;45(7):613–20.
- [20] Liese JG, Stojanov S, Zink TH, Froeschle J, Klepadlo R, Kronwittner A, et al. Safety and immunogenicity of Biken acellular pertussis vaccine in combination with diphtheria and tetanus toxoid as a fifth dose at four to six years of age. *Munich Vaccine Study Group. Pediatr Infect Dis J* 2001;20(10):981–8.
- [21] Liese J. Clinical trial report TRI05, EUDRACT-number 2005-003765-17; 2007.
- [22] Wirsing von König CH, Gounis D, Laukamp S, Bogaerts H, Schmitt HJ. Evaluation of a single-sample serological technique for diagnosing pertussis in unvaccinated children. *Eur J Clin Microbiol Infect Dis* 1999;18(5):341–5.
- [23] Zepp F, Knuf M, Habermehl P, Schmitt JH, Rebsch C, Schmidtke P, et al. Pertussis-specific cell-mediated immunity in infants after vaccination with a tricomponent acellular pertussis vaccine. *Infect Immun* 1996;64(10):4078–84.
- [24] Baughman AL, Bisgard KM, Edwards KM, Guris D, Decker MD, Holland K, et al. Establishment of diagnostic cutoff points for levels of serum antibodies to pertussis toxin, filamentous hemagglutinin, and fimbriae in adolescents and adults in the United States. *Clin Diagn Lab Immunol* 2004;11(6):1045–53.
- [25] Liese J, Rieber N, Malzer T, Ocak M, Johnson DR, Decker MD. Safety of a sixth consecutive dose of tetanus–diphtheria–acellular pertussis vaccine in adolescents. Abstract for ICAAC 2007.
- [26] 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(17):1604–10.
- [27] Knuf M, Zepp F, Meyer C, Grzegowski E, Wolter J, Riffelmann M, et al. Immunogenicity of a single dose of reduced-antigen acellular pertussis vaccine in a non-vaccinated adolescent population. *Vaccine* 2006;24(12):2043–8.
- [28] Cassone A, Ausiello CM, Urbani F, Lande R, Giuliano M, La Sala A, et al. Cell-mediated and antibody responses to *Bordetella pertussis* antigens in children vaccinated with acellular or whole-cell pertussis vaccines. The Progetto Pertosse-CMI Working Group. *Arch Pediatr Adolesc Med* 1997;151(3):283–9.
- [29] Ausiello CM, Lande R, Urbani F, Di Carlo B, Stefanelli P, Salmasso S, et al. Cell-mediated immunity and antibody responses to *Bordetella pertussis* antigens in children with a history of pertussis infection and in recipients of an acellular pertussis vaccine. *J Infect Dis* 2000;181(6):1989–95.
- [30] Vieira P, Rajewsky K. Persistence of memory B cells in mice deprived of T cell help. *Int Immunol* 1990;2(6):487–94.
- [31] Mills KH, Barnard A, Watkins J, Redhead K. Cell-mediated immunity to *Bordetella pertussis*: role of Th1 cells in bacterial clearance in a murine respiratory infection model. *Infect Immun* 1993;61(2):399–410.
- [32] Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. *Clin Microbiol Rev* 2005;18(2):326–82.
- [33] Guiso N, Njamkepo E, Vie le Sage F, Zepp F, Meyer CU, Abitbol V, et al. Long-term humoral and cell-mediated immunity after acellular pertussis vaccination compares favourably with whole-cell vaccines 6 years after booster vaccination in the second year of life. *Vaccine* 2007;25(8):1390–7.
- [34] Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat Rev Immunol* 2006;6(10):741–50.
- [35] Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 2002;298(5601):2199–202.
- [36] Raman VS, Lind EF, Benson MJ, Noelle RJ. Strategies for selective priming of memory B cells. *Immunol Lett* 2007;109(2):93–100.