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T-Cell Responses before and after the Fifth Consecutive Acellular Pertussis Vaccination in 4-Year-Old Dutch Children

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Immunization with acellular pertussis vaccine (aP) induces higher specific antibody levels and fewer adverse reactions than does immunization with the whole-cell vaccine (wP). However, antibody levels in infants induced by both types of pertussis vaccines wane already after 1 year. Therefore, long-term T-cell responses upon vaccination might play a role in protection against pertussis. In a cross-sectional study (ISRCTN65428640), we investigated T-helper (Th) cell immune responses in wP- or aP-vaccinated children before and after an aP low-dose or high-dose preschool booster at 4 years of age in The Netherlands. T cells were stimulated with pertussis vaccine antigens. The numbers of gamma interferon-producing cells and Th1, Th2, Th17, and interleukin-10 (IL-10) cytokine concentrations were determined. In addition, pertussis-specific IgE levels were measured in plasma. Children being vaccinated with aP vaccinations at 2, 3, 4, and 11 months of age still showed higher pertussis-specific T-cell responses at 4 years of age than did wP-vaccinated children. These T-cell responses failed to show a typical increase in cytokine production after a fifth aP vaccination but remained high after a low-dose booster and seemed to decline even after a high-dose booster. Importantly, elevated IgE levels were induced after this booster vaccination. In contrast, wP-vaccinated children had only low prebooster T-cell responses, and these children showed a clear postbooster T-cell memory response even after a low-dose booster vaccine. Four high-dose aP vaccinations in infancy induce high T-cell responses still present even 3 years after vaccination and enhanced IgE responses after preschool booster vaccination. Therefore, studies of changes in vaccine dosage, timing of pertussis (booster) vaccinations, and the possible association with local side effects are necessary.

Recently, during a large pertussis outbreak in California 10 infants have died (19), and 9,154 cases of whooping cough have been reported by the California Department of Public Health (4). Already in the 1990s, many developed countries replaced the whole-cell pertussis component (wP) with the acellular pertussis component (aP) in the DTP-IPV-Hib combination vaccine in order to achieve higher antigen-specific antibody levels and fewer side effects. This, however, did not stop the reemergence of pertussis in these countries (8, 38, 39). The high incidence of pertussis worldwide can be (partly) explained by adaptation of the circulating bacterial strains to vaccine pressure as well as waning immunity after vaccination and natural infection (2, 27).

In The Netherlands, wP vaccines have been used since the early 1950s, resulting in a decline of pertussis disease. Despite a high vaccination coverage, the incidence of pertussis increased again after 1996, and for this reason, an aP preschool booster vaccination (aP) was introduced at 4 years of age in 2001. From 2005 onwards, the infant wP vaccine component administered at 2, 3, 4, and 11 months of age has also been replaced by the aP vaccine.

Pertussis-specific antibody levels are induced by vaccination and natural infection and protect against disease; however, these levels decline very rapidly after vaccination (9, 13). Several studies have shown that protection against disease also relies on T-helper (Th) cells, as well as antibodies (7, 26). Multiple Th cell lineages may be involved, like the Th1, Th2, and Th17 cells, and each lineage is characterized by specific cytokine repertoires (6). However, the induction of long-term T-cell memory responses and the relative contribution of each Th cell lineage upon vaccination are largely unknown. Previous studies have shown that aP may lead to Th2 cytokine repertoires in infants and children, whereas wP rather primes for Th1 immune responses (1, 23). The aP vaccines consist of some purified pertussis proteins that may differ from wP vaccines in the induction of Th cell responses, which include many other biological components. Information about T-cell immunity after pertussis vaccination and comparison between wP- and aP-primed infants is scarce. Moreover, Th2 responses might be associated with atopic reactions (31), and pertussis-specific IgE has been found after aP vaccinations in infants (28). The aim of this study is to assess the Th1, Th2, and Th17 as well as interleukin-10 (IL-10) cytokine responses to pertussis vaccine antigens in children 4 years of age who received either a low-dose or a high-dose antigen aP preschool booster vaccination. We compared groups of children who have been primed either by wP or by aP in infancy. Apart from T-cell kinetics, also pertussis antigen-specific IgE responses in these groups of children are studied.

MATERIALS AND METHODS

Subjects and study design. In this study, a cohort of children 4 years of age forms a subset of a cross-sectional observational study in The Netherlands

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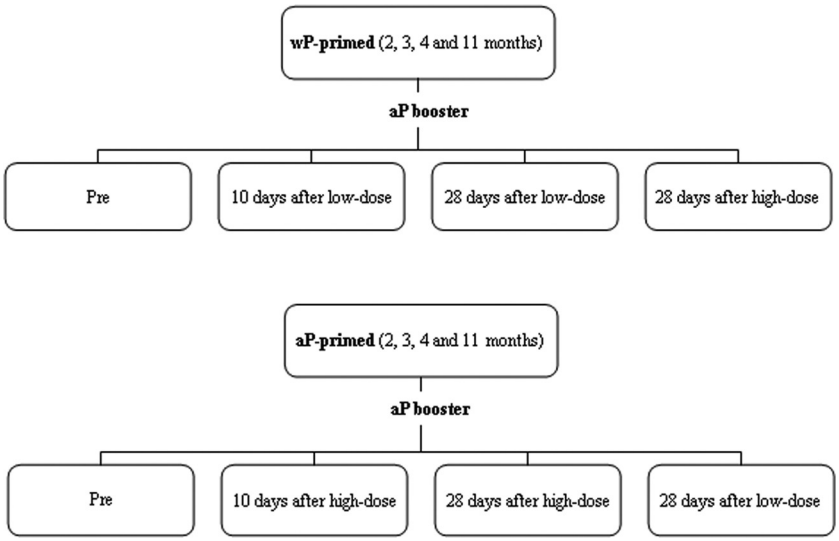

FIG 1 Different groups of children were used in this study: wP-primed and aP-primed children at 2, 3, 4, and 11 months of age received a booster at 4 years of age with either a low-dose or a high-dose vaccine. Groups of children were studied before booster and at 10 and 28 days after booster. The numbers of individuals used varied in the different assays as indicated.

(ISRCTN65428640) performed from 2007 (wP-primed children) onwards until 2008 (aP-primed children), which aimed to investigate pertussis-specific immunity in children 3 to 9 years of age. The pertussis vaccine-specific IgG antibody responses in these 4-year-old children have been published previously (13). Now, we evaluated T-cell immune responses in a randomly selected subset of these children ($n = 92$). As previously described (13), we divided the children into 8 different groups, according to (i) the vaccination history (wP or aP priming), (ii) the type of preschool booster vaccine (low-dose or high-dose aP), and (iii) time of blood sampling, i.e., before the booster or 10 or 28 days after the booster (Fig. 1). This study was conducted according to the Declaration of Helsinki, Good Clinical Practice Guidelines, with the approval of the relevant ethics review committee. Written informed consent was obtained from either parents or legal representatives.

Vaccines. All wP-primed children had received the DTwP-IPV-Hib (NVI, Bilthoven, The Netherlands) vaccine and all aP-primed children had received the DTaP-IPV-Hib (Infanrix-IPV-Hib; GlaxoSmithKline Biologicals S.A., Rixensart, Belgium) vaccine at 2, 3, 4, and 11 months of age according to the Dutch National Immunization Programme (NIP). The wP vaccine contained, among other bacterial proteins, about 0.16 μg pertussis toxin (PT), 2.6 μg filamentous hemagglutinin (FHA), and an unknown amount of pertactin (Prn), while the aP vaccine contained 25 μg PT, 25 μg FHA, and 8 μg Prn. During the inclusion period of this study, children received randomly either a low-dose or a high-dose preschool booster vaccine due to a shortage in supply of the vaccine. Therefore, we included children who had received a low-dose booster vaccine, Triaxis (Sanofi Pasteur, Lille, France), containing 2.5 μg PT, 5 μg FHA, 3 μg Prn, and 5 μg fimbria types 2 and 3, or a high-dose booster vaccine, Infanrix, in wP- and aP-primed children, at 28 days postbooster. At 10 days postbooster, we were able to include only wP-primed children who received a low-dose booster and aP-primed children who received a high-dose booster, because the booster vaccine in the NIP had switched during the period of our study.

T-cell stimulation. Peripheral blood mononuclear cells (PBMCs) were isolated from blood as described earlier and frozen (3). After thawing, 3.0×10^5 viable cells per well were cultured in AIMV medium (Gibco Invitrogen, Grand Island, NY) containing 5% human AB serum (Harlan Laboratories, Leicestershire, United Kingdom) (AIMV+) for 5 days at 37°C and 5% CO_2 in 96-well round-bottomed culture plates (Greiner, Invitrogen, Breda, The Netherlands) and stimulated in triplicate with 2 μg/ml inactivated PT, FHA (Kaketsuken, Kumamoto, Japan; endotoxin content, < 1.17 endotoxin units [EU]/ml), 4 μg/ml of recombinant Prn (22), and 5 μg/ml pokeweed mitogen (Sigma Chemicals, St. Louis, MO) as a positive control. Nonstimulated (NS) cells served as negative controls. The purified PT and FHA batches were heat inactivated by incubation for 10 min at 80°C. The batch of Prn was controlled for endotoxin contents by use of the *Limulus* amebocyte lysate assay (17). One batch of each antigen was used for the whole study. In preliminary experiments, we determined the optimal pertussis antigen concentrations for cell stimulation and the culture period needed to measure optimal numbers of gamma interferon (IFN-γ)-producing cells. After both 1 day and 5 days of stimulation, culture supernatants were collected and stored at −80°C to be able to measure early and late cytokine responses. T-cell responses were measured in culture supernatants, and 5 to 13 samples in all groups indicated in Fig. 1 were analyzed.

IFN-γ ELISpot assay. Enzyme-linked immunosorbent spot (ELISpot) plates (MSIP4510; Millipore, Danvers, MA) prewetted with 70% ethanol were coated overnight with 5 μg/ml anti-human IFN-γ (Mabtech, Nacka Strand, Sweden) in phosphate-buffered saline (PBS). After three washes with PBS and blocking the plates for 2 h with AIMV+, 1.4×10^5 stimulated cells per well were serially diluted 2-fold and cultured for 18 h at 37°C and 5% CO_2. After four washes with PBS-0.05% Tween 20 (PBST) (washed) and cell lysis with water, plates were incubated with 1 μg/ml anti-human IFN-γ (Mabtech) for 2 h in combination with peroxidase-labeled extravidin (Sigma) for 1 h. Spots were developed as described previously (14).

Multiplex bead-based immunoassay for cytokines. The cytokines IFN-γ, tumor necrosis factor alpha (TNF-α), IL-5, IL-13, IL-17, and IL-10 were determined in supernatants after 5 days, and IL-2 was measured after 1 day of stimulation by commercial multiplex bead-based immunoassay kits according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). All samples presented in this study have been stored just for a few months before testing and have been randomly divided over the different tests to avoid interassay variation between the samples. Measurements were performed with a Bio-Plex 200 in combination with Bio-Plex Manager software (Bio-Rad).

***Bordetella pertussis*-specific IgE.** Plasma samples were depleted of IgG by adding Gulsorb (1:10, vol/vol) (Meridian Bioscience Inc., Cincinnati, OH). Afterwards, the concentration of pertussis-specific IgE was measured using goat anti-human IgE-phycoerythrin (PE) (Epsilon;

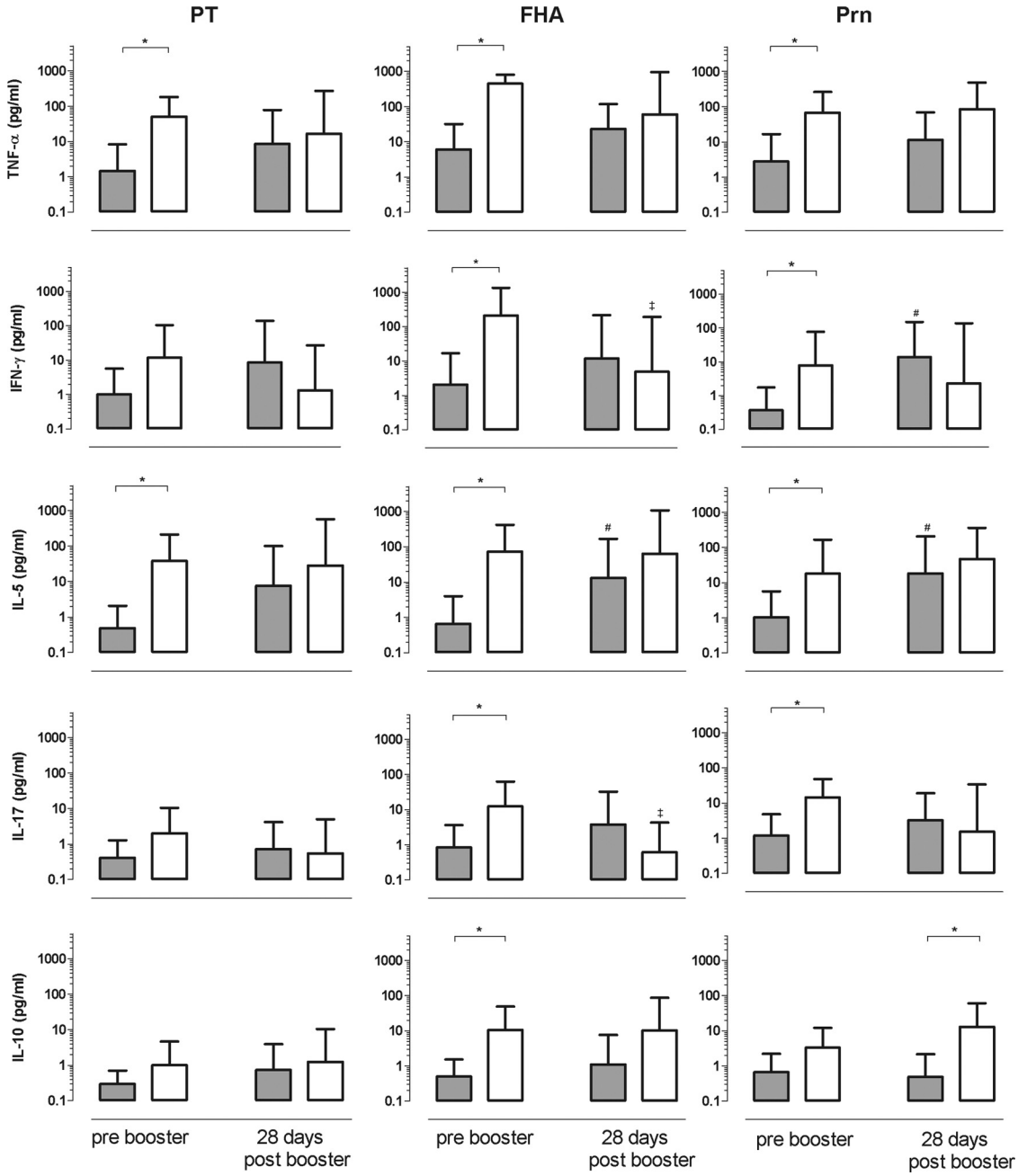

FIG 2 Concentrations of Th1 (TNF-α and IFN-γ), Th2 (IL-5), Th17 (IL-17), and IL-10 cytokines in supernatants of PBMCs of wP- (gray bars) and aP-primed (white bars) children specific for the pertussis proteins PT, FHA, and Prn. Bars represent GMs and 95% confidence intervals of data prebooster and at 28 days after booster vaccination with a high-dose aP vaccine. Groups consisted of 13 individuals before booster vaccination and 5 to 8 persons at 28 days after booster vaccination. *, significantly increased values in aP-primed children compared to wP-primed children; #, significant increase at 28 days after booster vaccination compared to prebooster values in wP-primed children; ‡, significant decrease at 28 days after booster compared to prebooster values in aP-primed children.

Fisher Scientific) in a multiplex bead-based assay as described previously (15, 37). IgE responses were measured in 16 to 52 plasma samples of all groups presented in Fig. 1.

Definitions, data presentation, and statistical analysis. All individual data or geometric mean (GM) values and 95% confidence intervals per group were presented. Background values of negative-control cells were deducted from each sample. Significant differences between groups ($P < 0.05$) were determined as indicated.

RESULTS

Induction of T-cell cytokines in wP- and aP-primed children in infancy. The concentrations of the T-cell cytokines TNF-α, IFN-γ, IL-5, IL-17, and IL-10 specific for the pertussis proteins PT, FHA, and Prn in PBMCs of aP- and wP-primed children before and 28 days after a high-dose aP preschool booster are illustrated in Fig. 2. At 4 years of age, 3 years after four aP

TABLE 1 IL-2 and IL-13 produced by PBMCs of wP- and aP-primed children pre- and postbooster upon stimulation with PT, FHA, and Prn[a]

Cytokine with stimulant	Cytokine concn (pg/ml)							
	wP primed				aP primed			
	Prebooster ($n = 10$)	10 days, low dose ($n = 8$ or 9)	28 days, low dose ($n = 9$)	28 days, high dose ($n = 8$)	Prebooster ($n = 9$)	10 days, high dose ($n = 9$)	28 days, high dose ($n = 6$)	28 days, low dose
IL-2								
PT	0.01 (−)	0.04 (7.2)	1.5[c] (501)	0.05 (1.1)	7.0[b] (55.2)	3.0 (15.4)	19.6 (36.6)	ND
FHA	0.01 (−)	0.6[c] (74.2)	6.0[c] (401)	1.6[c] (44.1)	36.1[b] (250)	22.5 (116)	37.9 (340)	ND
Prn	0.01 (−)	0.1[c] (2.4)	0.5[c] (12.3)	0.3[c] (6.4)	10.6[b] (42.0)	5.1 (66.8)	7.1 (20.6)	ND
	($n = 13$)	($n = 10$ or 11)	($n = 9$ or 10)	($n = 8$ or 9)	($n = 13$)	($n = 11$ or 12)	($n = 5$ or 6)	($n = 5$ to 7)
IL-13								
PT	0.5 (28.4)	15.1[c] (143)	8.4 (111)	13.8[c] (443)	132[b] (3,877)	99.7 (948)	65.5 (255)	82.3 (693)
FHA	1.9 (79.0)	16.9 (267)	7.0 (152)	47.7[c] (230)	502[b] (7,322)	326 (2,754)	45.8 (799)	644 (1,543)
Prn	1.6 (45.1)	39.3[c] (174)	43.4[c] (157)	39.8[c] (212)	158[b] (450)	43.3 (999)	38.7 (270)	135 (148)

[a] Children 4 years of age were vaccinated with either a low-dose (Triaxis) or a high-dose (Infanrix) aP booster vaccine. Data are presented as geometric means (standard deviations). ND, no data.
[b] Significantly higher prebooster values in aP-primed children than in wP-primed children.
[c] Significantly increased postbooster values compared to prebooster values in wP-primed children.

vaccinations in the first year of life, most prebooster T-cell cytokine levels specific for all three pertussis proteins (11/15) were significantly higher than those of wP-primed children. The PT-specific IFN-γ, IL-17, and IL-10 as well as the Prn-specific IL-10 were also higher in aP-primed children but did not reach significance (Fig. 2).

In wP-primed children, the aP booster did enhance the pertussis-specific TNF-α, IFN-γ, and IL-5 production, an effect which was significant for FHA- and Prn-specific IL-5 as well as the Prn-specific IFN-γ. In contrast, the aP booster in aP-primed children did not enhance the T-cell cytokine concentrations. Moreover, after administration of a fifth high-dose aP vaccine, the FHA-specific IFN-γ and IL-17 response even showed a decline. In general, the pertussis-specific IL-17 and IL-10 values were low compared with the other cytokine values, especially for PT (Fig. 2).

To compare the production levels of the Th2 cytokine and the Th1 cytokines, the geometric mean values (GMs) of the IL-5 levels per group before and after the booster were compared to those of IFN-γ. The GM of the Th2 cytokine IL-5 specific for PT was about 6-fold higher in aP-primed children than was the Th1 IFN-γ GM before booster vaccination. At 28 days after a fifth, high-dose aP vaccine, the IL-5 GM was about 10- to 20-fold higher than that of IFN-γ for all three pertussis antigens. In contrast, similar values of Th1 and Th2 cytokines were found in wP-primed children (Fig. 2).

Kinetics of the T-cell cytokine production in wP- and aP-primed children after a preschool aP booster vaccination. To illustrate the kinetics of the T-cell cytokine production after an aP booster vaccination at 4 years of age, the concentrations of two other cytokines are presented in Table 1, showing data for day 10 after an aP booster vaccination, too. Also, for the Th1 cytokine IL-2 and the Th2 cytokine IL-13, prebooster T-cell cytokine levels specific for all pertussis antigens were significantly higher in aP-primed children than in wP-primed children, as shown for the other cytokines (Table 1).

In general, the T-cell cytokine concentrations of wP-primed children specific for all pertussis antigens showed increased values already at day 10 after a low-dose booster vaccination. These values were significantly higher for FHA- and Prn-specific IL-2 as well as PT- and Prn-specific IL-13. During the next 18 days, no significant differences in cytokine production were observed. In contrast, no increased values or a tendency toward lower values was observed at day 10 after even a high-dose aP booster in aP-primed children (Table 1).

Due to the high prebooster values, the IL-13 cytokine levels for all three proteins in aP-primed children at day 28 after a low-dose booster were still higher than those found in wP-primed children after a low-dose booster vaccination. This was also found for PT-specific IL-2 and IL-13 at 28 days after a high-dose booster, although the differences were not significant due to the high variation in the relatively small amount of samples (Table 1).

Induction of IFN-γ-producing T cells in wP- and aP-primed children. The numbers of IFN-γ-producing T cells in wP- and aP-primed children before and at 10 and 28 days after the booster showed the same pattern as that found for the cytokine values in the T-cell supernatants. At 3 years after four aP vaccinations in the first year of life, the numbers of IFN-γ-producing cells specific for all three pertussis antigens were about 10-fold higher than those in children vaccinated with wP in infancy (Fig. 3).

In wP-primed children, the numbers of IFN-γ-producing cells specific for PT increased significantly at both day 10 and day 28 after a low-dose booster ($P = 0.02$ and $P = 0.04$, respectively) as well as after a high-dose booster ($P = 0.02$). Those numbers specific for FHA and Prn were significantly increased at day 10 ($P = 0.04$ and $P = 0.03$, respectively) and were just slightly increased at day 28 after either a low-dose or a high-dose booster. At day 28, a high-dose booster in wP-primed children induced numbers of IFN-γ-producing cells comparable with those induced by a low-dose booster for all three antigens (Fig. 3).

The already high numbers of IFN-γ-producing cells at 4 years of age prebooster in aP-primed children did not further increase after children received either a low-dose or a high-dose booster. Surprisingly, at 28 days after a high-dose aP booster vaccine, these numbers even tended to decrease for all three antigens, which resulted in significantly lower numbers ($P = 0.04$) for Prn than those after a low-dose booster (Fig. 3). Furthermore, at 28 days after a low-dose preschool booster, the numbers of pertussis-specific IFN-γ-producing cells in aP-primed children were higher than those in wP-primed children, which reached significance for

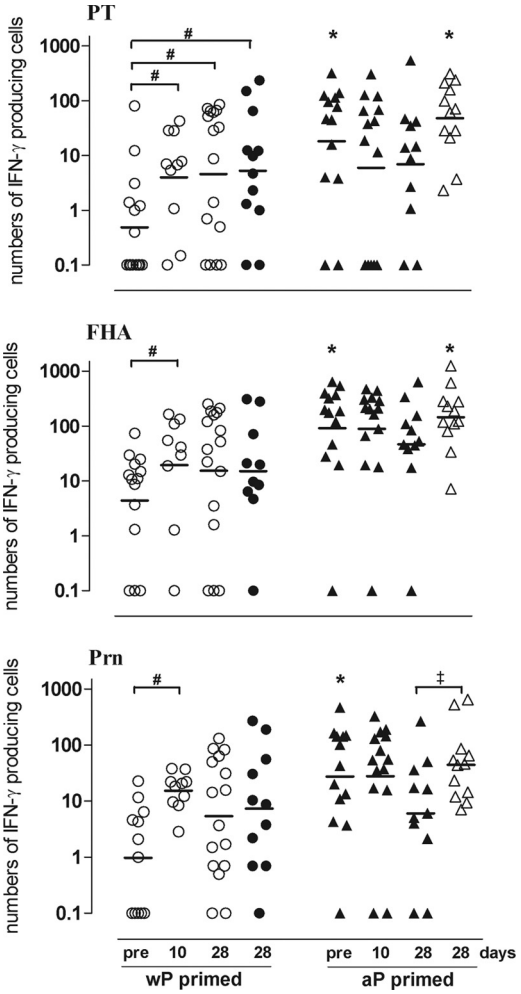

FIG 3 Numbers of IFN-γ-producing cells prebooster and at 10 and 28 days after booster vaccination in wP- (circles) and aP-primed (triangles) children specific for the pertussis proteins PT, FHA, and Prn. At 4 years of age, both wP- and aP-primed children have been administered an aP booster with either a low-dose (open symbols) or a high-dose (closed symbols) vaccine. Horizontal lines indicate the geometric mean per group. #, significantly increased numbers postbooster compared to prebooster values in wP-primed children; *, significantly increased numbers in aP-primed children compared to wP-primed children; ‡, significantly higher numbers after a low-dose booster compared to a high-dose booster in aP-primed children at 28 days postbooster.

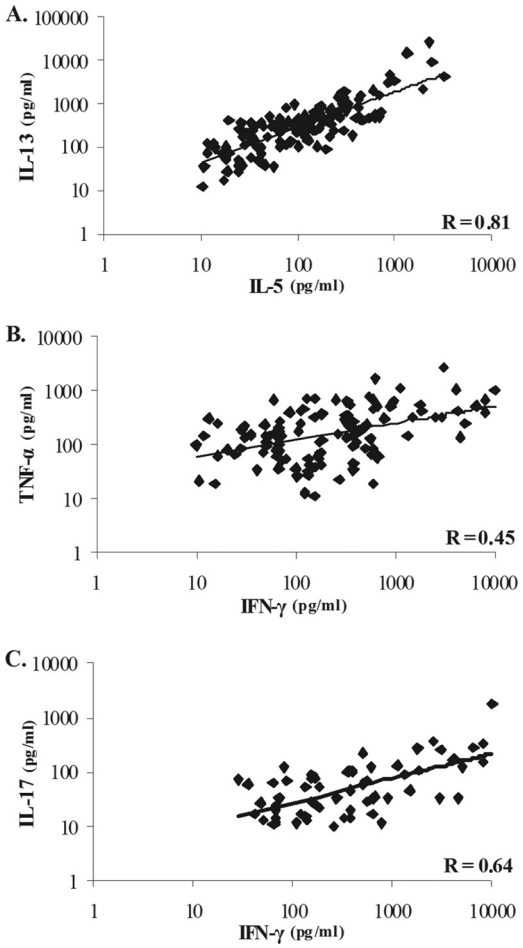

FIG 4 Correlation of concentrations of the Th2 cytokines IL-5 and IL-13 (A), the Th1 cytokines IFN-γ and TNF-α (B), and the Th1 cytokine IFN-γ and the Th17 cytokine IL-17 (C) present in T-cell culture supernatants of all wP- and aP-primed children used in this study.

the antigens PT and FHA ($P = 0.04$ and $P = 0.03$, respectively) and not just for Prn ($P = 0.06$) (Fig. 3).

Correlations of cytokine levels. Significant correlations were found for the concentrations of the Th2 cytokines IL-5 and IL-13 ($R = 0.81$), for the Th1 cytokines IFN-γ and TNF-α ($R = 0.45$), and even for the Th17 (IL-17) response with that of IFN-γ ($R = 0.64$) using the values (>10 pg/ml) of all wP- and aP-primed children in this study (Fig. 4). Additionally, the correlations of the Th1 cytokines IL-2, measured at 24 h, and IFN-γ, at day 5, were similar ($R = 0.48$). The correlation between Th1 and Th2 cytokines mutually was lower (data not shown).

Differences in plasma pertussis-specific IgE levels of wP- and aP-primed children. In addition to pertussis-specific IgG levels that were previously reported (6), we also evaluated pertussis-specific IgE values upon the preschool booster vaccinations of 4 years old children. Pertussis-specific IgE values were similar in wP- and aP-primed children before booster vaccination (Fig. 5). Both PT- and Prn-specific IgE values increased after either a low-dose or a high-dose booster vaccination in aP-primed and wP-primed children (Fig. 5). However, in aP-primed children IgE values specific for Prn were significantly higher after both booster doses ($P = 0.01$ and <0.0001, respectively) compared with wP-primed children, and this was also the case for the IgE values specific for PT after a high-dose booster ($P = 0.001$). Total IgE values were also higher in aP-primed children than in wP-primed children after a low-dose or a high-dose booster vaccination (data not shown).

DISCUSSION

We demonstrated that infant vaccinations with high-dose acellular pertussis vaccines resulted in high pertussis antigen-specific Th1 and Th2 T-cell responses that persisted in children at least until 4 years of age in aP-primed children, despite waning IgG levels. Importantly, a fifth aP preschool booster vaccine at 4 years of age did not show a typical memory response by increasing these T-cell responses. In contrast, an aP booster in wP-primed children did induce memory T-cell responses. Apart from inducing high

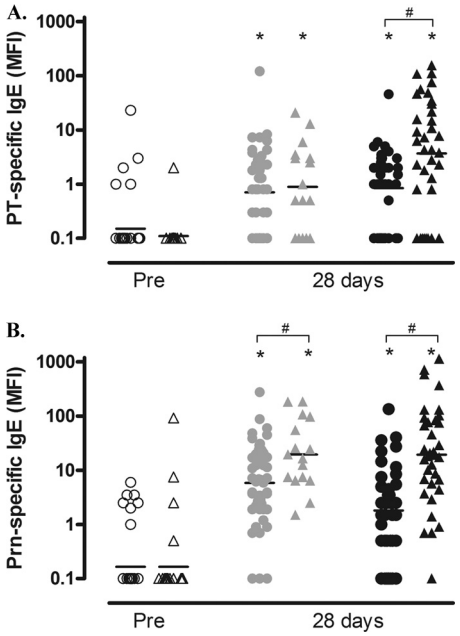

FIG 5 PT- (A) and Prn-specific (B) plasma IgE values in wP- (circles) and aP-primed (triangles) children prebooster ($n = 46$ and $n = 32$, respectively) and at 28 days after a low-dose booster (gray symbols) ($n = 46$ and $n = 16$, respectively) or a high-dose booster (black symbols) ($n = 52$ and $n = 35$, respectively). A horizontal line indicates the geometric mean per group. *, significantly increased values postbooster compared to prebooster; #, significantly increased values in aP-primed children compared to wP-primed children. MFI, mean fluorescence intensity.

IgG levels (13), the fifth aP vaccine elevated IgE antibody values, which are associated with Th2 and atopic responses. Moreover, pertussis-specific IgE values were increased in aP-primed children after both low- and high-dose preschool booster vaccination.

Still, limited information on T-cell cytokine responses in young children is available since most studies that evaluated pediatric DTaP-IPV-Hib (HebB) and adult TdaP vaccines have focused on antibody responses against the different vaccine components (13, 21, 24, 25). Pertussis-specific antibody levels found in the 4-year-old children in this study (13) vaccinated at 2, 3, 4, and 11 months of age were comparable with data from French children of the same age (12) vaccinated at 2, 4, and 18 months of age. In both countries, the prebooster anti-PT antibody levels were similarly low in 4-year-old children and those specific for Prn and FHA were higher after aP vaccination than those found after wP vaccination (12, 13). The comparison of the few studies on cell-mediated immune responses against pertussis and the replacement of wP with aP vaccines is complicated since different vaccines, various vaccination schedules, and different age groups have been used (1, 5, 12, 23, 32, 34). Similar Th1 responses have been found before and 5 weeks after the booster in 4- to 6-year-old aP-primed children, whereas increased Th2 responses were found after the fourth aP vaccination (34). However, we did not find such an increase after a fifth aP booster vaccine. This discrepancy probably results from the additional booster given after the primary series in the first year of life in our children, which might explain the already higher levels of prebooster IL-5 at 4 years of age. These IL-5 levels resemble the data of 4- to 6-year-old French aP-vaccinated children, although their IFN-γ data were higher. This can be explained by the comparison of mean values with geometric mean values in combination with a high variation between samples. The higher cytokine levels in French wP-vaccinated children could have been induced by the use of a different wP vaccine in France than the one used in The Netherlands (12).

The immunological basis of long-term vaccine-induced protection against pertussis is not clear yet. In the present study, we determined pertussis-specific Th1, Th2, Th17, and T-regulatory cytokine responses 3 years after infant aP or wP vaccinations but also shortly after a preschool booster with two aP vaccines containing different doses of proteins. After a fifth aP vaccination in our study, the already high levels of all T-cell cytokines remained elevated, whereas the IFN-γ levels and the numbers of IFN-γ-producing cells even slightly declined. The results for the IFN-γ-producing cells confirm the kinetics of the Th1 responses found in T-cell supernatants, corroborating the differences in pre- and postbooster T-cell responses between wP- and aP-primed children. Surprisingly, we did not find a typical T-cell memory response shown by an increase in cytokine production shortly after a fifth high-dose aP vaccine. This might suggest that the T-cell responses were still high enough to assist the T-cell-dependent recall antibody production upon an aP booster in aP-primed children, which has resulted in significantly increased postbooster antibody levels (13). Although we were not able to study the effects of a high-dose aP booster in wP-primed children, we clearly showed that even a low-dose aP booster vaccine did increase the postbooster T-cell cytokine responses in these children at 10 days postbooster. It can be expected that a high-dose aP booster vaccine will induce the same kinetics as those induced by a low-dose vaccine.

In aP-primed children, we did observe slightly higher Th2 responses than Th1 responses and low levels of IL-10 and IL-17 compared with the Th1 and Th2 cytokines. Although IL-17 plays a role in protection against intracellular pathogens (10) and in protection against *Bordetella* infection in mice (16), we do not know much about IL-17 induction in vaccinated children. In English infants vaccinated with *Mycobacterium bovis* BCG, also low IL-17 and Il-10 levels were found, whereas in infants from Malawi, higher levels of these cytokines have been shown, suggesting that this is influenced by the genetic background (20). In *Bordetella pertussis*-infected adults, however, we did measure higher levels of IL-17 (about 100 to 300 pg/ml) by using the same method as that in the present study (36) (R.-M. Schure, K. Öztürk, L. de Rond, E. A .M. Sanders, G. A. M. Berbers, and A. M. Buisman, unpublished data). Anyway, the exact mechanism of T-cell responsiveness or regulation in children is rather complex and needs to be the subject of further investigation.

After the fifth aP vaccination, at 4 years of age, the number of children reporting various side effects has doubled in comparison with that after an aP booster in wP-primed children. The local adverse effects range from nontender redness (<5 cm) to severe swelling (>5 cm) at the injection site (18). Moreover, the aP-primed children in this study showed increased pertussis-specific IgE levels after both a low-dose and a high-dose booster vaccine. This finding is in agreement with other studies (11, 34); however, the clinical relevance of these increased IgE responses is not clear. Since antihistamine was not effective in treating the adverse effects, the time course of these symptoms (mostly occurring within 1 or 2 days after vaccination [30]) reflects the possibility of a delayed-type hypersensitivity reaction caused by T cells and macrophages (29). We speculate that the four infant high-dose aP vac-

cinations might be responsible for the high prebooster Th1 and Th2 responses. The preschool booster vaccination additive to ongoing T-cell immunity may explain the adverse local reactions, which have been associated with Th2 responses by Rowe et al. (33) and T-cell responses by Scheifele et al. (35). Further research is necessary to assess the direct association between these high T-cell responses and local side effects, and at the moment, a study that recruits samples of children who actually report the local side effects is ongoing.

Our findings are in agreement with other studies (1, 23), showing that memory T cells persist for years while antibody responses wane rapidly after vaccination with aP or wP. This strongly suggests that memory T cells might play an important role in pertussis-specific long-term immunity. The T-cell responses in aP-primed children can be induced by vaccination and natural boosting due to the high circulation of pertussis in the population. However, since wP-primed children showed much lower T-cell responses than did aP-primed children of 4 years of age and the incidence of pertussis during the inclusion period of both groups was constant, the high T-cell responses were most likely induced by the aP vaccines administered early in life. The immune system of young infants is still under development and therefore very sensitive to stimulation. We propose that the high doses of proteins in the aP vaccines administered repeatedly within a short period of time, at 2, 3, and 4 months of age followed by a booster 7 months later, initiate high T-cell responses, which are maintained till even 3 years later.

The aP priming in infancy in combination with a preschool aP booster also induced high serum antibody levels (13) and memory B-cell responses (14). These responses together with epidemiological data indicate that protection against pertussis has improved in children after replacing the Dutch wP vaccine with aP vaccines. Since the effectiveness of vaccination is normally evaluated by antibody levels alone, we would like to stress that monitoring T-cell immunity in follow-up studies is necessary to better elucidate the efficacy and safety of infant vaccines. Moreover, the aP vaccines contain not only purified proteins but also an adjuvant stimulating the immune system to produce a Th2 response. In order to improve the immune response induced by aP vaccines in the future, the nature of the vaccine, the adjuvants, and the vaccination intervals need to be reconsidered. A vaccination schedule of 2, 4, and 6 months is employed by a third of the European countries (euvac.net) but also by the United States, Canada, and the South American countries. Therefore, we also recommend a more dispersed aP vaccination schedule starting at 2 months of age in The Netherlands. A better understanding of T-cell responses and their effect upon vaccination could result in a further improvement of the pertussis vaccination schedules for infants. The improved schedules should stimulate the immune system of infants in a better way to protect against pertussis.

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