

Immunogenicity of a single dose of reduced-antigen acellular pertussis vaccine in a non-vaccinated adolescent population

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Abstract

German adolescents ($n = 123$) without previous pertussis vaccination, no history of pertussis and low IgG-anti-pertussis-toxin (PT) levels received one dose of the Tdap vaccine BoostrixTM. Blood samples were taken before, and 5–12 days and 29–49 days after vaccination. IgG- and IgA-anti-PT, IgG- and IgA-anti filamentous hemagglutinin, IgG-anti-pertactin, IgG-anti-tetanus-toxin, and IgG-anti-diphtheria-toxin were measured by ELISA. 88.6% of subjects had an immune response to PT, and all vaccinees had an immune response to at least one pertussis antigen 29–49 days after vaccination. IgA-anti-PT and IgA-anti-FHA responses were found in 43 and 81% of subjects, respectively. This study shows that in unvaccinated German adolescents pertussis immunity can be achieved by a single dose of Tdap.
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1. Introduction

Childhood immunization against pertussis has successfully reduced the incidence of the disease [1]. Various countries, especially those with long-standing high vaccine coverage, have observed an increasing incidence of pertussis in older age groups in recent years. One reason for this may be waning immunity 4–12 years after the last vaccine dose of whole-cell vaccine and 5–6 years after acellular vaccines [2]. Thus, adolescent and adult immunization has been proposed to better control pertussis [3], and vaccination of adolescents aged 10–17 years and more recently 9–17 years has been recommended in Germany since 2000 and 2002, respectively [4]. In some Western parts of Germany, however, routine immunization of infants against pertussis has been reintro-

duced only after the licensure of acellular vaccines in 1995 [5].

Bordetella pertussis continues to circulate in vaccinated populations, and thus unvaccinated adolescents may have been in contact with pertussis antigens without clinical evidence of the disease [6,7]. It is unclear whether in these non-vaccinated individuals one dose of acellular pertussis vaccine with reduced antigen content could induce an immune response equivalent to that seen in vaccinated adolescents. A single dose of acellular pertussis vaccine with higher antigen content (infant formulation) would not be able to induce a measurable immune response in an immunologically naïve host.

Thus, we studied whether a single dose of acellular pertussis vaccine with reduced antigen content could induce an immune response in healthy adolescents 11–18 years of age, who were not vaccinated against pertussis, had no history of pertussis, and had low IgG-anti-pertussis-toxin (PT) levels. We used GSK Biologicals' reduced antigen content Tdap vaccine BoostrixTM, which is licensed for vaccination

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of immunized children and adolescents 4 years and above in Germany and various other countries.

2. Materials and methods

2.1. Study design

This was an open, non-randomized, multicenter study, conducted at 20 centres in Germany. It was conducted according to Good Clinical Practice and in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject/parent/guardian prior to entry into the study.

2.2. Study population

Male or female adolescents aged 11–18 years with no obvious health problems were included in the study, provided that they had IgG-anti-PT antibody concentrations <20 EU/ml at the time of screening. Exclusion criteria for enrolment included evidence of any history of vaccination against pertussis or known history of clinical pertussis, or administration of other vaccines, immunosuppressive drugs, or immunoglobulins. Acute diseases at the time of vaccination and/or fever of $\geq 37.5^{\circ}\text{C}$ were contra-indications for vaccine administration.

2.3. Study vaccine and administration

The vaccine was supplied as pre-filled syringes with each 0.5 ml dose containing ≥ 2 IU (2.5 Lf) of diphtheria toxoid, ≥ 20 IU (5 Lf) of tetanus toxoid, 8 μg of PT 8 μg of FHA, 2.5 μg of pertactin (PRN), 0.5 mg aluminium salts and 2.5 mg of 2-phenoxyethanol. All subjects received a single dose of vaccine on day 0. The vaccine was administered by deep intramuscular injection in the deltoid region of the non-dominant arm.

The following intervals between the four study visits were accepted: visit 1 (screening) could be done 1–21 days prior to vaccination, at visit 2 the vaccine was administered, visit 3 (first blood sampling) could be done 5–12 days after vaccination, and 92% of samples were actually drawn between day 6 and 8. Visit 4 (second blood sampling) could be done 29–49 days after vaccination, and 92% of samples were drawn between day 30 and 42.

2.4. Serological analysis

IgG-anti-diphtheria toxin, IgG-anti-tetanus toxin, IgG- and IgA-anti-PT, IgG- and IgA-anti-FHA and IgG-anti-PRN were measured at screening and 29–49 days after vaccination. Additionally, IgG- and IgA-anti-PT, IgG- and IgA-anti-FHA and IgG-anti-PRN were also measured 5–12 days after vaccination.

IgG- and IgA-antibodies against PT and FHA and IgG antibodies against PRN were measured by a standardized

ELISA procedure [8] and expressed in ELISA Units (EU/ml). The minimal level of detection for these assays was 1.7 EU/ml for IgG-anti-PT, 2.4 EU/ml for IgA-anti-PT, 1.8 EU/ml for IgG-anti-FHA, 1.7 EU/ml for IgA-anti-FHA, and 1.9 for IgG-anti-PRN [8,9]. The cut-off for all these tests was set to 5 EU/ml [9]. IgG-antibodies against diphtheria and tetanus toxoids were measured by a commercially available ELISA (Virion Serion, Wuerzburg, Germany). The cut-offs of these tests were set at 0.1 IU/ml.

2.5. Reactogenicity

Any adverse event occurring within 1 month following administration of the vaccine was recorded by the vaccinees or their guardians and the relationship of these symptoms to the vaccination was assessed by the investigator. Symptoms were graded by intensity, where grade 3 was the most severe category and represented prevention of normal, every-day activities.

2.6. Statistical methods

The primary objective of the study was to evaluate the proportion of subjects with an immune response 29–49 days after the vaccination. Based on the assumption that the response rate is 90% in this population, and that up to 15% of subjects would not be available for analysis, 120 subjects were planned to be enrolled. (One sided test, $\alpha = 5\%$, $\beta = 10\%$.)

Seropositivity rates and geometric mean concentration (GMCs) for antibodies against each component of the vaccine were calculated with 95% confidence intervals (CI). Antibody concentrations below the cut-off of the assay were given an arbitrary value of half the cut-off, and for concentrations with values above the last dilution tested, the last value was taken for GMC calculation.

2.7. Definitions: seropositivity

Defined as antibody concentrations ≥ 5 EU/ml for IgG- and IgA-anti-PT, IgG- and IgA-anti-FHA, IgG-anti-PRN antibodies or as anti-diphtheria-toxin and anti-tetanus-toxin antibody concentrations ≥ 0.1 IU/ml, respectively.

2.7.1. Immune response

No accepted protective antibody levels for pertussis antigens exist. Thus, an immune response against PT, FHA, or PRN was defined as post-vaccination concentration ≥ 5 EU/ml in subjects who were initially seronegative. In seropositive subjects it was defined as an at least 2-fold increase in antibody concentrations from pre-vaccination titers.

2.7.2. Booster response

An immune response to diphtheria toxoid and tetanus toxoid, defined as post-vaccination antibody concentrations of at least ≥ 0.4 IU/ml in previously seronegative subjects or

a 4-fold increase in antibody concentration in previously seropositive subjects.

3. Results

3.1. Demographics

All of the 127 subjects enrolled were Caucasians of whom 4 were eliminated from the immunogenicity analysis because of either seropositivity for anti-PT antibodies at pre-vaccination ($n = 2$) or non-compliance with blood sampling schedule ($n = 2$). The female/male ratio of the subjects included in the analysis was 1.2 (66/57) and the mean age of the cohort was 13.3 years.

3.2. Reactogenicity

A total of 11 subjects (8.7%) reported about symptoms. Five cases (4.1%) were considered by the investigator to be probably or possibly related to the study vaccine. The vaccinees reported injection site swelling (3), injection site pain (2) and fatigue (1). No serious adverse events were reported during the entire study period.

3.3. Immunogenicity

At 29–49 days post vaccination the booster response to diphtheria and tetanus toxoids were 61.8% (95% CI: 52.6–70.4) and 75.6% (95% CI: 67.0–82.9), respectively (Table 1). A response to vaccination was found in all subjects with initial levels of IgG-anti-diphtheria toxin of <0.1 IU/ml and 84.6% of subjects with initial levels of IgG-anti-tetanus toxin <0.1 IU/ml. No significant influence of gender could be found, and no significant difference in the response rate was observed, when the cohort was split into two age groups

along the median (≤ 13 years and >13 years). After vaccination, all subjects achieved concentrations for anti-diphtheria and anti-tetanus toxin antibodies that are considered to be protective (0.1 IU/ml). The increase in GMCs from pre- to post-vaccination was approximately 6-fold for anti-diphtheria toxin and 9-fold anti-tetanus toxin antibodies.

As shown in Tables 1 and 2, 90.2% of subjects had measurable IgG-anti-PT 29–49 days after vaccination, 100% had IgG-anti-FHA and 98.4% had IgG-anti-PRN. The increase in GMCs from pre- to post-vaccination was about 14-fold for IgG-anti-PT, 21-fold for IgG-anti-FHA and 42-fold for IgG-anti-PRN antibodies. Geometric mean concentrations (GMCs) at different time points are summarized as reverse cumulative distributions in Fig. 1.

At 5–12 days after vaccination an immune response to pertussis antigens was observed in 53.7% (IgG-anti-PT), 67.5% (IgG-anti-FHA) and 63.1% (IgG-anti-PRN) of vaccinees, respectively. At 29–49 days after vaccination 88.6% of the subjects showed an IgG response to PT, 91.9% to FHA and 96.7% to PRN (Table 2). Comparing the response of subjects with different pre-vaccination status showed no significant differences at day 29–49. Among 78 subjects primarily seronegative for IgG-anti-PT an immune response was observed in 66 vaccinees (84.6%). For the other antigens, the immune response was always more than 90% regardless of their pre-vaccination status (Table 2). Table 3 shows that the immune responses did not differ significantly between age groups, when the cohort was split along the median. All subjects in this study showed a response to at least one pertussis antigen at day 29–49 post vaccination.

Only one subject had IgA-anti-PT before vaccination, whereas 56.9% (70 subjects) had measurable prevaccination IgA-anti-FHA. 5–12 days post-vaccination, 28% of subjects had measurable IgA-anti-PT with a GMC of 3.8 EU/ml (95% CI: 3.3–4.3), and 81% of subjects had measurable IgA-anti-FHA with a GMC 22.8 EU/ml (95% CI: 17.6–29.6). After

Table 1
Seropositivity^a rates and GMCs in 123 vaccinees

Antibody	Time point	Seropositivity ^a % (95% CI)	GMCs Value (95% CI)
IgG-anti-PT	Pre ^b	36.6 (28.1–45.7)	4.1 (3.6–4.6)
	Early post ^b	63.4 (54.3–71.9)	13.1 (9.8–17.5)
	Post ^b	90.2 (83.6–94.9)	57.4 (43.2–76.3)
IgG-anti-FHA	Pre	91.9 (85.6–96.0)	26.1 (20.8–32.7)
	Early post	98.4 (94.2–99.8)	124.7 (97.4–159.6)
	Post	100 (97.0–100)	551.5 (449.1–677.2)
IgG-anti-PRN	Pre	43.1 (34.2–52.3)	6.3 (5.0–8.0)
	Early post	77.0 (68.6–84.2)	26.7 (18.5–38.5)
	Post	98.4 (94.2–99.8)	270.0 (199.8–364.9)
Anti-diphtheria-toxin	Pre	98.4 (94.2–99.8)	1.63 (1.35–1.98)
	Post	100 (97.0–100)	10.68 (9.16–12.46)
Anti-tetanus-toxin	Pre	89.4 (82.6–94.3)	0.43 (0.35–0.52)
	Post	100 (97.0–100)	3.97 (3.51–4.50)

^a Definition see text.

^b Pre = 28–0 days before vaccination; early post = 5–12 days after vaccination; post = 29–49 days after vaccination.

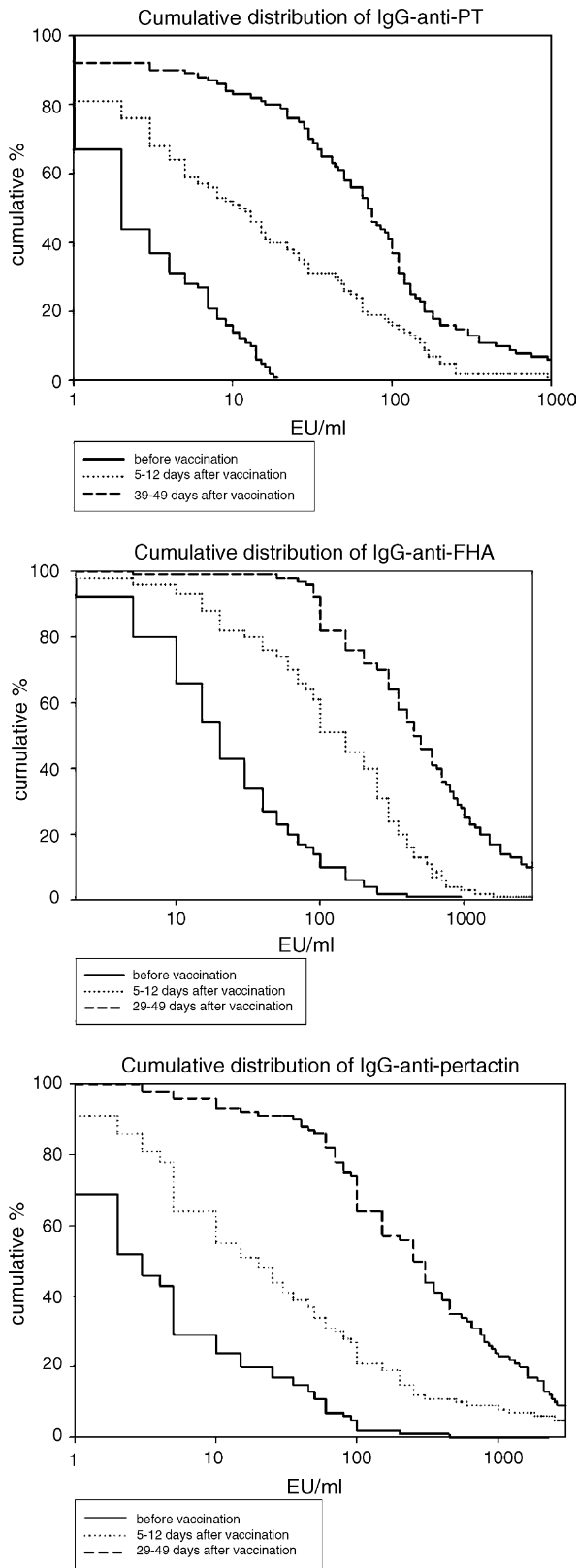


Fig. 1. Reverse cumulative distribution of pre- and post-vaccination levels of IgG-anti-PT, IgG-anti-FHA and IgG-anti-pertactin.

29–49 days after injection, 43.1% (95% CI: 34.2–52.3) and 81.3% (95% CI: 73.3–87.8) of subjects showed an immune response to IgA-anti-PT and IgA-anti-FHA, respectively, with GMC of 5.4 and 45.4 EU/ml for IgA-anti-PT and IgA-anti-FHA, respectively. From the 70 subjects who were initially seropositive for IgA-anti-FHA, a total of 55 (78.6%) demonstrated an immune response at day 29–49 post vaccination. The increase in GMCs from pre- to post-vaccination was about 2-fold in IgA-anti-PT and about 7-fold in IgA-anti-FHA.

4. Discussion

The present study was conducted to evaluate the immune response to one dose of reduced antigen content acellular pertussis vaccine in healthy adolescents who had not been previously vaccinated against pertussis, had no history of pertussis and who had anti-PT antibody titres below 20 EU/ml. Although there is no contraindication to vaccinate individuals with incomplete pertussis vaccination history or without a history of pertussis vaccination, available data on booster responses in this age group are limited to subjects who have been previously vaccinated with DTP vaccines. Consequently the study vaccine is currently recommended only to boost the immunity in subjects who have been previously primed with DTP vaccines. This study demonstrates that an immune response of IgG-anti-PT was induced in 88.6% of previously unvaccinated German adolescents. This is substantiated by the finding that 53.7% of vaccinees already showed a response 5–12 days after vaccination. Of the 78 primary seronegative subjects 84.6% had seroconverted 29–49 days after receiving a reduced dose of pertussis antigens.

The results obtained in this study cohort were similar to the responses observed in another study with previously vaccinated adolescents [10]. In that study, 92.1% of adolescents demonstrated an immune response in terms of anti-PT titres when they were given a dose of Tdap vaccine after being primed with the whole-cell pertussis vaccine (DTwP) at 3, 4, 5 and 24 months age. Other studies using this vaccine in adult populations again produced very similar overall results [11,12].

Regarding the response to the other pertussis components of the vaccine, all vaccinees had showed a response to at least one antigen. In this population of unvaccinated adolescents with no history of pertussis, a previous contact to pertussis antigens could be assumed by the finding that 91.9% of the study population had detectable IgG-anti-FHA before vaccination. Again, this is similar to the seroepidemiology of a vaccinated population [1]. The seropositivity rates in a study in which the same vaccine was given to 10–13-year old adolescents after they had been primed with a Finnish whole-cell vaccine, were 100, 94 and 90% for PT, FHA and PRN, respectively, and similar rates were found here with 90, 100, and 98%, respectively [13].

Table 2
Immune response rate (IgG) to three pertussis antigens

	Antibody	N	Day 5–12 % (95% CI)	Day 29–49 % (95% CI)
All subjects	Anti-PT	123	53.7 (44.4–62.7)	88.6 (81.6–93.6)
	Anti-FHA	123	67.5 (58.4–75.6)	91.9 (85.6–96.0)
	Anti-PRN	122	63.1 (53.9–71.7)	96.7 (91.9–99.1)
Subjects seropositive at pre-vaccination	Anti-PT	45	71.1 (55.7–83.6)	95.6 (84.9–99.5)
	Anti-FHA	113	66.4 (56.9–75.0)	91.2 (84.3–95.7)
	Anti-PRN	52	67.3 (51.7–78.5)	96.2 (87.0–99.5)
Subjects seronegative at pre-vaccination	Anti-PT	78	43.6 (32.4–55.3)	84.6 (74.7–91.8)
	Anti-FHA	10	80.0 (44.4–97.5)	100 (69.1–100)
	Anti-PRN	70	60.0 (47.6–71.5)	97.1 (90.0–99.7)

Definition of immune response and seropositivity see text.

Table 3
Immune response rate to pertussis antigens in different age groups

	Antibody	IgG % response (5–95% CI)	IgA % response (5–95% CI)
Subjects aged <13 years (n = 53)	Anti-PT	73.6 (59.7–84.7)	15.1 (6.7–27.6)
	Anti-FHA	92.5 (81.8–97.9)	52.8 (38.6–66.7)
	Anti-PRN	86.8 (74.7–94.5)	n.a.
Subjects aged >13 years (n = 70)	Anti-PT	82.9 (72.0–90.8)	12.9 (6.1–23.)
	Anti-FHA	90.0 (80.5–95.9)	68.6 (56.4–79.1)
	Anti-PRN	92.1 (84.1–97.6)	n.a.

Definition of immune response see text n.a.: not analysed.

A recently published paper describing a vaccine study in adults also measured IgA-antibodies to pertussis antigens in vaccinated adult populations after an injection of one dose of an acellular monocomponent pertussis vaccine with the same antigen content [14]. The concentration of IgA-antibodies found was again similar to the seroconversion rate and level of IgA-anti-PT and IgA-anti FHA observed here.

Our current study attempted to select a study population, which was as negative as possible with regard to pertussis immunity. Our results show that even in this adolescent population a sufficient immune response can be generated after a single dose of reduced antigen content acellular pertussis vaccine. As the reverse cumulative distribution (Fig. 1) of the antibody levels induced against all three antigens by this single immunization look very similar to those recorded after a three dose primary immunization in infants with acellular vaccines [15], it can be postulated that the protection against pertussis might also be similar to the degree of protection observed in the vaccine efficacy studies [16].

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