Immunity to Pertussis 5 Years after Booster Immunization during Adolescence

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(See the editorial commentary by Cherry on pages 1278–9)

Background. We conducted a 5-year follow-up study on the persistence of pertussis-specific antibody and cell-mediated immunity after booster immunization of adolescents aged 11–13 years with a tricomponent acellular pertussis vaccine (Boostrix; trials diphtheria-tetanus–acellular pertussis [Tdap]–004/030).

Methods. Cellular and humoral immunity to pertussis toxin (PT), filamentous hemagglutinin, and pertactin were measured in adolescents (age, 16 years) 5 years after booster immunization. Similar investigations were performed for control adolescents who had received only diphtheria and tetanus booster vaccination.

Results. Five years after pertussis booster vaccination, the geometric mean concentrations of immunoglobulin G (IgG) elicited by each of the 3 pertussis vaccine antigens decreased from 1-month and 3-year postvaccination levels, but with the exception of PT IgG, were still higher than the prevaccination levels. PT IgG levels were undetectable in 28% of the subjects, but 44% of those subjects still tested positive for cell-mediated immunity to PT. Filamentous hemagglutinin IgG and pertactin IgG levels were significantly higher in Tdap-boosted adolescents than in the control subjects. Antibody concentrations at 1 month after vaccination strongly predicted antibody persistence. Cell-mediated immunity levels to PT, filamentous hemagglutinin, and pertactin persisted above the prebooster levels measured 5 years earlier.

Conclusions. The results of the present study of adolescents indicate that the interval between acellular pertussis booster immunizations might be extended beyond 5 years.

Reemergence of pertussis among highly immunized populations has been reported in industrialized countries [1, 2], raising concern about the effectiveness of current vaccination strategies. Vaccine- and disease-induced immunity wane over time without boosting [3–6]. As a result, adolescents and adults are susceptible to pertussis [5, 7, 8] and potentially serve as sources of transmission to infants [9–12] who are at highest risk for complications [12, 13]. All of these factors favor the rationale for expanding pertussis vaccination programs beyond childhood to improve disease control [14].

Immunizing adolescents and adults against pertussis was not feasible in the past because of the unacceptable reactogenicity of whole-cell vaccines when administered after childhood. The decreased reactogenicity of acellular vaccines allows immunization of older age groups [15–17]. Furthermore, the ability of adult-type, reduced-antigen-content diphtheria-tetanus–acellular pertussis (Tdap) vaccines to induce antibodies to their antigenic constituents is well-established [16–19]. Thus, some countries have already introduced an adolescent booster in their vaccine programs [20, 21]. Although there are a number of publications concerning the immediate immune responses after adolescent or adult vaccination, scant information is available on long-term persistence of vaccine-induced humoral or cellular immunity during adolescence and adulthood.

We previously assessed the immediate humoral and cellular immunity after a Tdap booster in adolescents aged 11–13 years [16]. Three years after vaccination of this cohort, we demonstrated persisting cell-mediated immunity (CMI) and humoral immunity [22]. This article represents the persistence of CMI and humoral
immunity after 5 years of follow-up study of the original vaccinated cohort.

SUBJECTS AND METHODS

Subjects and study design. The present study was conducted among adolescents who were immunized 5 years earlier—when they were 11–13 years of age—with either Tdap (Boostrix, GlaxoSmithKline Biologicals; n = 450) or diphtheria and tetanus (dT) vaccine (n = 60), followed by acellular pertussis vaccine (pa) 1 month later, during a study performed in Turku, Finland, in 1997 [16]. All of the subjects had received 4 doses of whole-cell pertussis vaccine combined with tetanus and diphtheria toxoids (DTP) during their first 2 years of life. The quantities of pertussis antigens in the Tdap vaccine were 8 μg of pertussis toxin (PT), 8 μg of filamentous hemagglutinin (FHA), and 2.5 μg of pertactin (PRN). The first follow-up study was performed 3 years after administration of the Tdap booster, and the results were presented in a previous report [22].

All 510 adolescents in the initial study cohort who still resided in the study area were eligible for the 5-year follow-up study. The subjects were contacted by a letter, and of the 409 subjects who responded, 306 (75%) agreed to be reevaluated. No other pertussis vaccines were given during the follow-up period. During November 2002–February 2003, 303 subjects were enrolled (3 subjects did not attend their appointments). Of the 303 subjects, 267 belonged to the group that had received Tdap vaccine, and 36 were in the group that had received dT and pa vaccines (dT+pa). Seven subjects (6 who had received Tdap vaccine and 1 who had received dT+pa vaccine) were excluded (3 subjects had received an additional dose of dT vaccine, 2 subjects had no blood sample obtained, 1 subject had insufficient amount of blood sample for analysis, and 1 subject had received γ-globulin). All together, 296 subjects (mean age, 16.6 years; range, 15.8–17.9 years; 168 female subjects) were included; 261 had received Tdap vaccine, and 35 had received dT+pa vaccine. The subset of subjects in the 5-year follow-up study cohort was representative of the original cohort, because the prevaccination and 1-month postvaccination antibody levels for the current study cohort were similar to the levels observed for the Tdap vaccine recipients not participating in the 5-year study. The mean duration of time from the booster to the present study was 63 months (range, 52–74 months). Because the results of antibodies to pertussis antigens were similar between recipients of Tdap vaccine and dT+pa vaccine (data not shown), only the results for the group that had received Tdap vaccine are reported.

A group of control subjects who were immunized 5 years before our study with a commercial dT booster was included for comparison. These adolescents were chosen from the same cohort that participated as control subjects in the previous 3-year follow-up study. Fifty subjects from the same geographic region were contacted by a letter, of whom 38 subjects (mean age, 16.6 years; range, 16.1–17.2 years; 16 male subjects) agreed to participate. None of the study adolescents or control subjects had received a clinical diagnosis of pertussis during the follow-up years.

The study protocol(s) were approved by the joint commission on ethics of the Turku University and the Turku University Central Hospital, and written, informed consent was obtained from the study subject and the parent/guardian prior to enrollment. For each subject, the follow-up examination consisted of 1 study visit at which a blood sample was obtained. This study was conducted in accordance with the Somerset West, 1996 version of the Declaration of Helsinki.

Laboratory assays. CMI was evaluated in every subject previously studied for CMI and, in addition, (randomly) in every fifth participant. CMI assays were performed at the Pertussis Reference Laboratory, National Public Health Institute in Turku, according to previously described procedures [16, 23]. For the evaluation of CMI, blood samples were obtained and processed within 3 h. Triplicate cultures of a suspension of fresh peripheral blood mononuclear cells (10^5 cells/well) were incubated with 1 μg of heat-inactivated PT, 1 μg of FHA, or 2.5 μg of PRN. Pokeweed mitogen was used for positive mitogenic control, and RPMI 1640 medium alone was used for negative control. After 5 days of incubation at 37°C in an atmosphere with 5% CO₂, ³H-thymidine (0.5 μCi/well) was added to each well, and the incorporation of radioactivity was measured 16 h later. Results are expressed as mean counts per minute for triplicate cultures. As a criterion for cell reactivity, the proliferative response to pokeweed mitogen had to be >1000 counts/min. A stimulation index was used for final expression of CMI results. A CMI response was defined positive when the antigen-induced proliferation was >4-fold higher than the spontaneous proliferation (stimulation index, >4).

IgG antibodies to PT, FHA, and PRN and antibodies to diphtheria and tetanus toxoids were measured by ELISA at the laboratory of GlaxoSmithKline Biologicals (Rixensart, Belgium). The assay cutoff for pertussis antibodies was 5 IU/mL, and the cutoff for diphtheria and tetanus antibodies was 0.1 IU/mL. Serum samples with an ELISA antipertactin antibody level <0.1 IU/mL were retested using an in vitro neutralization assay on Vero cells with a cutoff of 0.016 IU/mL.

Statistical analysis. Statistical analyses were performed using SPSS for Windows. Serological results that were lower than the assay cutoff were assigned an arbitrary value of one-half the assay cutoff. Calculations of geometric mean antibody concentrations (GMC) and stimulation indexes were performed on log_{10}-transformed data, reposting the antilogarithm. Comparisons between geometric means were determined by the Wilcoxon rank sum test. Analysis of correlation was performed using the Spearman rank correlation coefficient. Comparisons
manifesting a 2-tailed $P$ value $<.05$ were considered to be statistically significant.

**RESULTS**

**Antibody persistence.** Table 1 shows the percentages of adolescents with detectable antibodies and GMCS of IgG to PT, FHA, and PRN before receipt of Tdap booster and at various time points after vaccination. Although IgG antibody levels to pertussis antigens decreased as time elapsed after the booster, the GMCS of FHA IgG and PRN IgG continued to be significantly higher, compared with those measured before Tdap booster ($P<.001$), whereas PT IgG approached prevaccination significantly higher, compared with those measured before Tdap booster ($P<.001$), whereas PT IgG approached prevaccination levels. GMCS of IgG to PT, FHA, and PRN were 1.2-fold, 2.1-fold, and 4.3-fold greater after 5 years, respectively, compared with the prevaccination levels. Overall, antipertussis IgG antibodies decreased only moderately since the previous follow-up study that was 3 years after vaccination (table 1). For 95% of the subjects, the observed PT IgG level was less than that measured at 1 month after vaccination, and the mean decrease in the PT IgG level was 88% (range, 20%–99%). Similarly, the mean decreases in FHA IgG and PRN IgG levels were 84% (range, 26%–96%) and 85% (range, 10%–98%), respectively. During the period of time from 3 to 5 years after vaccination, the mean reductions in PT IgG, FHA IgG, and PRN-IgG levels were 36% (range, 3%–78%), 33% (range, 2%–70%), and 34% (range, 3%–84%), respectively.

Five years after receiving the booster, all subjects had detectable FHA IgG, and 98% had detectable PRN IgG. Only 2 adolescents (0.8%) had undetectable antibodies to both PT and PRN. Of 253 subjects with available results, 193 (76%) had detectable PT IgG (table 1). Those 60 subjects who were seronegative for PT IgG at the 5-year follow-up study had a significantly lower PT IgG level 1 month after receiving the booster ($P<.001$), compared with those with detectable PT IgG. A significant correlation was found between antibodies obtained 1 month after vaccination and 5 years later (Spearman correlation coefficients, 0.795, 0.707, and 0.845 for PT, FHA, and PRN, respectively; $P<.001$) (figure 1).

We further examined the relationship between the prebooster prevalence of antibody and postvaccination and follow-up antibody concentrations and found that subjects with detectable prebooster antibodies to PT or PRN had higher concentrations at both 1 month and 5 years after vaccination, compared with subjects with undetectable prebooster antibodies.

Among control subjects, detectable antibodies to PT, FHA, and PRN at the second follow-up visit were observed in 61%, 97%, and 87% of subjects, respectively (table 1). Although the proportions of subjects with detectable IgG to PT, FHA, and PRN did not differ between recipients of Tdap vaccine and control subjects, the recipients of Tdap vaccine had significantly higher antibody levels to FHA and PRN, compared with the control subjects ($P<.001$ for both). No significant difference in PT IgG levels was observed between the groups ($P = .852$).

A $\geq$2-fold increase in the concentration of PT IgG, FHA IgG, or PRN IgG was observed between the 3- and 5-year follow-up studies in 4 (2.2%) of 184, 1 (0.5%) of 190, and 3 (1.6%) of 192 Tdap vaccine recipients, respectively. Only 1 subject had a clear increase in IgG concentration to all 3 pertussis antigens. A $\geq$2-fold increase in PRN IgG concentration was seen in 4 (14.3%) of 28 control subjects, whereas corresponding increases in PT IgG and FHA IgG concentrations were each seen in 1 of 27 control subjects. Significantly fewer Tdap vaccine recipients than control subjects experienced a $\geq$2-fold increase in PRN IgG concentration between year 3 and year 5 ($P = .006$). In the control group, 1 subject had a $\geq$2-fold increase in the concentration of IgG antibodies to every antigen. This subject also experienced increases in CMI to all pertussis antigens.

At the 5-year follow-up study, all of the Tdap vaccine recipients sustained seroprotective levels of antitetanus antibodies, and 99.2% of the subjects continued to have seroprotective antidiphtheria antibodies revealed by ELISA or neutralization assay on Vero cells.

**CMI.** CMI to any of the 3 pertussis antigens was detected in the majority (79%) of Tdap-boosted adolescents 5 years after vaccination (table 2). Five years after booster, CMI positivity rates among Tdap vaccine recipients for PT, FHA, and PRN were 57%, 69%, and 52%, respectively. The geometric mean values of stimulation indexes against pertussis antigens were significantly higher, compared with the prevaccination levels ($P = .045$ for PT and $P<.001$ for FHA and PRN). Although the geometric mean values were significantly lower, compared with 1-month postvaccination levels, they did not differ from geometric mean values observed at the follow-up study visit that occurred 2 years previously.

Of the 120 Tdap vaccine recipients who had both CMI and IgG antibodies tested 5 years after receiving the vaccine, 34 subjects (28%) had undetectable antibodies to PT. Despite having undetectable PT IgG, 44% of the subjects had positive proliferative responses to PT. This figure does not differ significantly from the PT CMI positivity rate of 63% that was observed in subjects who had detectable PT IgG at the 5-year follow-up study visit.

Among the 37 control subjects, the CMI positivity rates to PT, FHA, and PRN at the 5-year follow-up study were 57%, 51%, and 51%, respectively (table 2). Although these positivity rates did not differ from those of the Tdap vaccine recipients, the quantitative CMI to FHA was significantly higher in Tdap vaccine recipients than in control subjects ($P = .019$), whereas CMI to PT and PRN were similar in both groups. Among the control subjects, 8.1% had both undetectable PRN IgG and...
Table 1. The rates of antibody detection and geometric mean antibody concentrations (GMCs) of IgG to pertussis antigens at different time points of evaluation.

<table>
<thead>
<tr>
<th>Group, time of evaluation</th>
<th>PT IgG</th>
<th>FHA IgG</th>
<th>PRN IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects with available serologic results</td>
<td>Percentage of subjects with a PT IgG level ≥5 IU/mL</td>
<td>GMC (95% CI)</td>
</tr>
<tr>
<td>Tdap vaccine recipients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before immunization</td>
<td>437</td>
<td>54</td>
<td>9.7 (8.4–11.2)</td>
</tr>
<tr>
<td>1 month after immunization</td>
<td>439</td>
<td>98</td>
<td>117.5 (105.3–131.1)</td>
</tr>
<tr>
<td>3 years after immunization</td>
<td>258</td>
<td>82</td>
<td>16.0 (13.7–18.7)</td>
</tr>
<tr>
<td>5 years after immunization</td>
<td>253</td>
<td>76</td>
<td>11.7 (10.2–13.4)</td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st follow-up</td>
<td>39</td>
<td>62</td>
<td>9.7 (8.0–15.6)</td>
</tr>
<tr>
<td>2nd follow-up</td>
<td>36</td>
<td>61</td>
<td>11.5 (7.0–18.8)</td>
</tr>
</tbody>
</table>

NOTE. FHA, filamentous hemagglutinin; PRN, pertactin; PT, pertussis toxin.
negative CMI to PRN, whereas only 0.8% of Tdap vaccine recipients lacked both cellular and humoral immunity to PRN.

Five years after receiving the booster, 48 (38%) of 126 Tdap vaccine recipients tested positive for CMI to all 3 pertussis antigens. This was comparable to the corresponding figure (35%) among the control subjects. The proportion of CMI negativity to all pertussis antigens varied from 21%, among Tdap vaccine recipients, to 27%, among control subjects ($P = .41$). We did not detect any clear correlation between pertussis antibodies and CMI responses 5 years after receipt of the booster.

**DISCUSSION**

In the present study, the prolonged follow-up of a cohort that was enrolled in a previous Tdap vaccination trial [16, 22] allowed us to assess the persistence of immune responses after adolescent Tdap immunization over a mean follow-up period of 63 months. Instead of using antibodies as a sole marker for vaccine-induced immune persistence, we also studied the long-term persistence of CMI. Furthermore, we compared these follow-up findings with available data on a similarly aged cohort of adolescents who had not received a pertussis booster at 11 years of age. Therefore, the results of our study can be viewed from 2 different perspectives: a study of persistence of both CMI and humoral immunity that was induced by a recently licensed booster vaccine and a study comparing long-term immunity in Tdap-boosted and unboosted adolescents.

According to the present study, antibodies to FHA and PRN were well preserved for a mean period of 63 months following Tdap booster. Several reports of the duration of immunity after acellular pertussis primary vaccination are available [24–27]. In these studies, the conclusions about the duration of immunity were derived from antibody levels measured before the first booster dose. Antibody levels substantially decreased during the years after primary immunization and generally were at low levels before the preschool booster [28, 29]. Thereby, the duration of humoral immunity after the adolescent booster is comparable to that achieved with primary immunization.

McIntyre et al. [30] assessed an adult population in Australia 3 years after booster immunization with the same vaccine used in the current study, and they found that all of the subjects remained seropositive for FHA antibodies and that >90% of adults remained seropositive for antibodies to PT and PRN. Compared with the results we observed in adolescents, antibodies to PRN and FHA showed similar persistence in both studies, although detection rates of PT IgG were lower in adolescents, even when compared with the first follow-up visit, which was 3 years after vaccination. It should be kept in mind that the differences observed in PT IgG may be a result of a different circulation of *Bordetella pertussis* between the 2 countries. Our results correspond with those of the Adult Pertussis Trial conducted in the United States in which pertussis antibody persistence was observed for 18 months after booster vaccination of adolescents and adults with a monovalent acellular pertussis vaccine with the same pertussis components and antigen amounts as the Tdap vaccine used in the present study [31]. In the Adult Pertussis Trial, 65% of subjects had PT IgG levels greater than the limit of quantitation, whereas antibody levels to FHA and PRN were preserved at greater than that threshold in 99% and 89% of subjects, respectively. Furthermore, it seems that the duration of immunity after pertussis...
booster vaccination is not substantially different from that after infection in adults [32].

The rate of decrease of antibody levels appears different among antigens, with PT showing the most rapid decrease. Similarly rapid reductions in PT IgG levels have been documented after pertussis infection [32, 33]. Interestingly, our study revealed that the PT IgG level achieved 1 month after booster vaccination with the combined Tdap vaccine was strongly predictive of persistence of immunity.

Protection against pertussis is mediated by both humoral and cellular immunity. In the present study, 28% of Tdap vaccine recipients had lost measurable PT IgG 5 years after the booster. Of the subjects without PT IgG, 44% had maintained CMI to PT. These subjects could still be protected against pertussis because of the persistence of CMI and antibodies to other pertussis antigens. Therefore, both types of immune response after vaccination should be evaluated, especially when the mechanism of protection is not completely understood, and because the kinetics of both responses seem to differ.

The detection rate of FHA IgG remained high. This is in agreement with the findings of Grimprel et al. [34], showing longer persistence of anti-FHA than other pertussis antibodies after whole-cell immunization. The possibility that FHA antibodies observed in this study were boosted by immunologically cross-reactive antigens cannot be ruled out as originally suggested. It is known that antibodies to FHA and PRN can be induced by infections with other *Bordetella* species [35, 36] and that FHA antibodies may also be the result of cross-reacting antibodies to *Mycoplasma pneumoniae* and other unidentified agents [36]. In the present study, there were only 2 vaccine recipients with detectable FHA IgG who were without PT IgG or PRN IgG.

Although the level of PRN IgG was lower in control subjects than in Tdap vaccine recipients, control subjects possessed equal stimulation indexes against PT and PRN. The similarity in CMI detection between Tdap vaccine recipients and control subjects in the present study might indicate evidence of exposure that would naturally increase the CMI in the adolescents who did not receive a pertussis booster. It will be of interest to study further whether the subclinical exposures are the major mechanism for preserving CMI to pertussis.

Acellular pertussis vaccine protects against pertussis in adolescents and adults [37]. The combination of pertussis antigens with dT vaccine does not impair antibody responses against these toxoids [18, 38], although the Tdap vaccine tested in the study by Halperin et al. [18] was different from that used in the present study. To reduce morbidity due to pertussis in adolescents, it therefore seems to be preferable to offer the Tdap vaccine as a substitute for the routinely recommended dT booster.

Vaccination of adolescents, in whom the largest increases in disease incidence have been documented [39, 40], is clearly a step towards enhanced control of pertussis. A crucial question remains as to how long the interval between pertussis booster immunizations should be extended. This is, to our knowledge, the first long-term follow-up study that evaluated CMI, in addition to serological responses, after administration of the Tdap booster to adolescents. Pertussis-specific immunity persisted for 5 years after receipt of the booster in the majority of vaccinated adolescents. Antibodies to pertussis antigens were maintained at greater than preimmunization levels and generally exceeded levels in unboosted adolescents. Similarly, 5 years after receipt of the booster, pertussis-specific CMI levels were higher in vaccinated subjects, compared with preimmunization levels. The results of the present study, therefore, indicate that the interval between routine acellular pertussis booster immunizations might be extended beyond 5 years.

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