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Frequent failure of adolescent booster responses to tetanus toxoid despite infant immunization: Waning of infancy-induced immune memory?

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ABSTRACT

To define the capacity of a tetanus toxoid booster to reactivate infant-triggered immunity, anti-tetanus antibodies were assessed before and after boosting 162 adolescents and 219 children from Mfou (Cameroon). Among 63 adolescents with 3 recorded dose of infant DTP, 29/63 (46%) responded with a ≥4-fold increase of antibody titers, 35/63 (55%) reaching the 0.10 UI/ml threshold. Response rates were slightly higher (62%) in children aged 10–11 years. Responders and non-responders only differed significantly in their baseline anti-tetanus antibodies. Thus, early life immune immaturity may limit the persistence of infant-induced immunity and subsequent boosters may be required for sustained protection.

1. Introduction

Tetanus remains an important public health problem, with a total number of deaths estimated at 213,000 cases in 2002 [1]. Although most deaths occur among neonates or their mothers, tetanus may occur following injuries at any age [1]. Protection relies upon the presence of neutralizing IgG antibodies, which are readily elicited by a few doses of detoxified tetanus toxin (tetanus toxoid, TT). The main goals of tetanus control have been to eliminate maternal and neonatal tetanus through maternal immunization and to achieve a high coverage of 3 infant doses of tetanus toxoid-containing vaccines, usually given in combination with diphtheria and whole-cell pertussis vaccines (DTwP) [1]. Infant DTwP has been part of WHO's Expanded Programme on Immunization (EPI) since its inception in 1974 [1]. In contrast, tetanus boosters have only recently been officially recommended [1] and limited resources have delayed their implementation in a majority of developing countries.

Consequently, millions of adolescent and young adults were only immunized against TT in infancy and should have their immunity reactivated. A single booster is currently considered as sufficient to reactivate TT immunity even several decades after the last dose [1]. However, evidence is lacking that this recommendation applies to immune memory elicited in infancy as well as later in life. To generate evidence in support of this single adolescent booster recommendation, we designed a field study in the health district of Mfou (Cameroon), where EPI was implemented in 1981. In Mfou, TT has been recommended at 6, 10 and 14 weeks and available free of charge for infants in public and nongovernmental health centers for the last 25 years [2]. Childhood boosters have only been available through private providers who generally charge for immunizations, such that most have not received such booster. We assessed the capacity of one TT booster to elicit anamnestic anti-TT antibodies in primary and secondary school students primed in infancy.

2. Materials and methods

2.1. Study population

This study was approved by the National Ethical Committee and the Ministry of Secondary Education of Cameroon, and by the Ethical Committee of Children Action. Students were recruited in January 2008 in 6 schools and 13 classes (9 classes in 4 primary schools, 4 classes in 2 secondary schools) of the district of Mfou (Cameroon). Students were eligible if they provided an immunization record and written parental consent. Financial incentives were not given. Exclusion criteria included children who were sick at the

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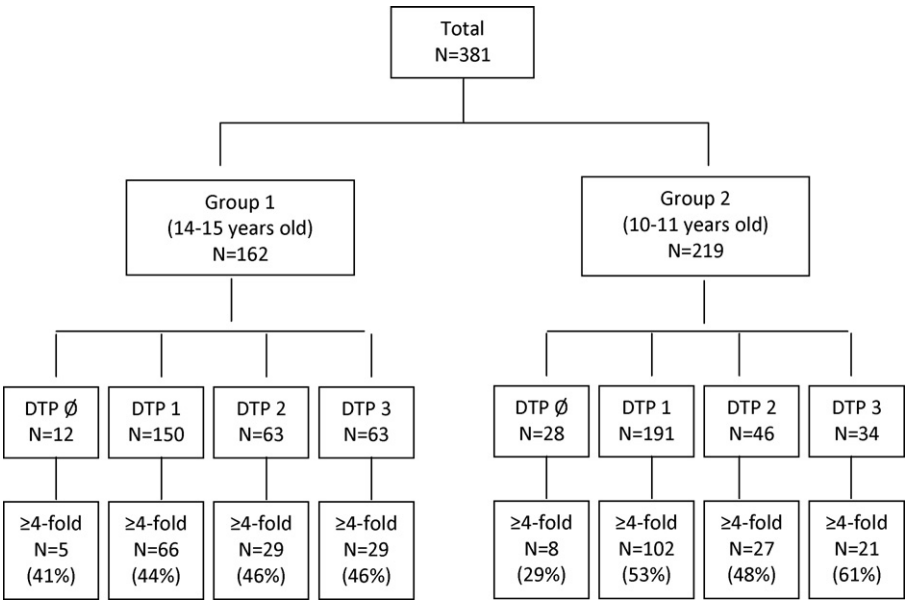

Fig. 1. Distribution of study participants.

time of the study (chronic fever with weight loss, cough, sickle cell children in crisis, and children outside of the 2 targeted age groups (14–15 years, Group 1; 10–11 years, Group 2).

We attempted to enroll at least 150 students per group, calculating that if 60% of Group 1 and 80% of Group 2 had residual anti-TT immune memory, this difference of 20%, with an alpha of .05, would allow the detection of a significant difference among the groups with a power >90%. We accounted for a maximal loss of 10% given the short duration of the study, agreeing to exceed this number to avoid declining participation within a class. An oral interview collected information from the students on their recall of previous severe illnesses (including episodes of tetanus, malaria, meningitis, pulmonary infections, typhoid fever, sickle cell anemia and allergy), hospitalizations and surgical interventions—to identify potentially immunodeficient children. Immunization records were assessed by trained field nurses who collected dates of recorded BCG and any DTP immunization. According to local practices of record keeping, all recorded immunizations were considered as having been administered. All students had received WHO prequalified aluminium-adsorbed tetanus toxoid vaccines.

2.2. Blood sampling and immunization

Dried whole blood adsorbed on filter paper was collected for the determination of anti-TT antibodies, as previously validated [3,4]. At the first visit, fingers were cleansed with alcohol, allowed to dry and punctured with a capillary lancet (*BD Microtainer® Contact-Activated lancet*, *REF*: 366593). Four 4 separate drops of blood were collected on Whatman's 903 Protein Saver Card (Ref No. 10 531 018), air dried at ambient temperature for at least 3 h and stored in individual plastic zip lock bags prior to shipment to the laboratory [5]. Participants were then given a single dose of WHO prequalified aluminium-adsorbed tetanus toxoid vaccine containing ≥5 Lf tetanus toxoid (*Serum Institute of India, Lot No. EU10702-C*) by intramuscular injection in the deltoid. To discriminate between primary and secondary anti-TT responses [6,7], participants were invited to return 7 days later for repeat blood sampling.

2.3. Serological analyses

A 1-hole puncher was used to take 6.0 mm circles from the center of 4 spots, standardizing the volume of blood [5]. Two circles were added in a flat-bottomed 96-well microtiter plate (NUNC MaxiSorp™) with 150 μl of PBS-Tween 0.5% elution buffer, as described [8]. Blank filter papers were used as controls. Plates were shaken during 10 min at room temperature and incubated overnight at 4 °C. Eluates were transferred to Eppendorf tubes after a brief shaking of the plates, and used at 8-fold serial dilutions starting at 1:5. The determination of anti-TT IgG was performed by indirect ELISA using TT-coated plates (sanofi pasteur), as described [9]. Briefly, the incubation of serum samples was followed by successive additions of biotinylated goat anti-human IgG (Sigma Chemicals), streptavidine–peroxidase (Zymed) and ABTS substrate. Positive and negative controls were included on each plates. Ab concentrations were calculated with the Softmax PRO software (Molecular Devices, Sunnyvale, CA) by comparison with standard curves (4-parameter fitting) using WHO calibrated international standards of reference. The cut-off of this assay, in routine use in our laboratory, is 0.01 UI/ml. Samples with titers below this cut-off were arbitrarily given a value of 0.005 UI/ml to allow calculation of geometric mean titers (GMTs). Preliminary experiments compared anti-TT serum titers in venous and capillary blood samples simultaneously harvested from a panel of adult volunteers, confirming the direct correlation of TT antibody concentrations and defining the diameter–volume correction factor as corresponding to a 2.5 pre-dilution (not shown).

2.4. Definitions

A threshold of 0.01 UI/ml was used to define seropositivity against TT. As ELISA assays overestimate antibody concentrations of low-titer sera [10] and ELISA titers ≥0.10 UI/ml best correlate with neutralizing activity [1,11], seroprotection was defined by antibody concentrations ≥0.10 UI/ml. In seronegative individuals, an anamnestic response was defined by reaching a post-immunization titer ≥0.10 UI/ml, which is reached by > 98% infants at the end of a primary immunization series. For seropositive participants, a ≥4-fold increase in IgG anti-tetanus antibodies was defined as evidence of an anamnestic response. A ≥2-fold increase was defined as suggestive of anamnestic responses, taking into account the short delay (7 days) between boosting and bleeding.

2.5. Statistical analyses

Standard descriptive statistics were used to describe socio-demographic characteristics. Comparison between different serologies was performed using Student's *t*-test, while categorical data were compared using chi-square tests or Fisher's exact test when appropriate ($n < 5$ in any cell of the 2×2 table). Serologies were compared as GMTs. ANOVA was used to examine continuous variables. Logistic regression analysis was used to assess the proportion of vaccine responders, controlling for any significant demographic variables that might function as confounders (gender, age group, etc.). All variables were examined at univariate level. After this, all the variables with a *P*-value <.25 by univariate analysis were included in the multivariate model.

For all statistical tests, a two-sided *P*-value <.05 was considered statistically significant or when the 95% confidence interval (CI) did not include 1.0. SPSS (version 15.0, Chicago, IL) statistical-software program was used for analyses.

3. Results

3.1. Study population

A total of 381 healthy students were enrolled (Fig. 1), including 211 girls (55.4%) and 170 boys (44.6%) similarly distributed in the 2 age groups. When interviewed, 268 (70%) did not recall having been hospitalized and 16 (4.2%) mentioned a surgical intervention. Episodes of malaria were mentioned by 346 (90.8%), pulmonary infections by 14 (3.7%), typhoid fever by 22 (5.8%) and meningitis by 2 (0.5%) students. None recalled having suffered from tetanus. These figures were similar in both groups and no event suggestive of immune deficiency or immunosuppression was identified. Immunization records indicated that 341/381 (89.5%) students had at least one recorded dose of infant DTP (Fig. 1). All had been immunized with BCG. Appropriate blood samples were provided by all but 7 students (Group 1: 4, Group 2: 3) who were excluded from immunogenicity analyses.

3.2. Anamnestic anti-tetanus antibody responses

3.2.1. Anamnestic anti-tetanus responses in 14–15-year-old adolescents

Among Group 1 adolescents, 63/162 (38.9%) had 3 recorded doses of infant DTP (Fig. 1) and provided appropriate blood samples, constituting the primary cohort (Gr1V3) of our analyses. Baseline anti-TT antibodies were low (GMT 0.019 UI/ml, 95% 0.012–0.026) and most (58/63, 92.1%) adolescents had very low (<0.1 UI/ml) anti-TT concentrations. One week after a single dose of aluminium-adsorbed TT vaccine, their mean anti-TT concentrations had increased by 11.31-fold, as expected for anamnestic responses. However, post-booster GMT remained low (0.086 UI/ml, 95% CI 0–0.195). Only 35/63 (55%) adolescents reached the 0.10 UI/ml threshold and 29/63 (46%) responded with a ≥4-fold increase of their anti-TT concentrations (responders). These responses followed a bimodal distribution, 24/63 (38.1%) adolescents failing to raise any significant anti-TT response (<2-fold, Gr1V3neg) (Fig. 2A). Responders and non-responders did not significantly differ in their clinical characteristics, as defined by the oral interviews.

A substantial proportion of Group 1 adolescents (86/162, 53%) had only 1 recorded dose of infant DTP (Gr1V1). Their anti-TT titers were low at baseline (Table 1) and most (81/86, 94%) had very low (<0.10 UI/l) titers. One week after boosting their anti-TT GMTs were similar to those of Gr1V3 students: 41/86 (47.7%) sera were ≥0.10 UI/ml and 37/86 (43%) adolescents had responded by a ≥4-fold increase of anti-TT concentration. A bimodal distribution of the booster responses was again observed (Fig. 2A). Comparing adolescents with at least 3 or only 1 recorded dose of infant DTP (Table 1) indicated similar anti-TT titers. Thus, fewer than 50% adolescents retained sufficient anti-TT immune memory cells to rapidly elicit anamnestic responses, regardless of the number of recorded doses of infant DTP.

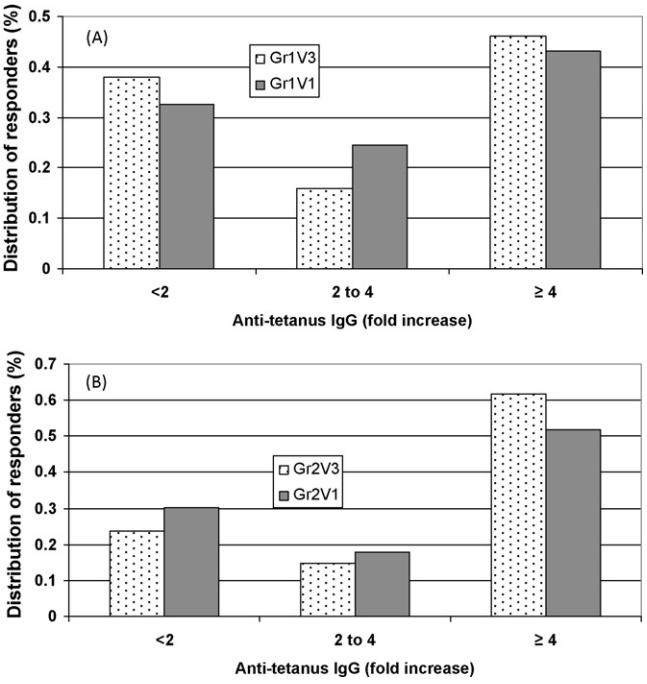

Fig. 2. (A) Distribution of vaccine responses among Group 1 adolescents. (B) Distribution of vaccine responses among Group 2 students.

3.2.2. Anamnestic anti-tetanus responses in 10–11-year-old children

We next asked whether anti-TT immune memory was better maintained in younger (10–11 years, Group 2) students. In this cohort, only 34/219 (15.5%) had 3 recorded doses of infant DTP (Fig. 1). In these Gr2V3 students, baseline anti-TT were low (Table 1) and 33/34 (97%) had anti-TT < 0.10 UI/l. Post-booster GMTs were not significantly higher than in Gr1V3 adolescents (0.114 UI/ml, 95% CI 0.005–0.248). The proportion of responders (≥4-fold increase) was higher (21/34, 62%), but not significantly than among Gr1V3 adolescents (29/63, 46%). One week after boosting, 18/34 (53%) had anti-TT concentrations above 0.10 UI/ml. Among 145 Group 2 students with only 1 recorded dose of infant DTP (Gr2V1), anti-TT titers were similarly low at baseline (Table 1). One week after boosting, only 65/145 (44.8%) sera were ≥0.10 UI/ml and 75/145 (51.7%) children had responded by a ≥4-fold increase of anti-TT concentration. Again, a bimodal pattern of response was observed (Fig. 2) and responders and non-responders did not significantly differ in their clinical characteristics. Thus, younger students did not respond significantly better than adolescents.

3.3. Determinant of the persistence of anti-TT immunity

In the absence of age-associated differences, the results of both age groups were pooled to assess the main determinants of the long-term persistence of anti-TT immunity. Thirteen students had baseline anti-TT antibodies (>0.10 UI/l). After boosting, anti-TT antibodies increased ≥4-fold in 9/13 (69%). The rate of anamnestic responses was similar (77.8%) among students with anti-TT antibodies between 0.05 and 0.10 UI/l prior to boosting but significantly

Table 1
Anti-TT titers at baseline and after a booster dose.

	N[a]	Baseline, UI/ml (SD)	Post-booster, UI/ml (SD)	P-value[b]
Group 1 (all)	149	0.034 (0.067)	0.269 (0.466)	<.001
Gr1V3	63	0.025 (0.027)	0.263 (0.44)	<.001
Gr1V1	86	0.041 (0.084)	0.273 (0.486)	<.001
Group 2 (all)	179	0.026 (0.036)	0.367 (0.748)	<.001
Gr2V3	34	0.021 (0.019)	0.309 (0.396)	<.001
Gr2V1	145	0.027 (0.038)	0.380 (0.809)	<.001

SD: standard deviation; CI: confidence interval.
[a] Number of participants with valid samples.
[b] Baseline vs. post-booster.

Table 2
Proportion of participants with anamnestic responses (≥4-fold) according to baseline antibodies.

	Responders (n, %)	Responders (n, %)	Responders (n, %)
Baseline titers	<0.02 UI/l	0.02–0.05 UI/ml	≥0.05 UI/ml
Group 1: 14–15 years	26/76 (34.2%)	30/56 (53.6%)	10/17 (58.8%)*
Group 2: 10–11 years	35/95 (36.8%)	48/71 (67.6%)	13/13 (100%)°
Total	61/171 (35.7%)	78/127 (61.4%)	23/30 (76.7%)*

* $P < .001$ comparing response rates in participants with antibodies <0.02 UI/ml or ≥0.05 UI/ml at baseline.
° $P = .038$ comparing response rates in participants with antibodies <0.02 UI/ml or ≥0.05 UI/ml at baseline.

lower (47.7%, $P = .012$) in students with baseline antibodies below 0.05 UI/l (Table 2).

Multivariate analyses indicated that the proportion of vaccine responders (≥4-fold) was only significantly influenced by baseline antibody levels and not by age group or number of recorded doses of infant DTP.

4. Discussion

Antitoxin concentrations depend upon the magnitude of the primary responses and the time elapsed since the last immunization. To observe low anti-TT antibody levels in most adolescents primed with DTP in infancy was not unexpected [3,12–14]. In contrast to the long-term persistence of tetanus antibodies in adults or older children, the duration of immunity after a 3-dose primary infant vaccination was calculated to be 5 years (upper 95% confidence limit of estimated risk of serum antitoxin concentration below 0.01 IU/ml still <0.1%) [14]. Two studies in Tanzania and Nigeria have identified a higher proportion of children with detectable serum anti-TT antibodies [12,13]. Whether this reflects differences in age, ethnic groups or the unidentified administration of childhood boosters is unknown. Indeed, the number of vaccine doses controls anti-TT GMC such that anti-TT antibodies may persist for more than 30 years in adults who had received a childhood booster, even in the absence of adult boosters [15,16].

In contrast, we did not expect that such a large proportion of infant-primed adolescents would fail to raise anamnestic responses to a TT booster. Tetanus toxoid is a potent immunogen: almost all vaccinees reach antibody titers >0.10 UI/ml after 3 infant doses [1] and anti-TT anamnestic responses are best defined by the rapid induction of antibody titers >0.10 UI/ml, and/or by a greater than 4-fold increase of baseline antibodies. In our cohort, half of the participants did not generate anamnestic responses after boosting. Even among the younger participants with 3 recorded doses of infant DTP and who were still seropositive at baseline, only 60% raised anamnestic responses. Remarkably, the only identified determinant of anamnestic responses was to have sufficiently high residual anti-TT antibodies at time of boosting. Anti-TT antibodies at baseline result from the persistence of effector immune memory, i.e. long-lived antibody-secreting plasma cells. That persisting effector immune memory correlates with persisting central memory B cells, and thus boostability, is thus immunologically sound. Whether this reflects the administration of occult childhood boosters (15 students had detectable anti-TT antibodies despite no record of any tetanus-containing vaccine) or the induction of stronger primary infant responses is currently unknown. Similarly, the same response rate observed in children with 3 or only 1 recorded infant DTP may reflect the administration of unrecorded infant doses. We can also not exclude that a larger study would identify additional determinants such as age or number of prior vaccine doses. In any case, immune memory elicited by infant immunization against TT, one of the most potent vaccine antigens, did not persist at a sufficient level to allow its reactivation by a single booster in most 10–15-year-old students.

This conclusion has major policy implications, which requires a careful assessment of potential limitations and confounders. The use of capillary blood to facilitate bleeding in field studies of tetanus immunity was validated long ago [3–5]. Hematocrit may influence the volume of serum recovered, but this is unlikely to change within 1 week, leaving response rates unaffected. The amount of serum may increase by up to 13% as the volume of whole blood applied to a spot increases, but the volume of plasma excised from the center of a filter paper is relatively constant [5] and a 13% variation of antibody concentrations would not affect our conclusions. Last, our ELISA assay is validated and its accuracy periodically checked by internal and external GLP quality controls. Thus, assay-related confounders appear unlikely.

The lack of anamnestic responses in most adolescents did not reflect the use of poorly immunogenic primary or booster vaccines, as anti-TT titers increased by up to 74-fold in some vaccinees (not shown). Immune deficiency was not suggested by the medical history of these healthy students and would only affect a much smaller proportion than that of non-responders. Similarly, the prevalence of HIV/AIDS is estimated as very low in this young adolescent healthy population. Increasing the delay between boosting and blood sampling could possibly recruit additional adolescents among responders—as antibody titers continue to increase between days 7 and 30 after boosting [17]. However, TT is such a potent immunogen that it would have precluded the distinction between primary and memory responses, the main objective of this study. Indeed, 6 days were sufficient for anti-TT serum titers to increase above 0.10 UI/ml in all previously primed adult individ-

uals [7], reflecting the rapid differentiation of anti-TT memory B cells into potent antibody-secreting cells [6]. Anti-TT GMTs indeed increased by 18–20-fold within 1 week in responders, confirming the validity of this 7-day time point. Altogether, this indicates that a significant fraction of healthy adolescents fail to raise anti-TT anamnestic responses despite a 3-dose infant priming.

Why did such a large proportion of participants fail to respond to a TT booster? Some individuals may not have been previously immunized. Among 40 students whose immunization records included no tetanus vaccines (Fig. 1), 5/12 (41%, Group 1) and 8/28 (29%, Group 2) raised their anti-TT antibodies ≥4-fold responses, reflecting the administration of unrecorded vaccines. The proportion of "never immunized" participants is thus lower than 7% in our cohort. Primary vaccine failures could have occurred, but it normally affects fewer than 2–5% infants [18]. The potency of TT vaccines may be affected by improper production [19], but TT vaccines used in Cameroon in 1993–1994 and 1997–1998 were aluminium-adsorbed vaccines prequalified by WHO. Vaccine damage through freezing may not be retrospectively excluded. It is however unlikely that it would have similarly affected vaccine batches used several years apart. The strong recall responses observed in the responders also suggest an adequate quality of vaccines used at priming. Primary anti-TT immune responses could have been limited by the presence of maternal antibodies, but even high titers of maternal antibodies leave infant anti-TT booster responses essentially unaffected [20–22]. Last, neither vitamin A deficiency [23] nor infant malnutrition or malaria was reported to affect TT immunity. Thus, the failure to raise anamnestic anti-TT responses is more likely ascribed to secondary vaccine failure.

The observation that boosting failed to reactivate infant-driven anti-TT immune responses in most adolescents is against the current dogma that immune memory is life-long. It contradicts current official national and international recommendations which state that "booster responses can still be elicited after intervals of 25–30 years, demonstrating the persistence of immunological memory" [1] and that "even after many years, an interrupted schedule should simply be continued with the next dose that is due" [1]. These recommendations were based upon the demonstration that anti-TT immunity may indeed be boosted even decades after primary immunization [7,11,24,25]. However, these conclusions were reached in individuals primed after the age of 2 years [24] or with immunization schedules including a later booster dose. We demonstrate here for the first time that in contrast to immunization later in life, infant immunization in the absence of childhood boosting does not result into a long-term persistence of anti-TT immune memory.

It is now evidenced that infant antibody responses are limited by numerous factors [26,27]. We show here that these limitations do not only affect the induction of antibody-secreting cells but also that of immune memory. This conclusion is in accordance with emerging observations that the proportion of HBsAg responders declines as time elapses since infant priming. In Alaska, 95% of 5-year-old responded to an HBsAg booster, which was only observed in 60% of 14-year-old [28,29]. In Micronesia, an anamnestic response to an additional vaccine dose was only seen in half of 15-year-old participants [30] and similar reports are coming from Taïwan [31]. This indicates that although immune memory is readily elicited by neonatal or infant immunization, its long-term persistence is limited. Whether this results from the limitations of early life Germinal Center responses [32] reducing the size of the pool of memory B cells and/or from other factors limiting memory B and/or T cell persistence is now open for investigation.

As the risk of tetanus persists life-long, concluding that infant immunization with a vaccine as potent as TT may not induce immune memory persisting at a sufficient level to be reactivated 10–15 years later provides strong support to the recent WHO recommendation to implement childhood DTP boosters [1]. It indicates that such boosters should be given prior to the waning of immune memory. Critical issues are now to define the age groups in which infant-primed individuals should receive 1, 2 or perhaps even 3 doses of TT—as in unprimed individuals with no residual immune memory. By demonstrating that "infant immune memory may not be not life-long", this study thus calls for additional studies to support a careful reevaluation of current recommendations and catch-up immunization schedules.

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Contributors: KMPB, MK, PHL, CL and CAS were responsible for study design and implementation. JT, TE and MK performed the clinical study while laboratory analyses were set up, performed and analyzed by CS and SG. KMPB and CAS drafted the manuscript. All authors contributed to and approved the submitted manuscript.

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