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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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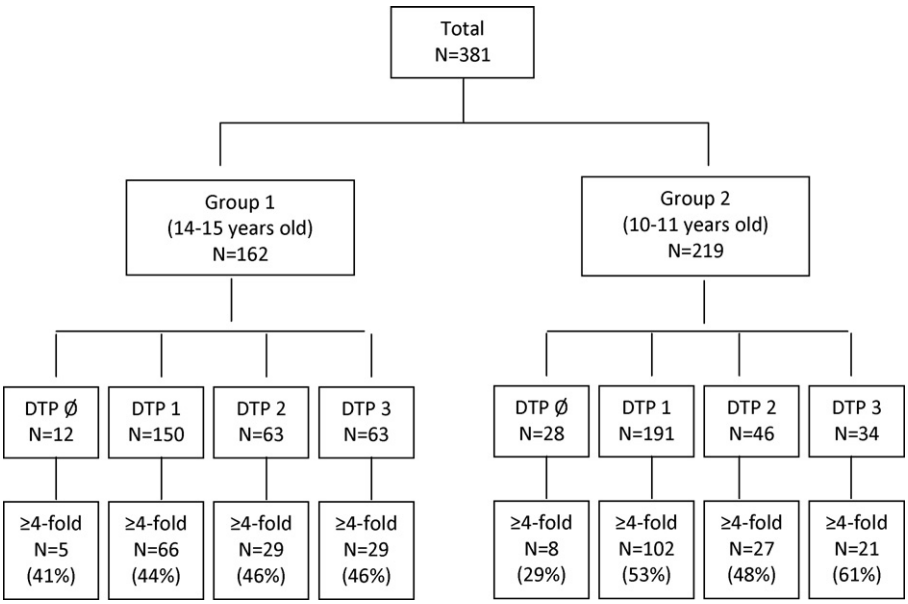

Fig. 1. Distribution of study participants.

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.