

RESEARCH LETTER

Repeated vaccination with tetanus toxoid of plasma donors with pre-existing specific IgE transiently elevates tetanus-specific IgE but does not induce allergic symptoms

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1 | INTRODUCTION

IgE responses against allergens have acquired much attention due to their pathogenic nature as mediators of allergic reactions. In contrast, IgE responses against vaccines such as diphtheria-tetanus-pertussis (DTP) and the potential persistence of IgE production have received relatively little attention, presumably because of the low prevalence of allergic symptoms.

In general, common early sensitivity reactions against vaccines, including tetanus toxoid, consist of mild local reactions and are not contraindicative of future vaccinations.^{1,2} These reactions might in part be mediated by IgE, but this remains unclear. Approximately 40% of the children develop IgE to tetanus and/or diphtheria.³ Also in adults, the IgE response rate is close to 50%.^{1,4,5} In these studies, no major tetanus-specific IgE-mediated allergic symptoms were reported. However, the high frequency of IgE responses may, despite the lack of clinical relevance, be a potential threat to the DTP vaccination programme in infancy.

To substantiate the safety of tetanus vaccination, we analysed the IgE anti-tetanus response of a special group of vaccine recipients: donors of anti-tetanus plasma used for the production of anti-tetanus immunoglobulin for passive immunization. These poly-vaccinated individuals are extreme examples, but are informative to investigate a potential risk of tetanus-specific IgE because they are frequently examined for side-effects. Our aim was to characterize the induction and persistence of tetanus-specific IgE responses in these subjects and to investigate the presence of IgE-mediated allergic symptoms upon revaccination.

2 | METHODS

2.1 | Anti-tetanus plasma donors and inclusion criteria of hyperimmunized donors

Donors were immunized with a tetanus toxoid (TT) preparation containing AlPO₄, NaCl and thiomersal (Bilthoven Biologicals, The Netherlands). One immunization dose contains ≥ 40 IU of TT and is injected intramuscularly. Voluntary blood donors may be asked to become anti-tetanus plasma donors when donors fit the general requirements as stated in our donor evaluation and infectious diseases guidelines and when TT-specific IgG levels exceed 5 IU/mL. Anti-tetanus plasma donors are re-immunized yearly or when TT-specific IgG levels drop below the sampling threshold. Plasma samples retrieved for analyses of TT-specific Ig titres were collected from immunized donors prior to plasmapheresis and anonymized. For the purpose of the study, donors were selected who received at least 4 booster vaccinations within the previous 5 years. Forty anti-tetanus plasma donors met this criteria, of which 17 had donated plasma at three consecutive time-points (pre-boost, 1 month and 6 months after boost). The Medical Ethics Committee of Sanquin Blood Supply approved of the study, and all donors gave written informed consent to usage of retrieved plasma samples for research purposes.

Evaluation of all adverse reactions in relation to TT revaccination in the period between January 2013 and December 2016, during which 2112 TT immunizations were administered to 1253 individuals, resulted in one reported adverse reaction. This donor experienced nausea, dizziness, vomiting and headache, but no fever, the morning after TT immunization. After 24 hours, all the symptoms had resolved. In our study, none of the IgE-positive donors

Unger and Makuch equally contributed to this study.

reported allergic reactions following vaccination. One donor experienced having had a warm and painful arm after vaccination, but this was years before inclusion in our study and during our study, it did not occur.

2.2 | Tetanus toxoid-specific IgE radio-immune assay

TT-specific IgE antibodies were measured as described previously,⁶ with some modifications. Briefly, 66 Lf TT (360 µg TT) (Lot. 6014079, RIVM, Bilthoven, The Netherlands) was coupled to 300 mg CNBr-activated Sepharose 4B beads (GE Healthcare, Eindhoven, Netherlands). Washed TT-Sepharose was then suspended at 2 mg/mL in PBS pH 7.4 supplemented with 0.3% BSA, 10 mmol/L EDTA and 0.25% Tween-20 (v/v). Serum samples (50 µL each) were then incubated overnight with 250 µL Sepharose suspension (600 ng TT/test), washed and incubated again overnight with [¹²⁵I]-labelled affinity-purified sheep anti-human IgE antibody (SH25, Sanquin, approximately 1 ng/test). The amount of bound [¹²⁵I] was measured using a gamma counter, and IgE antibody levels were converted to kU_A/L using a chimeric IgE antibody standard.⁷ We used 0.35 kU_A/L as cut-off value. Values below this cut-off were assigned 0.175 kU_A/L. Of note, the amount of TT coupled to the Sepharose beads is possibly insufficient for donors with very high IgG levels and may have led to an underestimation of tetanus-specific IgE levels due to interference of tetanus-specific IgG in the assay. Decreasing the amount of Sepharose-bound TT in this assay markedly decreased the amount of Sepharose-bound IgE from serum that contains high tetanus-specific IgG levels (data not shown). This decrease was less steep with serum with low tetanus-specific IgG levels.

2.3 | Tetanus toxoid-specific IgG and IgG4 reversed type radio-immune assay

TT-specific IgG and IgG4 antibodies were measured in a reversed type radio-immune assay using Protein G-Sepharose (GE Healthcare, Eindhoven, Netherlands) or monoclonal anti-IgG4 antibodies (MH164-1, Sanquin) coupled to CNBr-activated Sepharose 4B beads, respectively. First, 50 µL of 1:1000 or 1:40 diluted serum was added to the Sepharose beads to adsorb IgG or IgG4, followed by overnight incubation with [¹²⁵I]-labelled TT. After washing, the amount of bound [¹²⁵I] was measured using a gamma counter and data were calculated using a reference curve (derived from a serum pool of 50 anti-tetanus donors, containing 51 IU/mL anti-TT IgG). A cut-off value of 0.016 IU/mL for the IgG4 assay was used.

2.4 | Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 software. Relevant statistical tests used were one-way ANOVA and Mann-Whitney test and are indicated in the figure legends.

3 | RESULTS AND DISCUSSION

To analyse IgG and potential IgE response in donors that received multiple vaccinations with TT, 40 anti-tetanus plasma donors met the criteria of having received at least 4 booster vaccinations within 5 consecutive years (see methods for inclusion details). We retrospectively analysed the kinetics and persistence of tetanus-specific antibody responses and assessed whether tetanus-specific IgE responses resulted in allergic reactions. Anti-TT IgE levels above 0.35 kU_A/L before booster vaccination were present in 24 of 40 (60%) donors (Figure 1A), fitting with the published observation that 63% of tetanus-immunized individuals presented with immediate skin test reactivity.¹ No immediate hypersensitivity reactions after revaccination were observed in our donors. To follow individual IgE

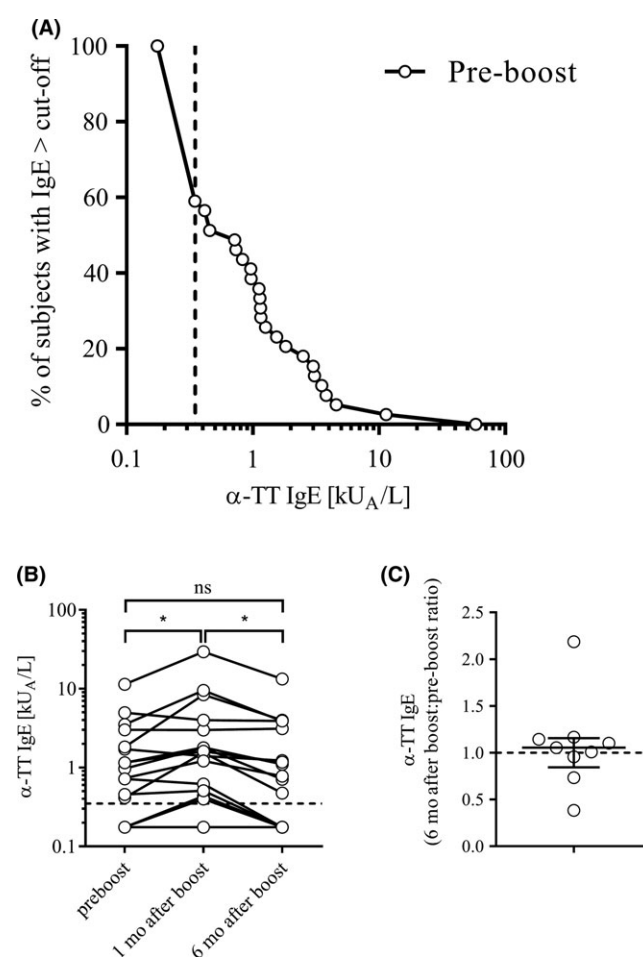


FIGURE 1 Prevalence of tetanus toxoid (TT)-specific IgE before and after revaccination in donors repeatedly vaccinated with TT. A, Cumulative curve showing the prevalence of TT-specific IgE equal to or higher than the cut-off values indicated on the x-axis before boost ($n = 40$; white circles). The IgE cut-off value of assay set at 0.35 kU_A/L (dotted line). B, TT-specific IgE levels over time ($n = 17$). One-way ANOVA with Friedman multiple comparisons test (paired, non-parametric) was used for statistical analyses. * $P < .05$. C, IgE anti-TT 6 months after boost relative to pre-boost in the nine donors with a rise in anti-TT IgE levels one month after boost

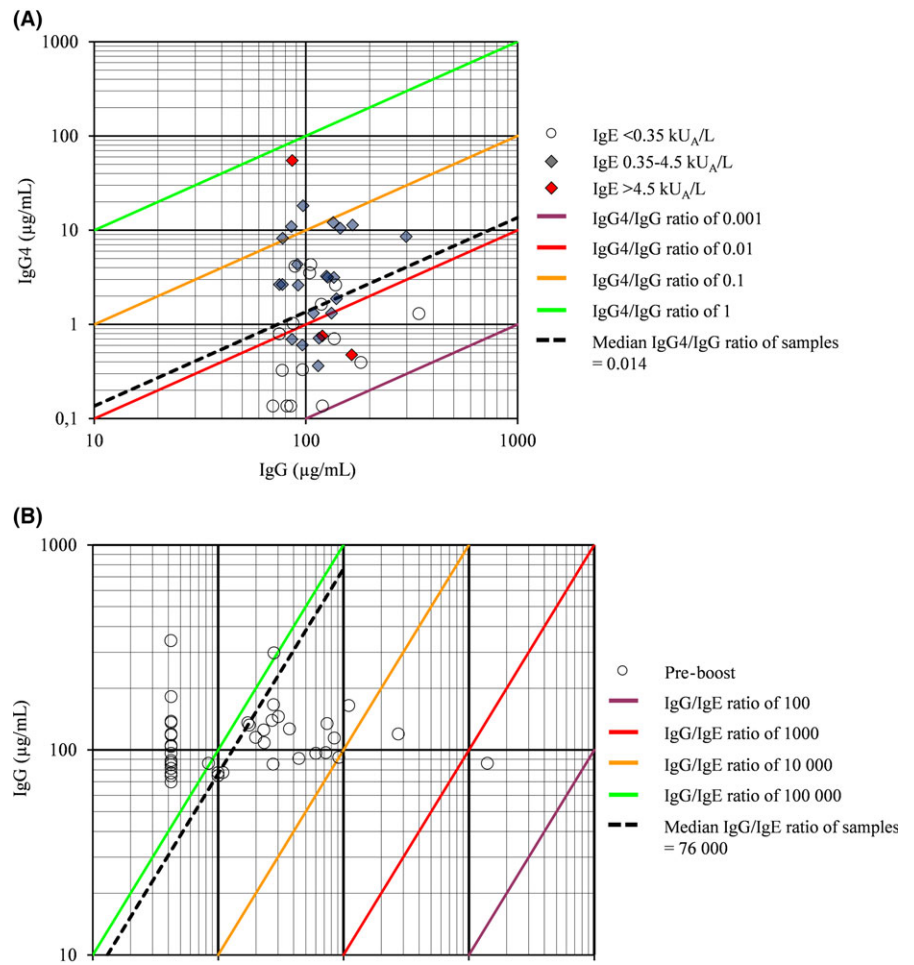


FIGURE 2 Comparison between IgE, IgG4 and IgG responses in donors repeatedly vaccinated with tetanus toxoid (TT). A, Anti-TT IgG4 (y-axis) and anti-TT IgG levels (x-axis) before boost in IgE-negative ($n = 16$; white dots) vs IgE-positive subjects ($n = 24$; grey and red diamonds). The lines (in different colours) indicate IgG4/IgG ratio borderlines. The dotted black line indicates the median IgG4/IgG ratio. B, Anti-TT IgE (x-axis) and anti-TT IgG levels (y-axis) before boost ($n = 40$). The plotted lines (in different colours) indicate IgG/IgE ratio borderlines. The dotted black line indicates the median IgG/IgE ratio

responses, longitudinal analyses were performed in 17 of 40 donors (Figure 1B). This showed that 13 of 17 donors (76%) had existing pre-boost titre of TT-specific IgE. Nine of these thirteen donors with pre-existing IgE (69%) showed a median 1.8-fold increase, which might be an underestimation (see methods section). Six months after boost, the increased IgE levels declined to baseline levels before boost in 8 of 9 donors, with a median post-6 months/pre-boost ratio of 1.1 (interquartile range of 0.84 and 1.16; Figure 1C) with only one donor showing a possibly more persistent increase. Together, these data show that TT revaccination in donors that had received multiple booster vaccinations before induces a transient rise in IgE, which might suggest the induction of short-lived TT-specific IgE plasma cells from TT-specific memory B cells. In addition, long-lasting TT-specific IgE is present in the majority of revaccinees, which might suggest that long-lived TT-specific IgE plasma cells have been generated at some point during the boosting regime.

We evaluated all adverse reactions reported in relation to TT revaccination in the period between January 2013 and December 2016 and in our study, none of the IgE-positive donors reported allergic reactions following vaccination (see methods for more details). The absence of IgE-mediated allergic reactions despite the presence of IgE at the time of vaccination is remarkable. One

possible and likely explanation is protection by TT-specific IgG that competes with IgE for binding to TT.

We assessed whether induction of potential harmful TT-specific IgE by revaccination was accompanied by induction of potential protective TT-specific IgG4. Indeed, specific IgG4 levels in the IgE-positive subgroup of hyperimmune donors (median 0.17 IU/mL) are significantly increased ($P < .01$) compared to the IgE-negative subgroup (median 0.04 IU/mL) before the boost with TT (Figure 2A), but the concurrent TT-specific IgG4 levels are very low compared to TT-specific IgG_{total} levels (Figure 2A). However, one donor with a high anti-TT IgE level ($58 \text{ kU}_A/\text{L}$) showed a IgG4/IgG ratio of 0.64 (Figure 2A). A more likely candidate for neutralization of the TT-specific IgE in these donors is IgG1 because this is the major anti-tetanus isotype.⁸ To analyse the relative amount of TT-specific IgE compared to IgG, the ratio of TT-specific IgG/IgE was calculated using conversion factors of 2.4 ng per IU of IgE and $17 \mu\text{g}$ IgG per IU of anti-tetanus⁹ (Figure 2B). Before TT booster, the median IgG/IgE ratio was 76 000 (interquartile range 32 000–210 000). The donor with the lowest TT-specific IgG/IgE ratio (615) had a high TT-specific IgE level of $58 \text{ kU}_A/\text{L}$. This 500-fold excess of TT-specific IgE might explain why none of the tetanus hyperimmune donors have allergic reactions upon revaccination. This ratio might be an overestimation as high TT-specific IgG

levels interfere with IgE binding in our assay (see methods section). Nevertheless, this would suggest that for suppression of symptoms, an IgG/IgE ratio even lower than 500 might suffice.

Our observations confirm that the presence and level of TT-specific IgE antibodies are not predictive for IgE-mediated allergic reactions in adult subjects with a history of frequent vaccination. To some degree, this result might be influenced by selection bias at inclusion, but allergic reactions were also not reported by tetanus-vaccinated donors that did not fit our inclusion criteria. Nevertheless, protection against allergic reactions seems to be common. The most likely mechanism, but not the only one possible, explaining the lack of allergic reactions upon repeated vaccination with TT is competition between IgG and IgE as a consequence of limited induction of TT-specific IgE plasma cells compared to the strong induction of TT-specific IgG plasma cells. The presentation of this data is intended to minimize unnecessary worries in relation to tetanus vaccinations.

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CONFLICT OF INTEREST

Sanquin is a not-for-profit organization that generates anti-TT antibodies for prophylactic treatment through hyperimmunization of voluntary donors.

AUTHOR CONTRIBUTIONS

Peter-Paul A. Unger and Mateusz Makuch participated in analyses and interpretation of data, drafting and writing of the article and approved the final version. Marja Aalbers and Ninotska I.L. Derksen participated in acquisition of data, critically reviewed the article and approved the final version. Anja ten Brinke, Rob C. Aalberse, Theo Rispens and S. Marieke van Ham participated in interpretation of data and contributed to writing, reviewing and finalizing the article. S. Marieke van Ham and Rob. C. Aalberse initiated the study.

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REFERENCES

1. Facktor MA, Bernstein RA, Fireman P. Hypersensitivity to tetanus toxoid. *J Allergy Clin Immunol.* 1973;52:1-12.
2. Kelso JM, Li JT, Nicklas RA, et al. Adverse reactions to vaccines. *Ann Allergy Asthma Immunol.* 2009;103(4 Suppl 2):S1-S14.
3. Gruber C, Lau S, Dannemann A, Sommerfeld C, Wahn U, Aalberse RC. Down-regulation of IgE and IgG4 antibodies to tetanus toxoid and diphtheria toxoid by covaccination with cellular Bordetella pertussis vaccine. *J Immunol.* 2001;167:2411-2417.
4. Dannemann A, van Ree R, Kulig M, et al. Specific IgE and IgG4 immune responses to tetanus and diphtheria toxoid in atopic and nonatopic children during the first two years of life. *Int Arch Allergy Immunol.* 1996;111:262-267.
5. Rowe J, Yerkovich ST, Richmond P, et al. Th2-associated local reactions to the acellular diphtheria-tetanus-pertussis vaccine in 4- to 6-year-old children. *Infect Immun.* 2005;73:8130-8135.
6. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol.* 1981;68:356-364.
7. Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. *J Allergy Clin Immunol.* 1997;99:545-550.
8. van der Giessen M, Groenboer-Kempers O. The subclasses of human IgG antibodies against tetanus toxoid. *Clin Exp Immunol.* 1976;25:117-121.
9. Schauer U, Stemberg F, Rieger CH, et al. Levels of antibodies specific to tetanus toxoid, Haemophilus influenzae type b, and pneumococcal capsular polysaccharide in healthy children and adults. *Clin Diagn Lab Immunol.* 2003;10:202-207.

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