

Further investigations of the IgE response to tetanus and diphtheria following covaccination with acellular rather than cellular *Bordetella pertussis*

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Abstract

Background: It has previously been shown in an uncontrolled study that the IgE response to vaccine antigens is downregulated by co-vaccination with cellular *Bordetella pertussis* vaccine.

Methods: In the present study, we compared in a controlled trial the humoral immune response to diphtheria toxoid (D) and tetanus toxoid (T) in relation to co-vaccinated cellular or acellular *B pertussis* vaccine. IgE, IgG4, and IgG to D and T were analyzed at 2, 7, and 12 months of age in sera of children vaccinated with D and T (DT, N = 68), cellular (DTPw, N = 68), 2- or 5-component acellular *B pertussis* vaccine (DTPa2, N = 64; DTPa5, N = 65).

Results: One month after vaccination, D-IgE was detected in 10% sera of DTPw-vaccinated children, whereas vaccination in the absence of whole-cell pertussis resulted in 50%-60% IgE positivity. Six months after vaccination, the IgE antibody levels were found to be more persistent than the IgG antibodies. These diphtheria findings were mirrored by those for tetanus. Only minor differences between vaccine groups were found with regard to D-IgG and T-IgG. No immediate-type allergic reactions were observed.

Conclusion: Cellular (but not acellular) *B pertussis* vaccine downregulates IgE to co-vaccinated antigens in infants. We assume that the absence of immediate-type allergic reactions is due to the high levels of IgG antibodies competing with IgE antibodies.

KEYWORDS

downregulation, human, IgE, IgG4, lipopolysaccharide, persistence, vaccination

1 | INTRODUCTION

Immunization induces primarily antibodies of the IgG isotype. In addition, a potentially undesirable production of specific IgE (sIgE) has

been observed in response to vaccine antigens.¹⁻⁴ sIgE production is usually assumed to be largely restricted to subjects with an atopic background. However, an IgE response to vaccine components has also been observed in many non-atopic individuals.²⁻⁶

We have previously shown that cellular *Bordetella pertussis* (Bp) downregulates sIgE and sIgG4 (but not sIgG) to co-vaccinated diphtheria (D) and tetanus (T) antigens.⁵ We hypothesized that the bacterial cell wall was the major factor of this suppression. Its main component, lipopolysaccharide, is known to suppress sIgE in experimental models.⁷ A weakness of this initial study was that it

Abbreviations: AU, arbitrary IgG4 units; Bp, *Bordetella pertussis*; D, diphtheria toxoid; DTPa2 and DTPa5, diphtheria/tetanus/acellular pertussis vaccine (2 or 5 components, respectively); DTPw, diphtheria/tetanus/whole-cell pertussis vaccine; EU, ELISA IgG units; k_u, allergen-specific IgE; Lf, limit of flocculation units; P, Bp toxin; RAST, radioallergosorbent test; T, tetanus toxoid.

*The present analysis was initiated by Dr Christoph Grüber, who died on January 10, 2016, during the final stages of the preparation of this manuscript.

was based on a parental decision whether or not to have their child immunized with Bp. At that time (early nineties), this was a cellular Bp vaccine. The presence of bacterial cell wall components in this type of vaccine is likely to have an impact on the immune response. Currently, immunization practice has shifted from whole-cell Bp vaccines to acellular vaccines. The controlled clinical trials preceding the introduction of these acellular vaccines provided a unique opportunity to compare effects of acellular and cellular Bp vaccines on the humoral immune response to co-vaccinated D and T antigens in a randomized controlled setting. We here present the results of a retrospective analysis of the IgE responses of the Bp-randomized clinical trial, of which the primary outcomes were reported in 1996.⁸

2 | METHODS

2.1 | Study population

In a pertussis vaccine trial of 9829 children at 14 study centers, 788 infants in Linköping, Sweden, were randomized to a double-blind comparison of the effects of two acellular vaccines, a whole-cell vaccine, and a pertussis-free DT vaccine as placebo.⁸ For our study, available serial sera from 265 children with and without allergic sensitization to environmental allergens were included. All study individuals were fully vaccinated, and none experienced pertussis infection. The parents of all participating children gave their informed consent. The Human Research Ethics committee of the Medical Faculty at the University of Linköping approved the study.

2.2 | Vaccines and immunization schedule

Of the 265 children included, 68 received the whole-cell Bp vaccine, 64 the 2-component acellular vaccine, 65 the 5-component acellular vaccine, and 68 the DT placebo. There was no statistically significant group difference with regard to gender, hereditary risk for allergic disease, allergic sensitization to nutritive or inhalant allergens, exposure to environmental tobacco smoke or furred pets, or the duration of breastfeeding (data not shown). At ages 2, 7, and 12 months, diphtheria (D)-IgG was available from 264, 265, and 256 children, and D-IgE and D-IgG4 from 235, 254, and 253 children, respectively. Tetanus (T)-IgG was available from 264, 265, and 256 children, T-IgE from 215, 227, and 223 children, and T-IgG4 from 235, 254, and 253 children, respectively. Children were randomized to receive one of four vaccines: a 2-component acellular pertussis vaccine (DTPa2, SmithKline Beecham), a 5-component acellular Bp vaccine (DTPa5, Connaught Ltd), whole-cell Bp vaccine (DTPw, Connaught Laboratory Inc), and diphtheria and tetanus vaccine (DT, Swedish National Bacteriological Laboratory) used as placebo. The trial vaccines were given in a series of three intramuscular injections on the side of the thigh at ages 2, 4, and 6 months.

Key Message

IgE to diphtheria and tetanus > 0.35 kUa/L in close to 50% of the children vaccinated with any of the 3 vaccines without cellular pertussis, with 20% of the results > 1 kUa/L. IgE (and IgG4) induction is largely suppressed by the presence of cellular pertussis. No reports on IgE-related reactions were noted. The IgE response persisted for more than 6 months.

2.3 | Skin prick testing

Skin prick tests were performed in duplicate on the volar aspect of the forearm with milk, egg white, and cat dander antigens at the age of 7 months, and with egg, cat dander, dog dander, two mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), timothy, and birch antigens at 30 months. Undiluted cow's milk and hen's egg and for the other allergens Soluprick SQ extracts (10 histamine equivalent in prick testing; Allergologisk Laboratorium A/S) were used according to the recommendations by the European Academy of Allergy and Clinical Immunology.⁹ Tests were regarded as positive if skin wheals had a mean diameter of 3 mm or more after 15 minutes. Histamine dihydrochloride, 10 mg/mL, and blank lancets were included as positive and negative controls. No antihistamine should have been used during 3 days preceding the skin prick test. A child was considered as atopic if a skin prick test to any of these allergens was positive at 7 and/or 30 months.

2.4 | Analysis of antibodies to vaccine antigens

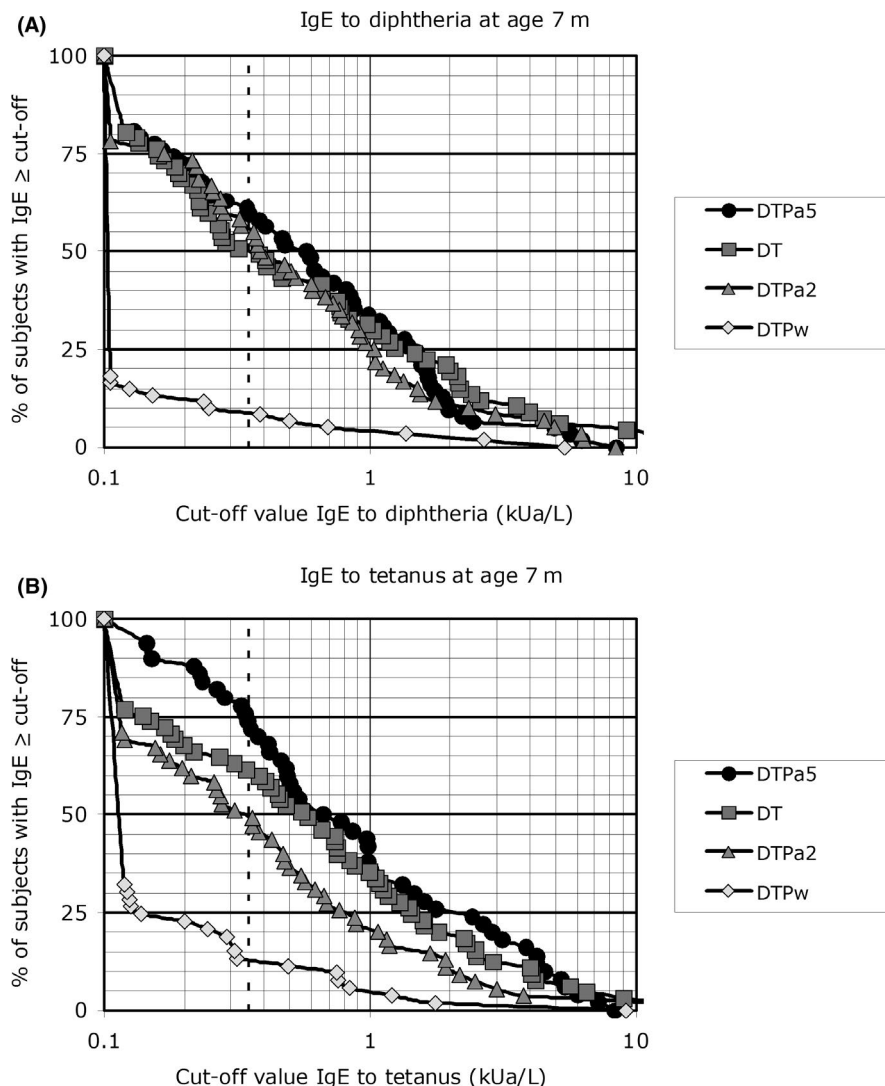
Antibodies to D and T were analyzed in serum samples obtained at ages 2, 7, and 12 months (before, +1 month, and +6 months after vaccination). If serum availability was limiting, diphtheria assay was given priority.

IgE and IgG4 antibodies to D and T were measured as described previously² using D and T toxoid coupled to CNBr-activated Sepharose and ¹²⁵I-labeled affinity-purified sheep anti-IgE antibodies and mouse monoclonal anti-IgG4 antibodies, respectively. The cutoff for a positive test was 0.35 kUa/L (D-IgE, T-IgE) and 5 AU/mL (D-IgG4, T-IgG4).

For graphical simplicity, the different antibody units are sometimes expressed as relative activity, that is, the potency as percent of a pool of 33 IgE-positive sera at age 7 months (slgE ≥ 1 for tetanus and/or diphtheria). This pool contained 4.0 kUa/L IgE antidiphtheria and 4.8 kUa/L IgE antitetanus; 49 and 52 AU/mL IgG4 antidiphtheria and antitetanus, respectively; and 1.8 EU/mL and 8.4 IU/mL IgG antidiphtheria and antitetanus, respectively.

Persistence of antibody levels was calculated as the (+6/+1) ratio. This is the ratio of the antibody level 6 months after the vaccination at age 6 months divided by the antibody level 1 month after the vaccination at age 6 months. To prevent skewing by the

FIGURE 1 Cumulative prevalence of sIgE to diphtheria (A) or tetanus (B) 1 mo after the 3rd vaccination at age 6 mo. The prevalence is plotted as a function of the sIgE cutoff value. The vertical dotted line indicates the commonly used cutoff value of a clinically relevant level of sIgE to airborne allergens (0.35 kUa/L). DT, DT vaccine without *B pertussis*; DTPa2, 2-component acellular pertussis vaccine; DTPa5, 5-component acellular pertussis vaccine; DTPw, whole-cell *B pertussis* vaccine



antibody level at +1, only children with sera with antibody levels at +1 that were higher than 3 times the cutoff value for the isotype were included (for tetanus $N = 50$, 212, and 263 for IgE, IgG4, and IgG, respectively; for diphtheria $N = 59$, 205, and 229, respectively; for all six tests: $N = 33$). Ratios lower than 0.33 were assigned a value of 0.33; ratios higher than 3 were assigned a value of 3.0.

2.5 | Statistics

For statistical analysis, we used SPSS for Windows 14.0 (SPSS Inc).

3 | RESULTS

3.1 | IgE response to diphtheria and tetanus toxoid in relation to pertussis vaccines

IgE to diphtheria toxoid was not detectable prior to vaccination but one month after vaccination 44.1% of the sera were positive (ie,

≥ 0.35 kU_a/L, see Figure 1). There was no statistically significant difference in the proportion of positive D-IgE tests between children vaccinated with DT (50.0%), DTPa2 (55.7%), or DTPa5 (60.3%), but the proportion was markedly lower among whole-cell pertussis co-vaccinated children (9.7%, $P < .001$).

While the correlation between IgE levels to tetanus and diphtheria was highly significant (Spearman $\rho = 0.59$, $P < .001$ with exclusion of the 80 double-negative children), 36/162 = 22.2% of the children with IgE to D and/or T were positive for T but negative for D. In 13 (8.0%) of the children, the reverse was found (Figure S1).

The IgE, IgG, and IgG4 responses to the four vaccines are shown in Figures 2 and 3. Among children vaccinated with cellular vaccine, the proportion of T-IgE-positive tests was significantly lower than among DT-vaccinated children at +1 month after three primary doses (13.0% vs 59.1%, $P < .001$) and +6 months after 3 primary doses (27.3% vs 67.2%, $P < .001$), whereas 2-component vaccinated children (+1 month after 3 primary doses, 50.0%; +6 months after 3 primary doses, 66.0%) and 5-component acellular vaccinated children (+1 month, 74.5%; +6 months, 72.2%) did not significantly differ from DT-vaccinated children. These results

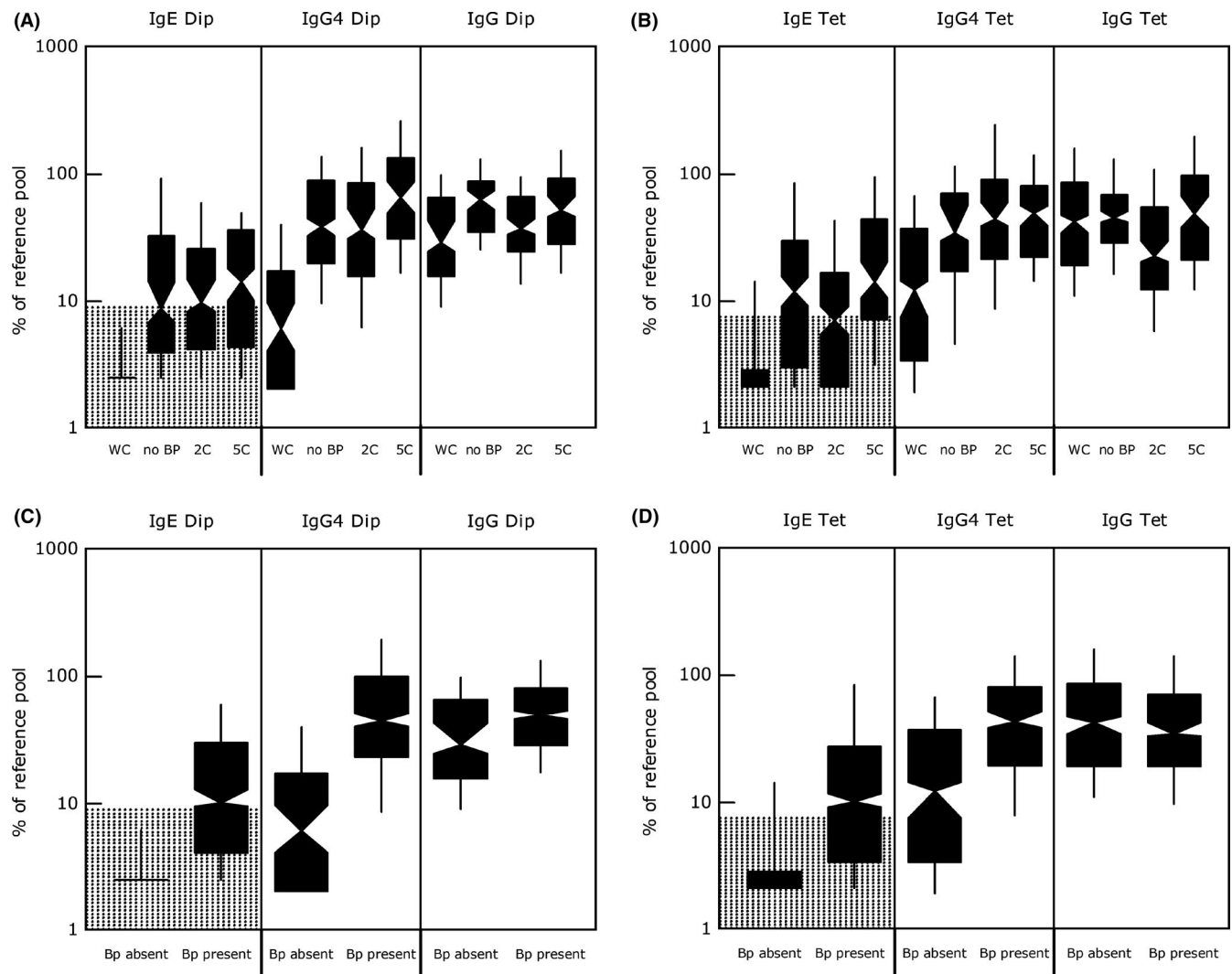


FIGURE 2 Serum levels of IgE, IgG4, and IgG to diphtheria toxin (A and C) and tetanus toxin (B and D) 1 mo after the 3rd vaccination at age 6 mo (DTPa5 = DT vaccine with 5-component acellular pertussis vaccine; DT = DT vaccine only; DTPa2 = DT vaccine with 2-component acellular pertussis vaccine; DTPw = DT vaccine with whole-cell *B pertussis* vaccine). In panels C and D, the results of 3 cell-free vaccines are pooled for comparison with the cellular vaccine. To facilitate the comparison between IgE, IgG4, and IgG, the results are expressed as percentage of a reference serum pool (see Section 2). The shaded block indicates the cutoff for a positive test for sIgE (0.35 kUa/L)

are in agreement with our previous observation that the levels of both D-IgE and T-IgE were downregulated in whole-cell pertussis-vaccinated group. Compared to the strong effect of whole-cell *B. pertussis* on the IgE response, the effect on the IgG response is weak (Figure 2).

In Figure 3, it is shown that children with an atopic background (established at age 3 yrs) have only a slightly higher IgE response, which is possibly less susceptible to suppression by *B pertussis*.

3.2 | Persistence of IgE antibody compared to IgG4 antibody

We analyzed the persistence of IgE and IgG antibodies by calculating the antibody ratio at +1 and +6 months after the vaccination at age 6 months (ratio +6/+1). The median ratio for IgG was 0.25,

that is, a 4-fold decrease, which is only slightly higher to what would be expected from the IgG half-life. Surprisingly, particularly in view of the much shorter half-life of IgE, the IgE levels among the IgE-positive cases had only rarely dropped more than 2-fold (see Figure 4).

3.3 | Absence of vaccination-related immediate-type allergic reactions

Among the many variables logged during the vaccination trial, we focused on erythema. Erythema within 24 hours was noted in 103/253 = 41% of the vaccinations at 6 months. Based on the relative prevalence of erythema in sIgE-positive versus sIgE-negative children (0.928, 95% confidence interval 0.689-1.252), sIgE has little or no contribution to post-vaccination erythema. However, a

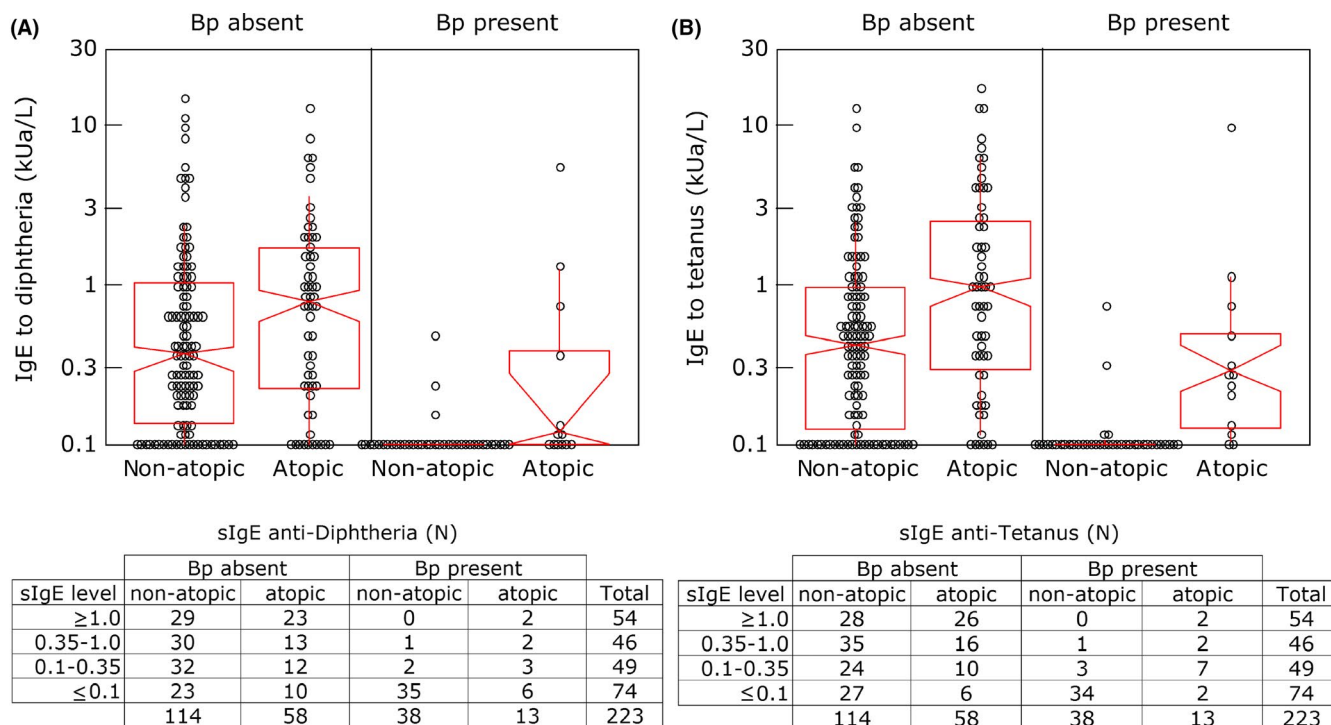


FIGURE 3 IgE to diphtheria toxoid (A) and tetanus toxoid (B) among non-atopic and atopic infants stratified by vaccine-type, that is, DTPw (DT vaccine with whole-cell *B pertussis* vaccine), versus the three vaccines without whole-cell Bp (DT, DTPa2, DTPa5) at age 7 mo. The box plots represent the 10, 25, 75, 90 percentiles and the median, with the wedge indicating the standard error of the median. The table below the graph shows the number of children with sIgE levels within the range indicated on the left of the table

post-test subgroup analysis suggested a sIgE effect in relation to tetanus vaccination in children with low IgG tetanus-specific IgG (see online repository Figure S2). For diphtheria, no such effect was observed.

4 | DISCUSSION

In earlier studies, we found that infants immunized against diphtheria/tetanus commonly produce IgE to vaccine antigens^{2,3} and that co-vaccination with cellular *B pertussis* vaccine downregulates IgE and IgG4 formation to D and T antigens.⁴ The findings of this study confirm these observations and add that co-vaccination with acellular Bp vaccines is ineffective in suppressing IgE and IgG4 formation to D and T antigens (Figures 1-3). The effect on the IgG4 response is stronger than on the overall IgG response, but not as strong as on the IgE response (Figure 2).

In a preliminary post-test analysis, we investigated whether the IgE-suppressing effect was different in atopic compared with non-atopic children. A trend toward a diminished suppressive effect was found in atopic children, but this did not reach statistical significance (Figure 3). This analysis protocol was based on the assumption that all antibody levels, including IgE antibodies, would be higher at 1 month after vaccination compared with 6 months after vaccination. When we noticed that in many children the IgE response was higher after 6 months, we additionally performed

the analysis with the 6 months time point. For IgE in non-atopic children, the pertussis effect was strong, with odds ratios of 0.052 (95% confidence interval: 0.015-0.178) and 0.048 (0.016-0.143) for diphtheria at 1 and 6 months after vaccination, respectively. For tetanus, the effect was slightly lower, but still highly significant with odds ratios of 0.068 (0.0120-0.235) and 0.109 (0.032-0.376). However, for atopic children the pertussis effect was less impressive, with 0.346 (0.113-1.059) and 0.550 (0.162-1.862) for diphtheria at 1 and 6 months after vaccination and even less for tetanus: 0.377 (0.120-1.184) and 0.839 (0.276-2.545). The statistical power of these post-test analyses is insufficient for strong conclusions, but if additional studies would confirm this negative interaction between atopy and anti-allergic microbial effects, this could mean that allergy prevention by pre- and probiotics might work less well in the children that would most need it.

Acellular Bp vaccines have been developed because they have fewer side effects such as fever.⁸ They lack the bacterial cell wall. Among the cell wall molecules, the lipid A fraction of lipopolysaccharides (LPS) has gained recent attention because of its immunomodulatory properties. Once released, it functions as a pathogen-associated molecular pattern and induces secretion of Th1-type cytokines including IL-12 by activation of toll-like receptor 4.¹⁰ As a key regulator of T-cell differentiation, IL-12 favors differentiation of naive T cells into the Th1 phenotype,¹¹ thus inhibiting IgE and IgG4 formation and inducing B cells to switch to IgG1. Experiments in mice demonstrate that systemic LPS exposure

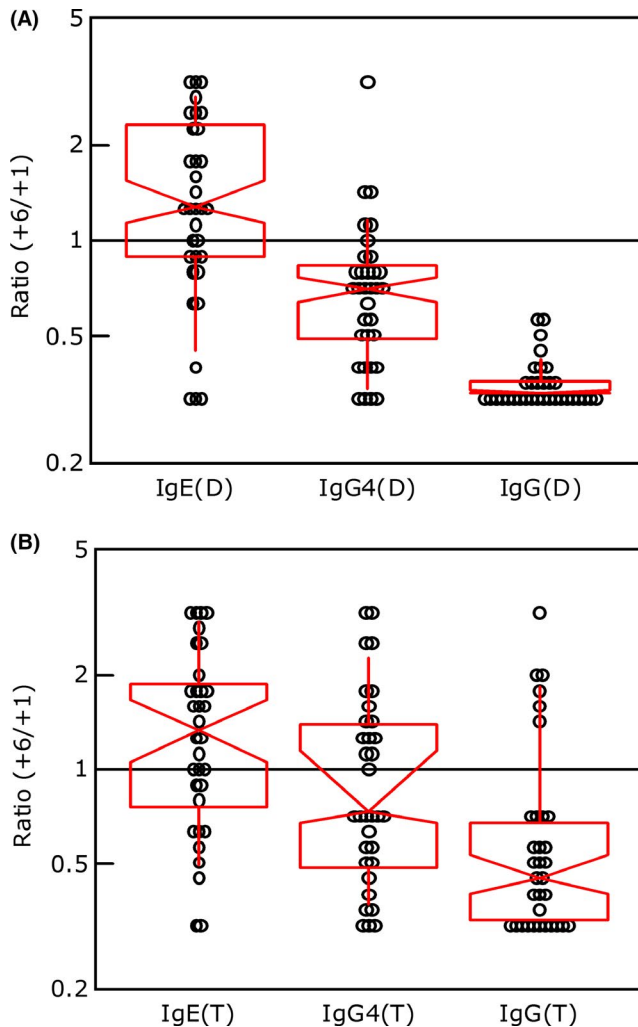


FIGURE 4 Difference in 6 mo persistence of the IgE, IgG4, and IgG antidiphtheria antibody levels in paired serum samples from 33 children. On the y-axis, the level at 6 mo after vaccination is compared to the level at 1 mo after vaccination. Ratios > 3 and < 0.33 were converted to 3 and 0.33, respectively. A, diphtheria; B, tetanus. The box plots represent the 10, 25, 75, 90 percentiles and the median, with the wedge indicating the standard error of the median

immediately prior to allergen sensitization inhibits Th2-type immune response through increased production of IL-12⁶ and inhibits IgE production but promotes specific IgG production.¹²

In humans, exposure to LPS early in life has been reported to be associated with a lower prevalence of allergic sensitization and atopic disease.^{13, 14} LPS is thus a hypothetic candidate for inducing in humans the observed suppression of Th2-driven Ig responses, including IgE and IgG4 to vaccine antigens. The timing of the presentation of immune-modulating agents in relation to the allergens in question is crucial. DT-immunized children co-vaccinated with cellular pertussis vaccine show downregulated IgE responses to D and T in a dose-dependent fashion. In the same cohort, however, antecedent BCG vaccination did not suppress IgE or IgG4 levels.⁵ Although BCG is a model inducer of a predominantly Th1-type immune response

in mouse and man,^{15–18} the time lag between BCG vaccination and DT vaccination may have been too long to effectively suppress the formation of Th2-associated immunoglobulins to DT.

5 | CONCLUSION

IgE to vaccine components might be more common than generally appreciated, but in the case of diphtheria and tetanus vaccination, it does not cause significant IgE-mediated reactions, possibly because of IgE-blocking effects of IgG antibodies.⁶

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CONFLICT OF INTEREST

None of the authors have a conflict of interest.

AUTHOR CONTRIBUTIONS

CG, LN, SK, UW, and RA contributed to the underlying concept and study design. RA and SK performed the experiments and serologic data analyses. CG, LN, ML, BN, and UW were responsible for the retrieval and analyses of the clinical data. CG and RA wrote the initial draft of the manuscript. RA finished the draft with input from LN, ML, SK, UW, and BN. The final version of the manuscript was approved by LN, ML, SK, UW, BN, RA, and Henrike Grüber (wife of the deceased Christoph Grüber).

INFORMED CONSENT

The parents of all participating children gave their informed consent. The Human Research Ethics committee of the Medical Faculty at the University of Linköping approved the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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**Further investigations of the IgE response to tetanus and diphtheria
following covaccination with acellular rather than cellular**

Bordetella pertussis

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Correlation between IgE to tetanus and to diphtheria.

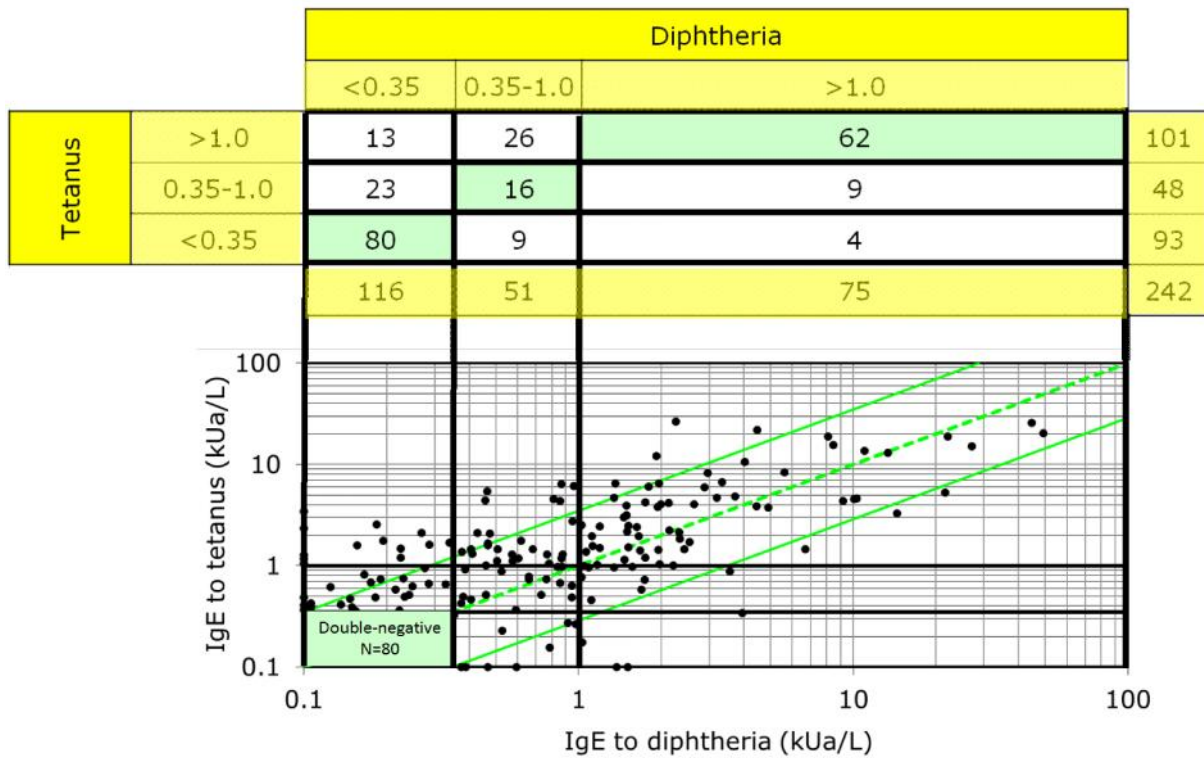


Figure S1. Correlation between IgE to tetanus and to diphtheria.

The dots in the figure indicate the association between IgE to diphtheria (x-axis) and IgE to tetanus (y-axis) for the 242 children for which both assays were performed. Sera below the green line on the bottom right have more than 3.5 times as much IgE to diphtheria than IgE to tetanus. Sera above the green line at the top left have more than 3.5 times as much IgE to tetanus than to diphtheria.

The numbers above the graph indicate the number of children in each of the nine groups indicated by the 9 black-lined boxes in the graph.

For each child we selected the time point (age 7 months or age 12 months) with the highest IgE antibody level to diphtheria and the highest IgE antibody level to tetanus, in order to minimize a possible bias by different IgE production kinetics between IgE to diphtheria and IgE to tetanus.

IgE suppression by cellular pertussis (Supplement)

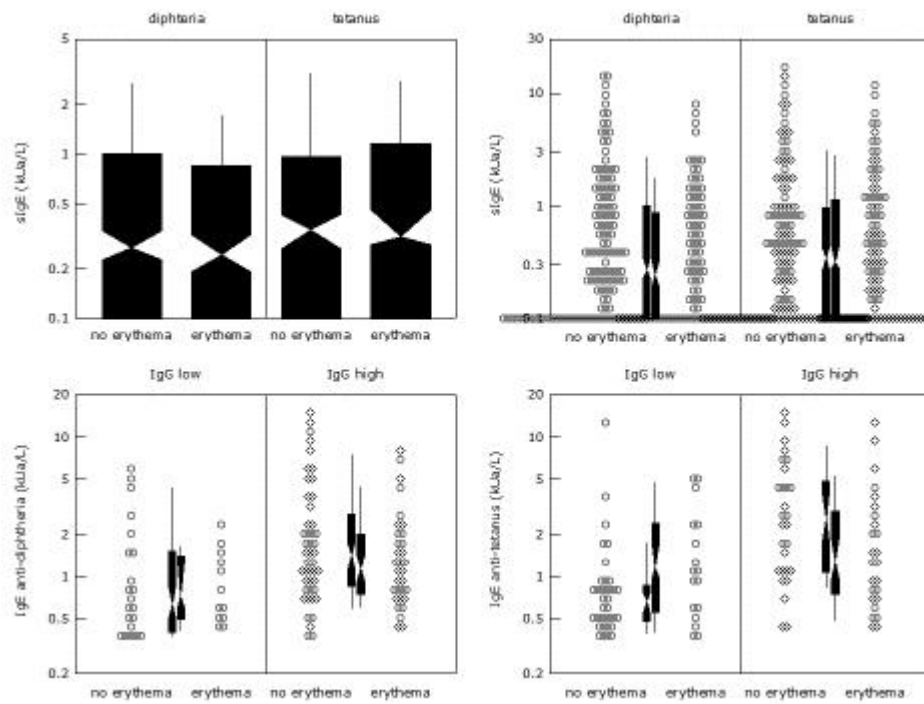


Figure S2. Subgroup analysis of a possible association between IgE to diphtheria or tetanus and post-vaccination erythema. The figures at the top show the apparent lack of association between post-vaccination erythema and the level of sIgE. The figures at the bottom show the analysis of the subgroup of sIgE-positive (≥ 0.35 kUa/L) children stratified by low or high sIgG. sIgE antibody levels to tetanus are possibly higher in the children with post-vaccination erythema, but only if sIgG to tetanus is low (i.e. lower than the median). These results would fit with an erythema-blocking effect of high IgG anti-tetanus. However, no such effect is found for IgG anti-diphtheria.