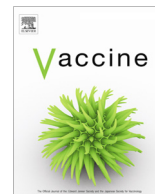




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Transiently increased IgE responses in infants and pre-schoolers receiving only acellular Diphtheria–Pertussis–Tetanus (DTaP) vaccines compared to those initially receiving at least one dose of cellular vaccine (DTwP) – Immunological curiosity or canary in the mine?

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ABSTRACT

Background: Several previous studies have highlighted the strong Th2-polarising and IgE-promoting activity of the DTaP vaccine, but there is no evidence that this has pathological consequences and accordingly there is no current interest amongst vaccine developers in reformulating DTaP to attenuate these properties. In light of an apparent resurgence in pertussis in many countries, and emerging evidence from other areas of paediatric immunology of IgE-mediated interference with host defence mechanisms, this issue requires more detailed clarification.

Methods: We have re-evaluated the impact of DTaP-only versus mixed DTwP/DTaP vaccination on Th2-dependent “bystander” IgE responses in three cohorts of children under different priming conditions, encompassing both vaccine-targeted and unrelated antigens including food allergens.

Results: We confirm the generalised IgE-trophic activity of the DTaP vaccine in pre-schoolers and demonstrate similar (albeit transient) effects in infants. We additionally demonstrate that use of an alternative mixed infant priming schedule encompassing an initial dose of DTwP significantly attenuates this property.

Interpretation: Central to our interpretation of these findings are studies demonstrating: (i) mixed DTwP/DTaP priming improves resistance to pertussis disease and attenuates the IgE-stimulatory component of long term vaccine-specific memory; (ii) IgE-mediated mechanisms can interfere with innate antiviral immunity and accordingly exacerbate airway symptoms in infected children. These observations, taken together with the data presented here, suggest a plausible mechanistic link between baseline pertussis-specific IgE titres in DTaP vaccinees and susceptibility to pertussis disease, which merits testing. Retrospective IgE analyses on sera collected from children at the time of presentation with pertussis could resolve this issue.

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1. Introduction

The whole cell diphtheria–tetanus–pertussis vaccine (DTwP) has been replaced in many countries since the 1990s by corre-

sponding acellular formulations (DTaP) due to their lower rates of severe adverse reactions. The relative merits of both vaccines continue to be debated from both the standpoints of safety and efficacy, fuelled in particular by recent epidemics of pertussis in regions including Australia and North America with high DTaP vaccine coverage [1,2]. Apart from extremely rare anaphylactic events [3], DTwP typically elicit immune responses in infants that are polarised towards production of the T-helper 1 (Th1) type cytokines that underpin resistance to microbial pathogens, whereas the

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DTaP formulation stimulates a more mixed response which includes a substantial component of T-helper 2 (Th2) cytokines [4–7]. These include IL-4 and IL-13 which drive IgE class switching [8]. This is considered to be the likely cause of co-production of Th2-dependent IgE antibody against vaccine antigens, in conjunction with specific IgG, amongst a subset of vaccinees [4–7,9–12].

Unlike the situation in parasitic and allergic diseases wherein Th2-dependent IgE contributes directly to pathogenesis via its role in triggering pro-inflammatory immediate hypersensitivity responses, this antibody has traditionally been considered to be benign in the context of the host response to DTaP immunization [9–11]. However, a forerunner study from our laboratories on the DTaP-specific memory response induced by the preschool booster implicated the production of the Th2 cytokines that drive IgE production as contributors to risk for injection site “delayed” reactions in children primed during infancy via a 4 dose DTaP schedule [11]. Moreover it has been shown in several studies that the preschool booster response in such children includes “bystander” stimulation of overall (total) IgE production, but there is no evidence that this includes individual food or inhalant allergen-specific IgEs relevant to expression of allergic disease in this age group [9,10,13].

There is also evidence that injection site reactions to the preschool booster are significantly attenuated if infant priming includes 1 or more doses of DTwP [13], or if a 3 dose DTaP priming schedule without a booster dose in the second year of life, is used [14]. However it is not clear whether these alternative priming schedules also influence susceptibility to booster-associated effects on vaccine-specific and/or “bystander” IgE production in pre-schoolers, nor whether similar effects occur at earlier stages of DTaP priming during infancy. Addressing these questions was the first aim of this study. Secondly, there is currently a resurgence of interest in the possible role of pathogen-specific IgE in interference with pathogen clearance mechanisms during infections, driven by the recognition that alternative forms of the high affinity IgE receptor (FcεR1) are expressed in functional form(s) on a range of bone marrow derived cell populations with immunomodulatory activity (reviewed [15]). Against this background, we have gone back to cryobanked serum samples collected here during a series of earlier studies on immune responses among DTwP and DTaP vaccinated children, to re-assess “bystander” effects of vaccination on specific and total IgE production and the degree to which this may be modified via variations in the infant priming schedule.

2. Methods

2.1. Study subjects

The three studies (designated A, B and C) described below complied with guidelines set by the National Health and Medical Research Council of Australia and were approved by local institutional human ethics committees. Study A was carried out among a group of infants ($n = 46$) who received diphtheria–tetanus–pertussis vaccination as part of a 4 dose infant vaccination schedule at 2, 4, 6 and 18 mths, in combination with one or more of the standard inactivated polio vaccine (IPV) or measles–mumps–rubella (MMR) vaccine, as detailed previously [13] and in Supplementary Table 1. The subjects were given the DTaP preschool booster around the age of 5 yrs and blood was collected for antibody assays before and 4 weeks after boosting [13]. Study B was carried out in subjects ($n = 81$) who received DTaP priming comprising 3 doses of DTaP–IPV at 2, 4 and 6 mths, without a dose at age 18 mths [14,16]; they were given the preschool DTaP–IPV booster at ~5 yrs and blood was again collected before and after boosting as in study A. Subjects in study C were from a cohort of infants ($n = 123$) who were vaccinated at 2, 4, 6 and 18 mths analogous to those in study A,

and blood was collected for antibody assays at those time points and at 12 mths when they received the MMR vaccine [5]; these subjects were not followed beyond 18 mths.

Studies A and C were initiated during the crossover period between local introduction of the DTaP vaccine and discontinuation of the DTwP vaccine. Post hoc re-examination of immunization records from both studies revealed that a subset of subjects had received at least one dose of DTwP vaccine during the infant pertussis priming schedule, in most cases only a single dose, usually at 2 mths of age. As detailed below, vaccine-associated immune response data were reanalysed after subject stratification on this basis.

2.2. Antibody assays

Serum was routinely collected and cryobanked for antibody assays at the time of receipt of infant vaccinations (2, 4, 6, 18 mths and in one study also at 12 mths), and in the case of the preschool booster both on the day of vaccination and 4 weeks later. IgE antibody assays against tetanus toxoid (TT), individual environmental allergens, and total IgE, were carried out employing the ThermoFisher (formerly Phadia) ImmunoCAP system in the Princess Margaret Hospital Pathology Department. Analytical software from ThermoFisher was utilised which enabled quantitation of IgE to a lower limit of 0.03 kU/L.

2.3. Statistical analyses

For studies A and B, IgE titres (total and/or tetanus toxoid specific) amongst pre-schoolers were almost all above assay detection limits; differences in IgE titres within these groups were analysed by Mann Whitney *U* test or Wilcoxon matched pairs signed rank test as appropriate. Because many infants in study C did not show IgE specific responses above the detection limit (0.03 kU/L), data were analysed in two distinct ways: (i) mixed effects logistic regression with any detection versus below detection limit as the response, and with subjects as random effects and measurement time (4 m, 6 m, 12 m, and for some 2 m and 18 m) and family history as fixed effects; and (ii) mixed effects tobit regression with values below the detection limit treated as censored and coming from the same log-normal distribution as the detected values. Further analyses were done on each measurement time separately using simple logistic and tobit models. Because of missing and sparse data, children were classified simply as vaccinated with DTwP at 2 mths ($n = 38$) or not ($n = 40$).

3. Results

We have previously reported group level differences in *in vitro* immune responses to the preschool DTaP booster, comparing children in study A previously vaccinated at 2, 4, 6 and 18 mths with DTaP alone versus those who had previously received mixed (DTwP/DTaP) priming, in which the first (and in some cases the first two) of the priming doses was DTwP [13]. These analyses demonstrated a positive association between the Th2 cytokine component of vaccine antigen specific immunological memory at the time of pre-school boosting and the magnitude of ensuing injection site inflammatory reactions, and subsequent expansion of Th2-memory. Moreover, in contrast to the recipients of mixed priming, the exclusively DTaP-primed injection site reactors manifested parallel increases in vaccine antigen (TT) specific IgE production [13]. The question of whether the IgE-stimulatory effects of pure DTaP priming may extend to antigens beyond those in the vaccine itself was not pursued in detail at that time, as an initial analysis did not detect differences in total IgE between the

different groups. As the first step in the present investigation we have re-analysed antibody response profiles in the sera of these children, comparing pre- to post-booster IgE levels in individual recipients of DTaP-only versus mixed DTwP/DTaP priming.

Fig. 1A illustrates total IgE levels pre- and post-boosting in individual children at age ~5 years in study A, grouped on the basis of type of priming (DTaP-only or mixed DTwP/DTaP). In the exclusively DTaP-primed children (but not in those who received DTwP/DTaP) the post booster titres were significantly increased ($p = 0.001$ by Wilcoxon), and as illustrated via the normalised data in Fig. 1B this increase was frequently >3fold. We have previously reported booster-induced increases in mean levels of TT-specific IgE in this same group [13], and for purposes of comparison we have re-expressed the data from individual children after normalisation as per Fig. 1B (Supplementary Fig. 1). These findings suggest that a subset of exclusively DTaP-primed children may be susceptible to stimulation of both vaccine antigen specific and overall IgE production in response to DTaP booster immunization, and these effects are attenuated if one or more doses of DTwP are given in place of DTaP as part of the initial infant priming schedule. This is consistent with earlier observations relating to the use of cellular versus acellular pertussis-only vaccines in infants, which also indicated protection against the IgE-stimulatory effects of early post-natal vaccination with acellular vaccine by inclusion of pertussis cell wall-derived components in the inoculum [17].

There were too few subjects in study A with measurable levels of IgE to specific food or inhalant allergens for more detailed assessment of possible bystander effects of DTaP. However we were able to pursue this question subsequently in a larger cohort. This study (designated study B) focused on preschool DTaP booster responses in children primed via a reduced DTaP schedule involv-

ing vaccination at the 2, 4, and 6 mth time points only (without the 18 mth booster dose which has been removed from the standard priming schedule in Australia). This regimen has been shown to reduce the frequency of injection site reactions following the preschool booster compared to the schedule which includes a DTaP dose at 18 mths [14]. However, as illustrated in Fig. 2A/B, a subset of these children still manifest relatively high level bystander boosting of total and vaccine-antigen-specific IgE ($p = 0.001$ and $p = 0.003$ respectively by Mann Whitney U test), comparable to that observed for the original DTaP priming schedule. Analysis of environmental allergen specific IgE revealed preschool booster-associated rises in titre in a small number of subjects (Supplementary Fig. 2), but the generally low magnitude and frequency of these events makes it difficult to gauge their significance.

A question remaining unanswered from these latter analyses is the degree to which the Th2-stimulatory effects of DTaP may influence IgE responses during the earlier infant priming period, when the immature immune systems of most children still manifest a fetal-like Th2-polarised state [18,19]. To address this question we turned to cryobanked samples from a second 4 dose vaccinated cohort comprising children with pure DTaP versus mixed DTwP/DTaP priming, analogous to the study group in Fig. 1. For these analyses (study C) we had available cryobanked serum samples from the subjects collected at the time of the 2, 4, 6 vaccinations plus at 12 and 18 mths, and these were assayed for total and specific IgE content.

The proportions with detectable specific IgE over the time course for the two groups are shown in Fig. 3A with mean total IgE in Fig. 3B. In addition to the 3 antigens displayed (egg, milk and TT), specific IgE responses were also measured at 4 m, 6 m, and 12 m for cat, house dust mite, and peanut allergens. The

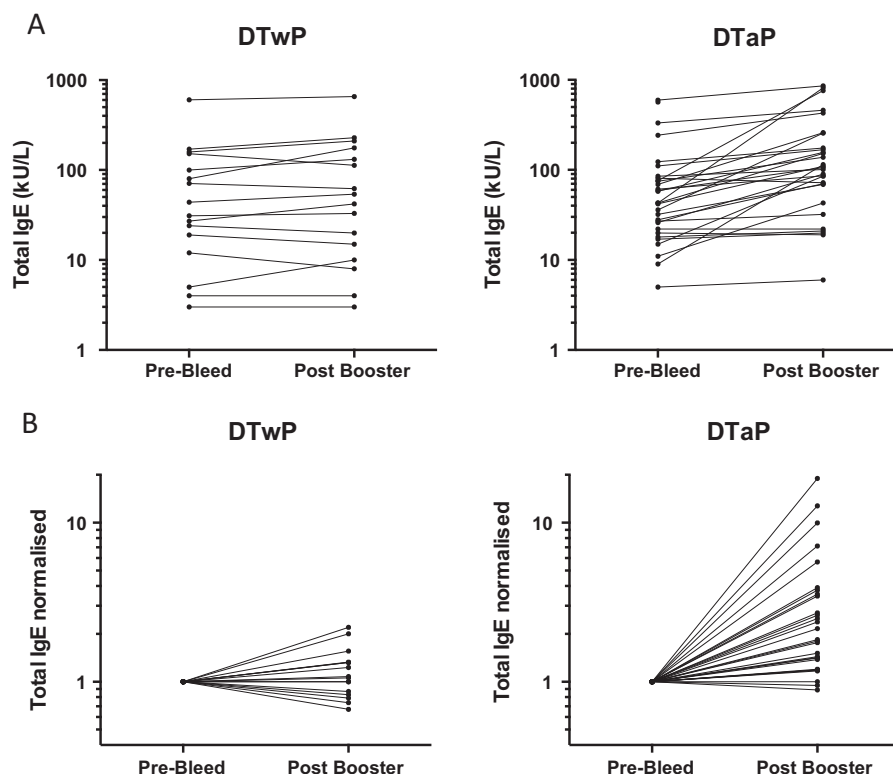


Fig. 1. Total IgE levels in subjects primed with DTaP alone or in combination with DTwP via the 4 dose infant vaccination schedule, before and 4 weeks after the preschool DTaP booster – study A. *Panel A:* Total IgE levels in individual children pre and post booster, expressed as kU/L; children who received mixed DTaP/DTwP priming are designated as DTwP (DTwP $n = 16$; DTaP $n = 33$; post-booster > pre-booster in DTaP group; $P = 0.001$ by Wilcoxon signed rank test). *Panel B:* Data for each child were normalised to respective pre-booster titres.

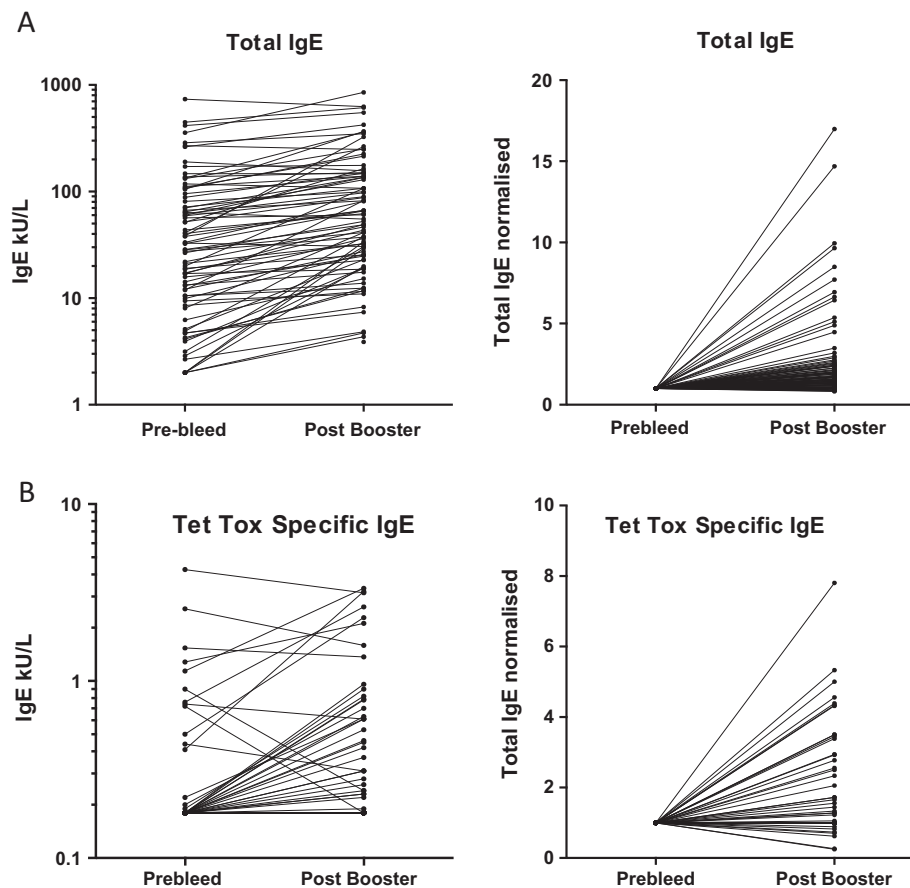


Fig. 2. Total and TT-specific IgE levels in subjects primed via the 3 dose infant DTP schedule, before and 4 weeks after the preschool DTaP booster – study B. *Panel A:* Total IgE levels (as kU/L and normalised) in individual children pre and post booster ($n = 81$; post-booster > pre-booster in DTaP group; $P = 0.001$ by Wilcoxon signed rank test). *Panel B:* TT-specific IgE levels in the same children as kU/L (post-booster > pre-booster; $P = 0.003$ by Wilcoxon signed rank test) and normalised against pre-booster titres.

proportions of children showing detectable IgE against the two major food allergens (egg and milk) and TT were transiently increased in the exclusively DTaP-primed group relative to those receiving mixed DTaP/DTwP-priming (Fig. 3A and Supplementary Fig. 3), and these differences disappeared from 6 mths onwards. In contrast no differences were detected in total IgE or IgE specific for the additional three allergens, across this age range. All the statistical analyses found no significant difference in total IgE between DTwP and DTaP vaccinated groups, and significant differences in specific IgE types at 4 mths only, whether measured as responding or not or on a continuous log scale, and either replacing non-detects by half the detection limit or using tobit regression. Adjustment for detection at 2 mths or for atopic family history made no material differences to these findings.

4. Discussion

The phenomenon of “bystander” stimulation of immunity during active vaccination is well recognised, and this process operates via a range of mechanisms including spillover of cytokines generated within vaccine-specific immune responses which can enhance non-specific T-cell and B-cell activation [20,21] with likely parallel effects on cytokine-sensitive functional programming of myeloid cell precursors in bone marrow [22]. In relation to the DTaP vaccine the focus of interest in this regard has been on Th2-associated bystander immunity at the time of booster administration to pre-primed children; while these booster responses include enhancement of total and vaccine antigen specific IgE production,

the present (Fig. 2; Supplementary Fig. 2) and earlier studies [11,13] have not detected significant effects on IgE production against individual clinically relevant allergens [10,13].

The situation at younger ages is less clear, and accordingly one focus of the present study was on the IgE-promoting effects of DTaP during early infancy when the immature immune system is first engaging with environmental allergens. Low level IgE production against food allergens and accompanying symptoms of mild gastrointestinal inflammation and eczema are relatively common during this period but are usually transient, as the immune system in most cases silences these responses via induction of protective immunological tolerance [23]. As shown in Fig. 3 early food allergen specific IgE responses were more common in exclusively DTaP-primed infants in our study group compared to those with mixed DTaP/DTwP priming, but this effect was only transient; data relating to their allergy-associated symptoms were not available to us. Comparable findings relating to the food allergen beta-lactoglobulin have been reported comparing infants receiving an acellular pertussis vaccine (Tetravac, Sanofi) at 2, 3 and 4 mths versus the corresponding cellular formulation (Tetracoq, Sanofi), notably that the Th2-skewing of allergen specific cytokine responses was significantly higher in recipients of the acellular formulation at 6 mths [24].

It is also well established that the bystander response to DTaP following the preschool booster immunization can include stimulation of IgE antibody directed against vaccine target antigens specific for tetanus, diphtheria and pertussis [10,12,17]. As shown in Fig. 3 in relation to the tetanus-derived antigen TT, induction of relevant IgE antibody also occurs during priming, and is transiently

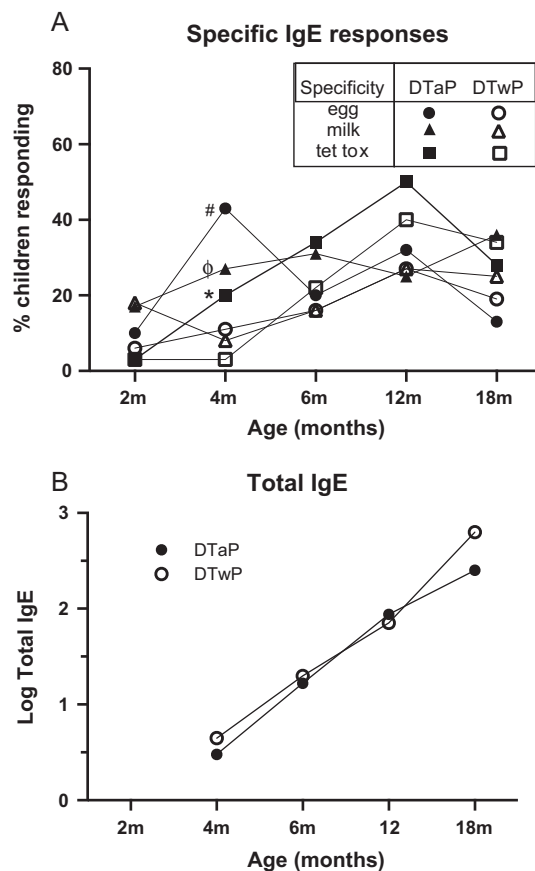


Fig. 3. Total and specific IgE levels in infants during priming with DTaP alone or in combination with DTwP via 4 dose infant vaccination schedule - study C. *Panel A:* Percentage of children (grouped as per Fig. 1) with specific IgE levels above the assay limits of detection (proportion in DTaP group > DTwP group: $^{\#}P = 0.003$, $^{\Phi}P = 0.06$, $^{\ast}P = 0.02$ by Fishers exact test test). *Panel B:* Log total IgE levels in the same children (no significant group differences at any time point).

enhanced in recipients of DTaP-only relative to those receiving mixed DTwP/DTaP priming. It has not previously been considered plausible that these vaccine-specific IgE antibodies are active in host responses to vaccine targets as there is no evidence of characteristic immediate hypersensitivity reactions occurring in immunized children during natural infection, or in children experiencing booster-associated injection site reactions which follow a much more delayed time course characteristic of T-cell mediated immunity [13]. However this conclusion may represent an over-simplification, in light of emerging findings of expression of the high affinity IgE-receptor (FcεR1) on cell populations other than the mast cells/basophils responsible for immediate hypersensitivity reactions. These include plasmacytoid dendritic cells (pDC) which are a major source of type 1 IFNs that mediate resistance to primary infection with respiratory pathogens [25,26], and also monocytes [27]. Cross-linking of IgE-loaded FcεR1 on pDC by binding of specific antigen inhibits the capacity of these cells to secrete type 1 IFNs [28,29], and cross-linking of the same receptors on monocytes has been shown to inhibit phagocytic functions that are central to pathogen clearance and eventual resolution of invasive infection [27]. The presence of pathogen-specific IgE at the time of infection may hence create risk for triggering both of these inhibitory mechanisms, thus slowing pathogen clearance.

Additionally, recent studies in animal models [30,31] and in children [32–34] have linked expression of FcεR1 on myeloid dendritic cells (mDC) in the airways and their precursors in blood during respiratory infection with increased severity and persistence of

ensuing lower airways inflammation. Mechanistic studies have demonstrated that the presence of FcεR1-bound IgE on mDC markedly enhances their capacity for uptake and subsequent presentation of locally available antigens to corresponding cytokine secreting Th2-memory cells [35,36]. In the context of host responses to respiratory pathogens, this provides a mechanism for mDC-mediated induction of Th2 cytokine signals at the infection site [15]. Based on experience with other human pathogens exemplified by dengue and herpes viruses [37,38], such Th2 signals are likely to antagonise local pathogen clearance mechanisms (a process termed immune deviation). Collectively, these findings suggest that a range of mechanisms operate in the context of anti-microbial immunity, which are potentially susceptible to covert IgE-mediated modulation.

The role of such mechanisms in the pathogenesis of airways inflammation associated with respiratory infections is an issue of major current interest in the paediatric asthma field, in particular the contribution of IgE-mediated inflammation induced during viral infections to the severity of asthma exacerbations triggered by these agents [31,33]. A series of studies confirming that IgE production against many common respiratory pathogens including HRV, RSV and influenza is a frequent consequence of infection [39–42], together with the recent demonstration of the efficacy of anti-IgE antibody as a prophylactic treatment to reduce the frequency of virus-associated asthma exacerbations in children [43], have added significant weight to these discussions.

To date however, the potential applicability of these IgE-dependent mechanisms to the pathogenesis of pertussis disease has not been seriously considered, but given findings indicating bystander boosting of pertussis-specific IgE following DTaP vaccination at levels comparable to those shown here for tetanus antigen [10,12], this issue merits re-examination. This view is reinforced by recent and more direct observations from Sheridan et al. [44]. These investigators conducted a retrospective data linkage analyses of pertussis notifications and vaccination records in a cohort of 58,233 children born in Queensland Australia in 1998 following the local introduction of DTaP, tracking their subsequent pertussis reporting rates spanning 1999–2011. Their findings which are strikingly complementary to those described here, indicate major variations in pertussis protection between subgroups who received different combinations of DTaP and/or DTwP priming [44]. Notably, exclusive DTaP priming was associated with a significantly higher risk of subsequent pertussis relative to exclusive DTwP priming; moreover most of the increased protection afforded by exclusive DTwP priming could be achieved by inclusion of a single (first) dose of DTwP in the 3 dose infant priming schedule [44]. The increased protection relative to exclusive DTaP persisted for at least a decade, supporting the key role of the first infant vaccine dose given in early infancy in programming effective long term vaccine specific immunological memory. The findings discussed above suggest that the balance between pertussis-specific Th1/Th2 cytokine production within the memory response may be key to this outcome, and further that downstream effects of Th2 cytokines in regulation of antibody class switching may also be important factors. In this regard, it is pertinent to emphasise that the high levels of booster-induced TT specific IgE reported here in some children up to 5yrs after priming (Fig. 2; Supplementary Fig. 1) are similar to those observed for pertussis-specific IgE in comparably boosted children in the same age group [10].

It is plausible based on the discussion above that high level pertussis-specific IgE in serum may serve as a biomarker of risk for breakthrough pertussis infection and disease amongst vaccinated children, and this possibility could be tested prospectively, or perhaps retrospectively with existing serum repositories. If this hypothesis is proven, it would provide a rationale for adjuvantising DTaP formulations, for example by the inclusion of a chemically

defined TLR agonist of the type shown to be capable of binding to the Th2-skewing alum component of the vaccine with resultant attenuation of its Th2-stimulatory properties [45]. An alternative possibility would be to reintroduce DTWP as the first vaccine in the infant DTaP priming schedule, provided regulatory approval could be obtained.

Since submission of this manuscript a retrospective analysis capturing all nationally reported US pertussis cases 1990–2014 has appeared (editorialised in <http://dx.doi.org/10.1001/jamapediatrics.2016.0157>) suggesting that the waning of resistance to pertussis in previously vaccinated children during adolescence is significantly reduced in those who received at least some DTWP dose(s) during infant priming.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2016.05.048>.

References

- [1] Octavia S, Sintchenko V, Gilbert GL, Lawrence A, Keil AD, Hogg G, et al. Newly emerging clones of *Bordetella pertussis* carrying prn2 and ptxP3 alleles implicated in Australian pertussis epidemic in 2008–2010. *J Infect Dis* 2012;205(8):1220–4.
- [2] Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. *Clin Infect Dis: Off Publ Infect Dis Soc Am* 2012;54(12):1730–5.
- [3] Leung AK. Anaphylaxis to DPT vaccine. *J R Soc Med* 1985;78(2):175.
- [4] Edwards KM, Berbers GA. Immune responses to pertussis vaccines and disease. *J Infect Dis* 2014;209(Suppl 1):S10–5.
- [5] Rowe J, Macaubas C, Monger T, Holt BJ, Harvey J, Poolman JT, et al. Heterogeneity in diphtheria–tetanus–acellular pertussis vaccine-specific cellular immunity during infancy: relationship to variations in the kinetics of postnatal maturation of systemic Th1 function. *J Infect Dis* 2001;184:80–8.
- [6] Ausiello CM, Urbani F, la Sala A, Lande R, Cassone A. Vaccine- and antigen-dependent type 1 and type 2 cytokine induction after primary vaccination of infants with whole-cell or acellular pertussis vaccines. *Infect Immun* 1997;65(6):2168–74.
- [7] Ryan M, Murphy G, Ryan E, Nilsson L, Shackley F, Gotheffors L, et al. Distinct T-cell subtypes induced with whole cell and acellular pertussis vaccines in children. *Immunology* 1998;93(1):1–10.
- [8] Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 2003;3(9):721–32.
- [9] Aalberse RC, van Ree R, Danneman A, Wahn U. IgE antibodies to tetanus toxoid in relation to atopy. *Int Arch Allergy Immunol* 1995;107(1–3):169–71.
- [10] Ryan EJ, Nilsson L, Kjellman N, Gotheffors L, Mills KH. Booster immunization of children with an acellular pertussis vaccine enhances Th2 cytokine production and serum IgE responses against pertussis toxin but not against common allergens. *Clin Exp Immunol* 2000;121(2):193–200.
- [11] Dannemann A, van Ree R, Kulig M, Bergmann RL, Bauer P, Forster J, 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(3):262–7.
- [12] Hedenskog S, Bjorksten B, Blennow M, Granstrom G, Granstrom M. Immunoglobulin E response to pertussis toxin in whooping cough and after immunization with a whole-cell and an acellular pertussis vaccine. *Int Arch Allergy Appl Immunol* 1989;89(2–3):156–61.
- [13] Rowe J, Yerkovich ST, Richmond P, Suriyaarachchi D, Fisher E, Feddema L, et al. Th2-associated local reactions to the acellular diphtheria–tetanus–pertussis vaccine in 4-to 6-year-old children. *Infect Immun* 2005;73(12):8130–5.
- [14] White OJ. Immunogenicity and reactogenicity of acellular pertussis vaccines in newborns and preschool aged children. PhD Thesis. University of Western Australia library; 2010.
- [15] Holt PG, Strickland DH, Hales BJ, Sly PD. Defective respiratory tract immune surveillance in asthma. *Chest* 2014;145(2):370–8.
- [16] Marshall H, Nolan T, Robertson D, Richmond P, Lambert S, Jacquet JM, et al. A comparison of booster immunisation with a combination DTaP-IPV vaccine or DTaP plus IPV in separate injections when co-administered with MMR, at age 4–6 years. *Vaccine* 2006;24(35–36):6120–8.
- [17] 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(4):2411–7.
- [18] Siegrist CA. Neonatal and early life vaccinology. *Vaccine* 2001;19(25–26):3331–46.
- [19] Adkins B. T-cell function in newborn mice and humans. *Immunol Today* 1999;20(7):330–5.
- [20] Donnenberg AD, Elfenbein GJ, Santos GW. Secondary immunization with a protein antigen (tetanus toxoid) in man. Characterization of humoral and cell-mediated regulatory events. *Scand J Immunol* 1984;20(4):279–89.
- [21] Fernandez V, Andersson J, Andersson U, Troye-Blomberg M. Cytokine synthesis analyzed at the single-cell level before and after revaccination with tetanus toxoid. *Eur J Immunol* 1994;24(8):1808–15.
- [22] Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;3(1):23–35.
- [23] Holt PG. Environmental factors and primary T-cell sensitisation to inhalant allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr Allergy Immunol* 1995;6:1–10.
- [24] Mascart F, Hainaut M, Peltier A, Verscheure V, Levy J, Locht C. Modulation of the infant immune responses by the first pertussis vaccine administrations. *Vaccine* 2007;25(2):391–8.
- [25] Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol* 2000;1(4):305–10.
- [26] Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 1999;284:1835–7.
- [27] Pyle DM, Yang VS, Gruchalla RS, Farrar JD, Gill MA. IgE cross-linking critically impairs human monocyte function by blocking phagocytosis. *J Allergy Clin Immunol* 2013;131(2):491–500. e1–5.
- [28] Durrani SR, Montville DJ, Pratt AS, Sahu S, DeVries MK, Rajamanickam V, et al. Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children. *J Allergy Clin Immunol* 2012;130(2):489–95.
- [29] Gill MA, Bajwa G, George TA, Dong CC, Dougherty II, Jiang N, et al. Counterregulation between the FcεpsilonR1 pathway and antiviral responses in human plasmacytoid dendritic cells. *J Immunol* 2010;184(11):5999–6006.
- [30] Al-Garawi A, Husain M, Ilieva D, Humbles AA, Kolbeck R, Stampfli MR, et al. Shifting of immune responsiveness to house dust mite by influenza A infection: genomic insights. *J Immunol* 2012;188(2):832–43.
- [31] Grayson MH, Cheung D, Rohlfing MM, Kitchens R, Spiegel DE, Tucker J, et al. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med* 2007;204(11):2759–69.
- [32] Bosco A, Ehteshami S, Stern DA, Martinez FD. Decreased activation of inflammatory networks during acute asthma exacerbations is associated with chronic airflow obstruction. *Mucosal Immunol* 2010;3(4):399–409.
- [33] Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. *Nat Med* 2012;18(5):726–35.
- [34] Subrata LS, Bizzintino J, Mamessier E, Bosco A, McKenna KL, Wikstrom ME, et al. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *J Immunol* 2009;183(4):2793–800.
- [35] Novak N, Kraft S, Bieber T. Unraveling the mission of FcεRI on antigen-presenting cells. *J Allergy Clin Immunol* 2003;111:38–44.
- [36] Novak N, Tepel C, Koch S, Brix K, Bieber T, Kraft S. Evidence for a differential expression of the FcεpsilonRIγ chain in dendritic cells of atopic and nonatopic donors. *J Clin Invest* 2003;111(7):1047–56.
- [37] Koraka P, Murgue B, Deparis X, Setiati TE, Suharti C, van Gorp EC, et al. Elevated levels of total and dengue virus-specific immunoglobulin E in patients with varying disease severity. *J Med Virol* 2003;70(1):91–8.
- [38] Weber KS, Grone HJ, Rocken M, Klier C, Gu S, Wank R, et al. Selective recruitment of Th2-type cells and evasion from a cytotoxic immune response mediated by viral macrophage inhibitory protein-II. *Eur J Immunol* 2001;31(8):2458–66.
- [39] Smith-Norowitz TA, Kusonruksa M, Wong D, Norowitz MM, Joks R, Durkin HG, et al. Long-term persistence of IgE anti-influenza A H1N1 virus antibodies in serum of children and adults following influenza A vaccination with subsequent H1N1 infection: a case study. *J Inflam Res* 2012;5:111–6.
- [40] Welliver RC, Sun M, Hildreth SW, Arumugham R, Ogra PL. Respiratory syncytial virus-specific antibody responses in immunoglobulin A and E isotypes to the F and G proteins and to intact virus after natural infection. *J Clin Microbiol* 1989;27(2):295–9.
- [41] Welliver RC, Sun M, Rinaldo D, Ogra PL. Predictive value of respiratory syncytial virus-specific IgE responses for recurrent wheezing following bronchiolitis. *J Pediatr* 1986;109(5):776–80.
- [42] Tam JS, Jackson WT, Hunter D, Proud D, Grayson MH. Rhinovirus specific IgE can be detected in human sera. *J Allergy Clin Immunol* 2013;132(5):1241–3.
- [43] Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *New Engl J Med* 2011;364(11):1005–15.
- [44] Sheridan SL, Ware RS, Grimwood K, Lambert SB. Number and order of whole cell pertussis vaccines in infancy and disease protection. *JAMA* 2012;308(5):454–6.
- [45] Bortolatto J, Mirotti L, Rodriguez D, Gomes E, Russo M. Adsorption of toll-like receptor 4 agonist to alum-based tetanus toxoid vaccine dampens pro-T helper 2 activities and enhances antibody responses. *J Immunol Res* 2015: PMC4562177.

534 **Supplementary Table 1: Vaccination Schedules**

Study Cohort	Vaccine Manufacturer Details	Ages at vaccination (months)					
		2	4	6	12	18	48-72
A	Reference 13	DTaP or DTwP	DTaP	DTaP	MMR	DTaP	DTaP/IPV/MMR
B	Reference 16	DTaP	DTaP	DTaP	MMR	-	DTaP/IPV/MMR
C	Reference 5	DTaP or DTwP	DTaP	DTaP	MMR	DTaP	Not followed

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Supplementary Figure 1: TT-specific IgE levels in subjects primed with DTaP alone or in combination with DTwP via the 4 dose infant vaccination schedule, before and 4 weeks after the preschool DTaP booster – study A.

Legend: TT-specific IgE titres for individual children were normalised to respective pre-booster titres. The number of subjects in whom titres did not change are shown in parentheses (overall group sizes DTwP n=16; DTaP n=33; post-booster>pre-booster in DTaP group; P=0.001 by Wilcoxon signed rank test).

Supplementary Figure 2: Allergen-specific IgE levels in subjects primed via the 3 dose infant DTP schedule, before and 4 weeks after the preschool DTaP booster – study B.

Legend: Data are shown for IgE titres against milk, peanut, egg, cat and HDM allergen. The dotted line designates the currently accepted threshold for clinically relevant sensitization (0.35 kU/L). There were no significant group differences between pre and post-booster IgE levels.

Supplementary Figure 3: Specific IgE levels in infants at 4mths following receipt of a single dose of DTaP or DTwP at 2mths - study C.

Legend: Data points for individual children show specific IgE titres as kU/L (DTaP > DTwP: egg P=0.001, milk P=0.02, TT P=0.02 by Mann Whitney U test).

Figures
Figure 1

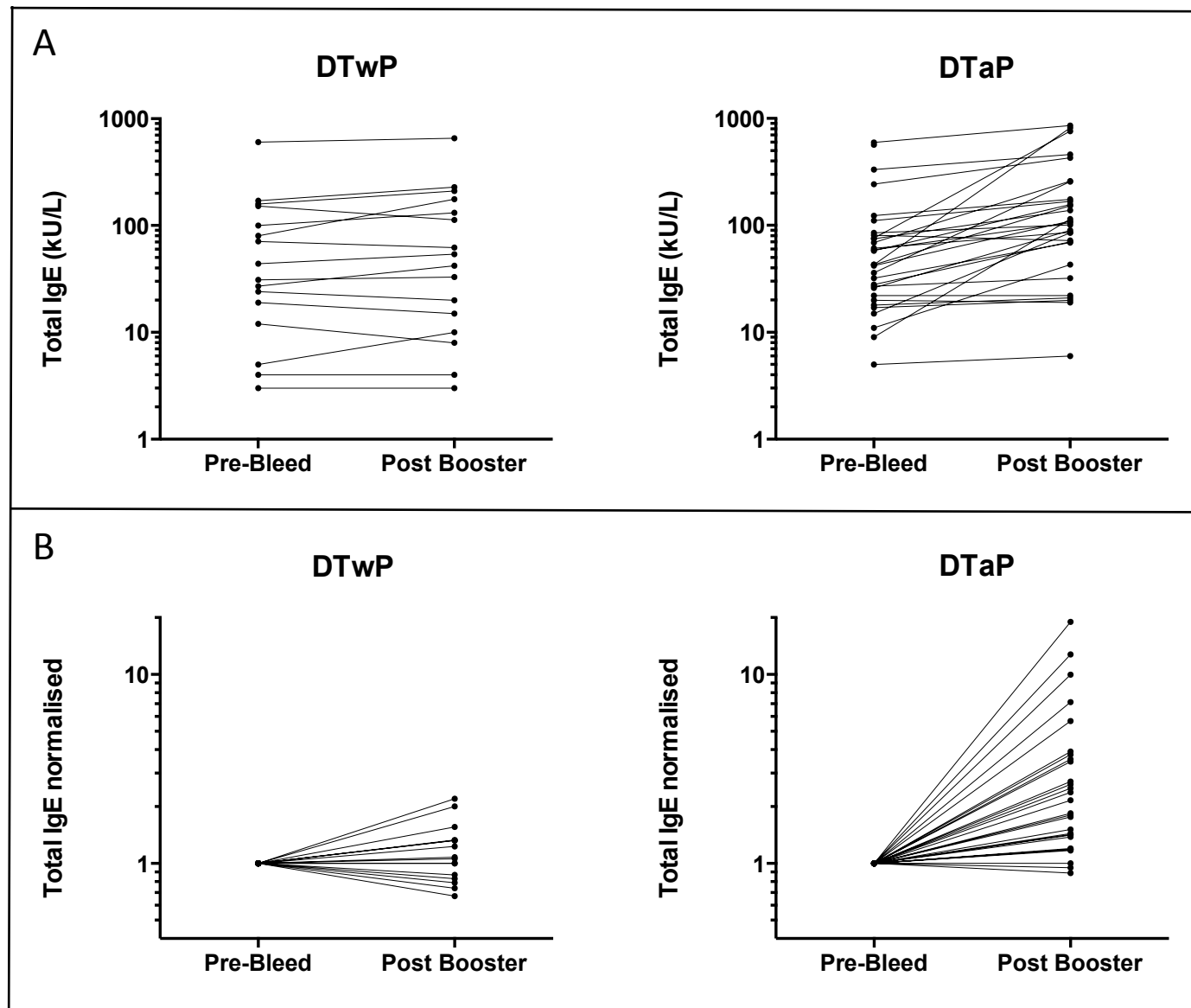


Figure 2

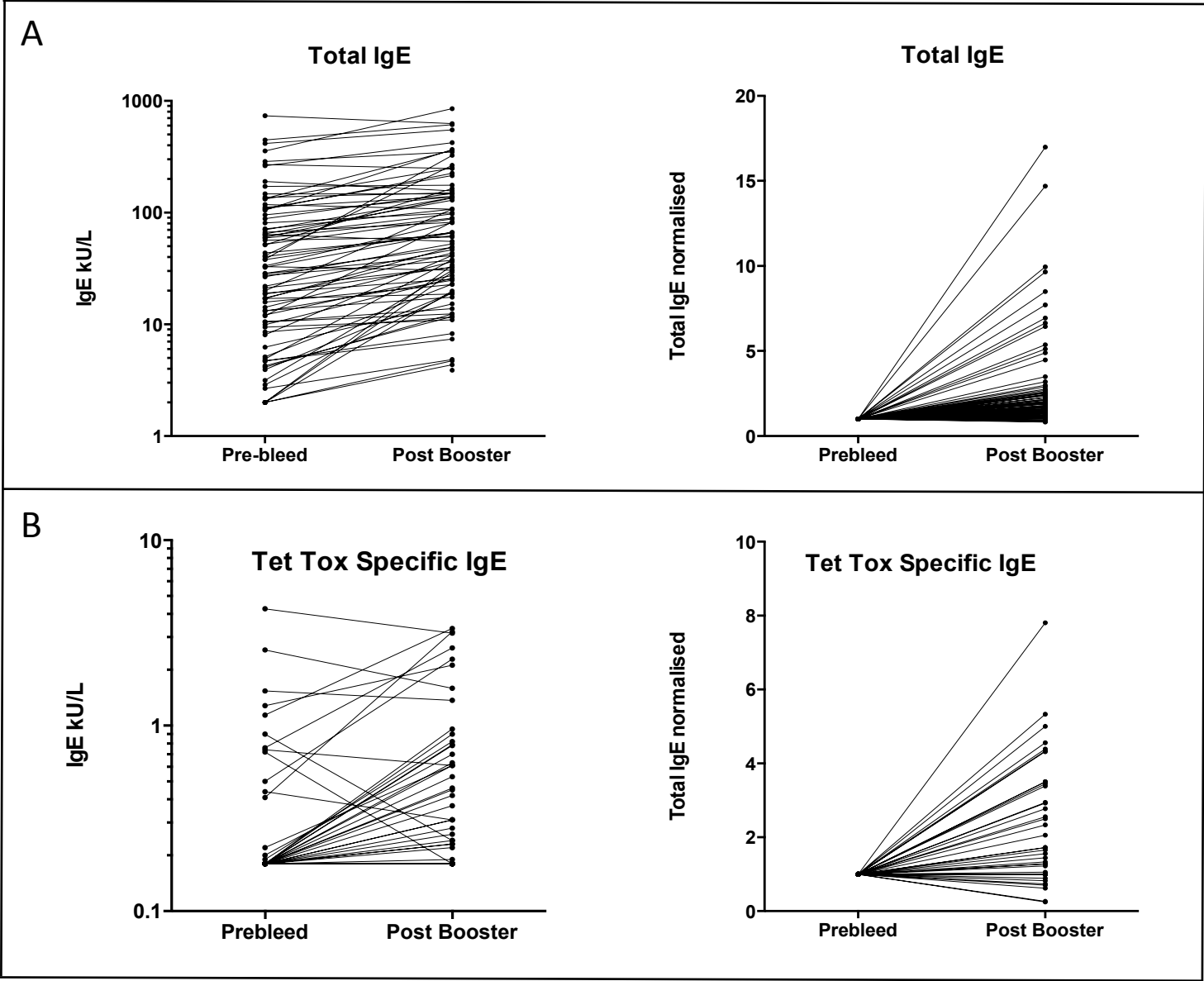


Fig 3A

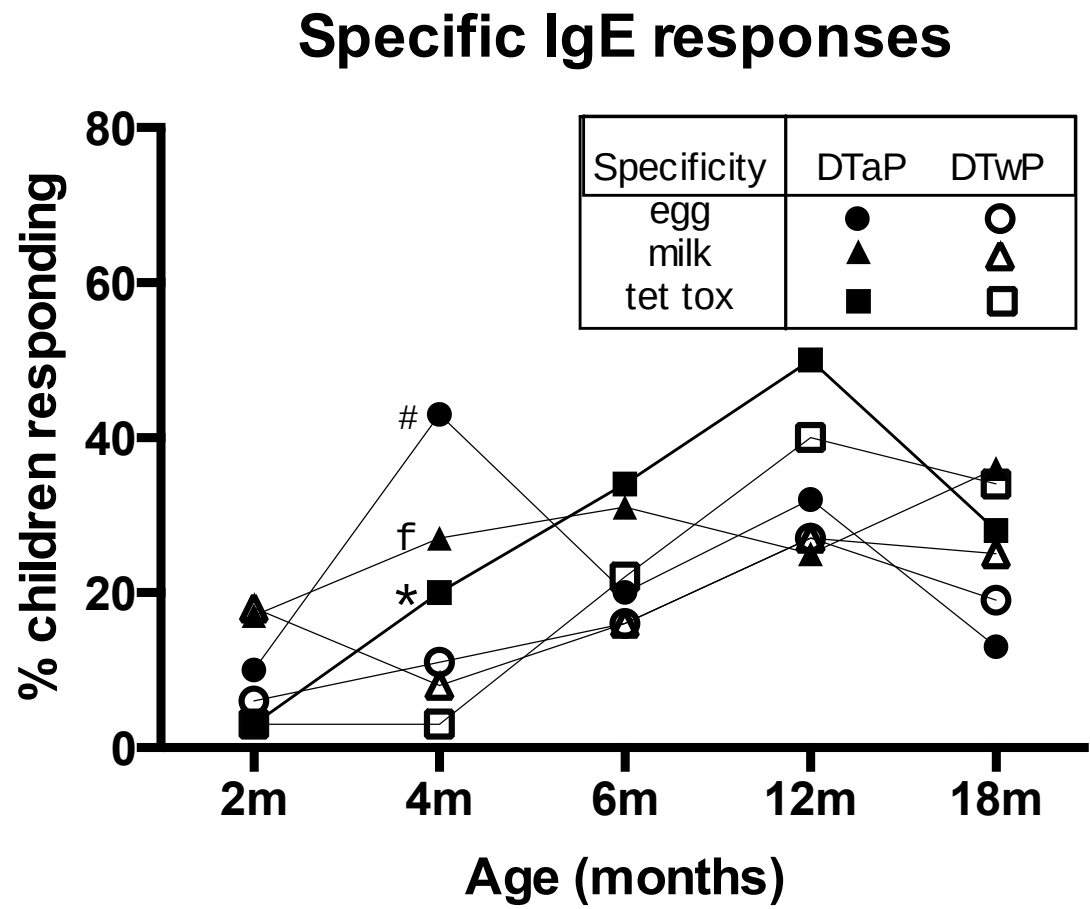
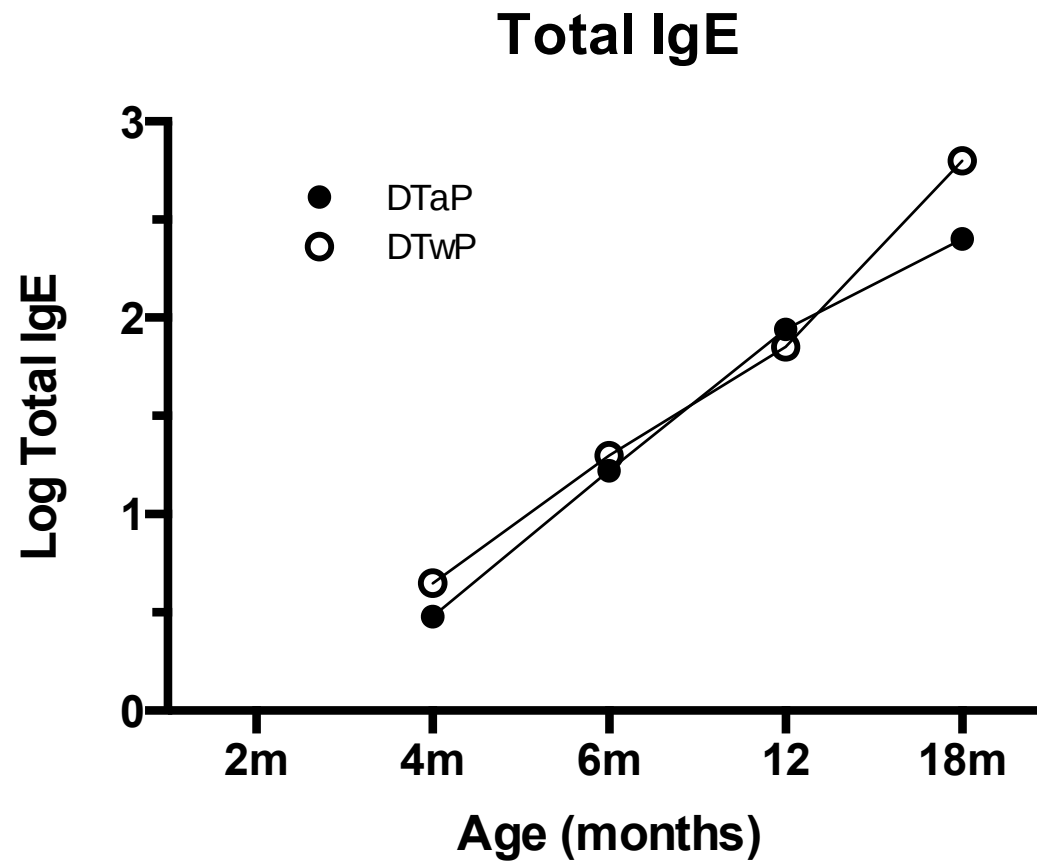
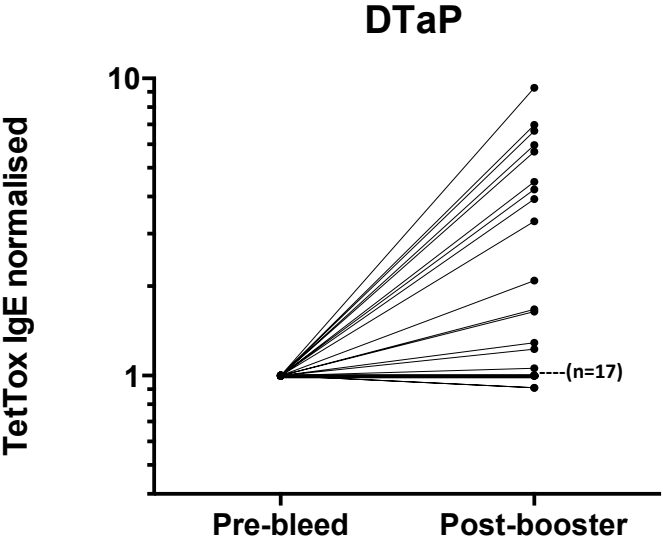
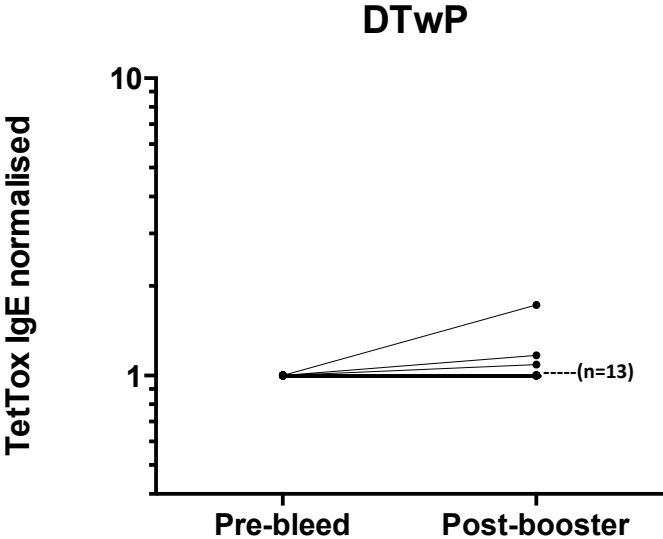


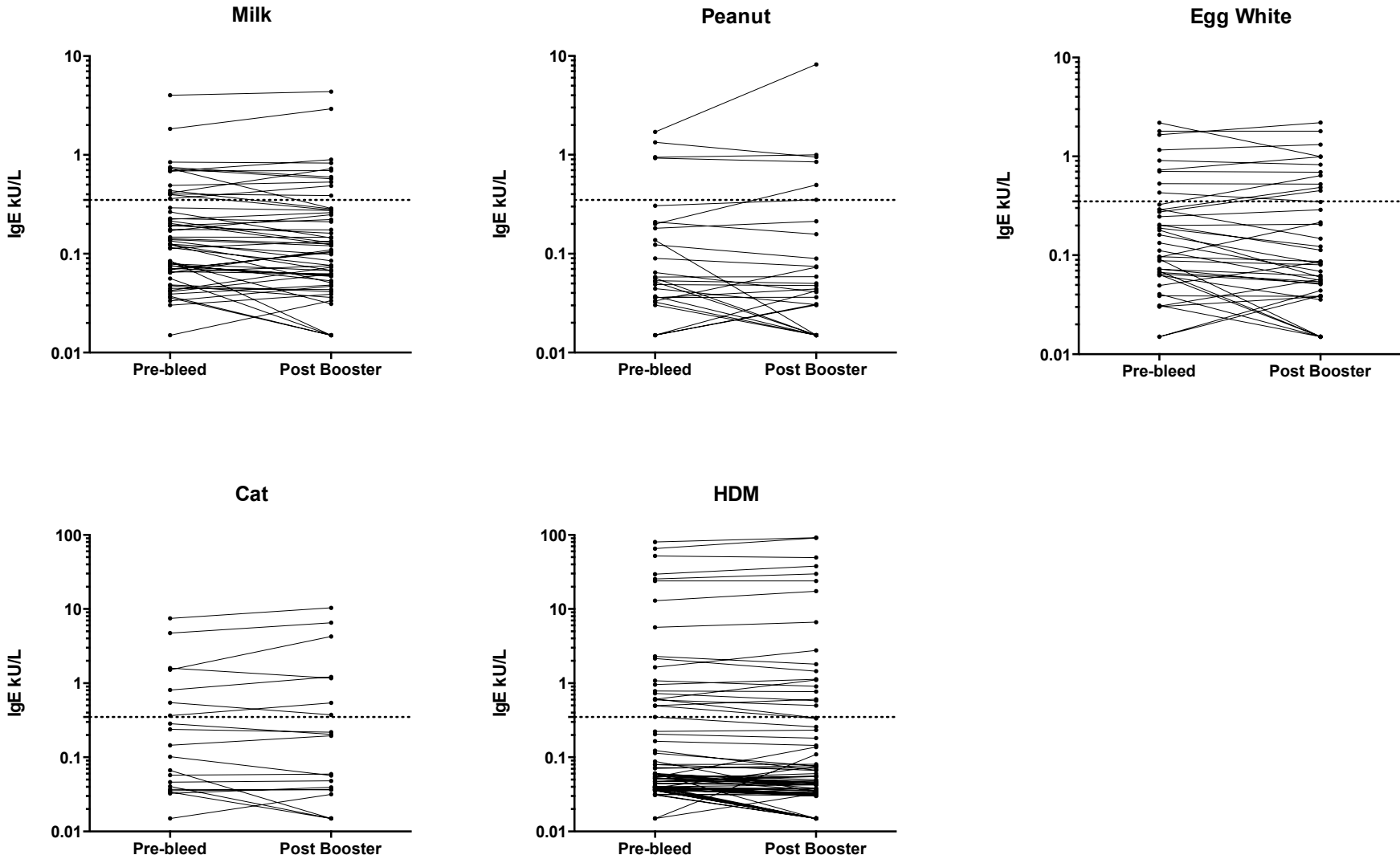
Figure 3B



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

