Effects of a Diphtheria-Tetanus-Acellular Pertussis Vaccine on Immune Responses in Murine Local Lymph Node and Lung Allergy Models

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Pertussis is a disease of the respiratory tract caused by Bordetella pertussis and among the infectious diseases with the highest morbidity and mortality worldwide. Although vaccination is efficacious against disease, pertussis has recently re-emerged despite high vaccine coverage (10). Antigenic divergence between clinical isolates and vaccines has been observed (23). Thus, there is an ongoing need to evaluate vaccine efficacy against clinical isolates (37). In such studies, mice vaccinated with both a whole-cell vaccine (WCV) and an acellular pertussis vaccine (ACV) showed increased lung pathology compared to nonvaccinated mice after challenge with B. pertussis. In addition, these mice showed an increase in the number of eosinophils in the bronchoalveolar lavage fluid (BALF), total serum immunoglobulin E (IgE), and ex vivo Th2 cytokine production by cells from the bronchial lymph nodes, collectively suggesting an immediate hypersensitivity (IH) response (36).

The possibility of an increased risk of atopic disorders in children vaccinated against pertussis or diphtheria-tetanus-pertussis has been the subject of debate for over a decade (for references, see reference 4). Since our findings of increased IH in vaccinated mice may possibly support such an increased risk, we sought to evaluate the effects of vaccination in two allergy models. The first allergy model is the local lymph node assay (LLNA) response against the respiratory allergen trimellitic anhydride and the contact allergen dinitrochlorobenzene. This assay measures effects on the sensitization (afferent) phase of the immune response (only), with a more Th2-directed response toward the respiratory allergen and a more Th1-directed response toward the contact allergen (8, 34, 35, 39). Immunosuppression due to measles virus infection (25) and exposure to the immunosuppressive drug cyclosporine (38) could be identified by using the LLNA, indicating that this assay can detect effects on immune function. The second allergy model is a lung allergy model with intranasal sensitization and challenge with ovalbumin (OVA). Whereas OVA alone induced OVA-specific IgE, lung pathology, and eosinophils in the BALF, coexposure of OVA and particulate matter (diesel exhaust particles, residual oil fly ash, and ambient particles) during the sensitization phase increased the size of these effects (33). Moreover, it has been described as a model for human lung allergy (14). This suggested to us that this model be also suitable to detect putative changes induced by vaccination.

Since many countries, including The Netherlands, have switched from WCV to ACV and since ACV induces a stronger IH response than WCV (36), we chose to study the vaccination effects of ACV. It may be expected that not all vaccine components similarly contribute to these effects. We therefore also analyzed the individual components.

Here we show that ACV vaccination (i) increased total serum IgE in BALB/c but not C57BL/6 mice; (ii) decreased the LLNA response to TMA, together with a small increase in the response to DNCB, with all vaccine components except fila-
mentous hemagglutinin (FHA) contributing to the decreased response to TMA; and (iii) did not further change the effects that were induced by sensitization with OVA alone.

MATERIALS AND METHODS

Animals. Male or female BALB/c mice, obtained from our own breeding colony, were used at 6 to 8 weeks of age. Female C57BL/6 mice were obtained from Jackson (Bar Harbor, ME). The diet consisted of ground standard laboratory chow (RMM-H; Hope Farms, Woerden, The Netherlands). Food and water were given ad libitum. All animal experiments were performed according to national and international guidelines.

Vaccines. The diphtheria-tetanus-acellular pertussis (DTaP) vaccine is a combined vaccine against diphtheria (D; 25 LF diphtheria toxoid), tetanus (T; 10 LF tetanus toxoid), and pertussis (25 μg of formaldehyde- and glutaraldehyde-detoxified pertussis toxin [PT], 25 μg of FHA, and 8 μg of pertactin [PRN; GlaxoSmithKline, Rixensart, Belgium) in 0.5 ml of saline. PT and FHA were detoxified pertussis toxin [PT], 25 μg of FHA, and 8 μg of pertactin [PRN; GlaxoSmithKline, Rixensart, Belgium) in 0.5 ml of saline. PT and FHA were previously prepared (16). DT vaccine (6.7 LF diphtheria toxoid and 5 LF tetanus toxoid in 0.5 ml of saline) was obtained from Sanofi Pasteur, Lyon, France. Adjuvant is 2% Al(OH)3 gel (Serva, Heidelberg, Germany). The amount of Al(OH)3 administered per mouse was 1.5 mg.

Total serum IgE. Mice received a subcutaneous (s.c.) injection with a one-fifth human dose (1/5 HD) of DTaP vaccine in 0.5 ml of adjuvant or with 0.5 ml of adjuvant alone at 28 and 14 days prior to autopsy. At autopsy, animals were anesthetized with ketamine, rompun, and atropine (KRA), and blood was collected from the orbital plexus. Blood was allowed to clot overnight at 4°C and then centrifuged for 2 min at 13,000 rpm. Total serum IgE was measured as described by Brewer et al. (7; see reference 36 for details).

LLNA. Mice received an s.c. injection with 1/5 HD of DTaP vaccine in 0.5 ml of adjuvant or with 0.5 ml of adjuvant alone at 28 and 14 days prior to sensitization. Alternatively, mice received an s.c. injection with 5 μg of PT, 5 μg of FHA, 1.6 μg of PRN, 1/5 HD of DT, PT-FHA-PRN-DT combined, or 1/5 HD of DTaP vaccine in 0.5 ml of adjuvant or with 0.5 ml of adjuvant alone at 28 and 14 days prior to sensitization.

The chemicals used were trimellitic anhydride (TMA; 97% purity; Sigma-Aldrich, Zwijndrecht, The Netherlands) and 2,4-dinitrochlorobenzene (DNCB; 98% purity; Sigma-Aldrich). TMA was dissolved in 4:1 acetic-acetone oil (AOO) to final concentrations of 10, 5, 2.5, and 1% (wt/vol). DNCB was dissolved in AOO to final concentrations of 1, 0.5, 0.25, and 0.1% (wt/vol). AOO was used as vehicle control. We have previously shown that 10% TMA and 1% DNCB induce a similar lymphocyte proliferation (34). For these consecutive days, 25 μl was applied to the dorsum of both ears. At 3 days after the last application, the local (auricular) lymph nodes (LN) were excised and weighed.

Preparation of cell suspensions, lymphocyte proliferation culture, and cell culture for cytokine measurements. Briefly, LN cell suspensions were prepared and the number of cells per two LN (per animal) established. The cell suspensions were used to measure the spontaneous ([3H]thymidine uptake, as well as the concanavalin A-induced production of cytokines (35).

Cytokine measurements. A five-plex panel containing beads for mouse interleukin-4 (IL-4), IL-5, IL-10, IL-13, and gamma interferon (IFN-γ; Bio-Rad, Hercules, CA) was used (36).

For transforming growth factor β (TGF-β), a separate enzyme-linked immunosorbent assay (ELISA) was performed as follows. MaxiSorp 96-well plates (Nunc-Immuno Plate, Roskilde, Denmark) were coated with 2 μg of coating antibody (Biosource, Camarillo, CA/ml) in phosphate-buffered saline (PBS). After overnight incubation at 4°C the plates were washed, and blocking buffer (1% bovine serum albumin [BSA; Sigma-Aldrich] plus 0.1% Tween 20 [Sigma-Aldrich]) was added. After incubation for 1 h, the plates were washed. Culture supernatants (100 μl) were pretreated by adding 4 μl of 1 N HCl and incubated for 15 min, and 3 μl of 1 N NaOH was added. Standards (Biosource) and pretreated culture supernatants (100 μl), as well as 50 μl of detection antibody (Biosource; 0.32 μg/ml), were added. After incubation for 2 h, the plates were washed, and streptavidin-HRP (R&D, Minneapolis, MN) was added. After incubation for 30 min, the plates were washed, and TMB solution (Sigma-Aldrich) was added. After incubation for 30 min, the reaction was stopped by adding 10% H2SO4, and the plates were read at 450 nm. Each washing step was performed six times with PBS plus 0.1% Tween 20. All steps except the overnight incubation were performed at room temperature.

OVA sensitization and challenge. Mice were randomly assigned to four groups (A to D). Groups A and B were s.c. injected with 1/5 HD of DTaP in 0.5 ml of adjuvant 14 days and 2 h prior to the first sensitization (day 0). At days 0, 14, 35, 38, and 41, the mice were slightly anesthetized with halothane; at the moment of awakening, they received into both nostrils 0.4 mg of OVA (grade V; Sigma-Aldrich)/ml in 50 μl of saline (groups A and C) or 50 μl of saline alone (group D). At autopsy (day 42), the animals were anesthetized with KRA.

OVA-specific IgE. Blood was collected from the orbital plexus and allowed to clot overnight at 4°C and centrifuged for 2 min at 13,000 rpm. OVA-specific IgE was measured as previously described (33). Briefly, MaxiSorp 96-well plates (Nunc) were coated overnight at 4°C with 2 μg of rat anti-mouse IgE (Zymed, South San Francisco, CA)/ml in 0.04 M Na2CO3 buffer (pH 9.6). Each incubation step was followed by extensive washing (six cycles) with PBS plus 0.1% Tween 20. The plates were blocked with Tris-buffered saline (TBS) plus 1% BSA (pH 8.0; Sigma-Aldrich) for 1 h at 37°C or overnight at 4°C. Serum samples (1:10 diluted) were incubated for 1 h at 37°C in TBS plus 0.05% Tween 20. OVA was labeled with digoxigenin (DIG) by using a commercial kit (see below). DIG-labeled OVA was diluted ~1,000 times (depending on the batch) in TBS plus 0.05% Tween 20, followed by incubation for 1 h at 37°C. Anti-DIG-PO (50 U/ml; Roche, Almere, The Netherlands) was diluted 625 times in TBS plus 0.05% Tween 20, followed by incubation for 2 h at 37°C. Finally, the plates were incubated with TMB for 3 min at 20°C. The reaction was stopped by adding 50 μl of 2 M H2SO4, and the optical density was read at 450 nm.

OVA was labeled with DIG by using a DIG antibody labeling kit (Roche). Unbound DIG was separated from OVA-DIG on a Sephadex G-25 column. The labeled protein was collected in 1-ml fractions. After determining the protein concentration using the BCA protein assay (Pierce, Rockford, IL), the fractions with the highest concentration were pooled, and the concentration was determined.

To obtain positive control serum, mice were sensitized by intraperitoneal injection of 20 μg of OVA emulsified in 2.25 mg of aluminum hydroxide in a total volume of 100 μl at day 0 and then challenged by intranasal administration of 1.4 mg of nebulized OVA/ml in PBS at days 35, 38, and 41. Sera from different mice were pooled and arbitrarily set at 1,000 U. The extinction values of the samples were divided by the extinction value of the positive control and are expressed in units.

Histological examinations. Perfusion of the lungs was performed with 2 ml of PBS plus 3.5% heat-inactivated fetal calf serum in the right heart ventricle. The lungs were fixed intratracheally using 4% formalin for 24 h. After overnight dehydration, they were embedded in paraffin. Sections (5 μm) were cut and stained with hematoxylin-eosin. Histological lesions were semiquantitatively scored as absent, minimal, slight, moderate, strong, or severe (0, 1, 2, 3, 4 or 5, respectively). These scores incorporate the frequency as well as the severity of the lesions.

BALF cells. Perfusion of the lungs was performed with 2 ml of PBS plus 3.5% heat-inactivated fetal calf serum in the right heart ventricle. The lungs were excised and used to obtain BALF cells. A cannula was placed intratracheally and fixed by using a suture. The lungs were placed in a 50-ml tube filled with PBS. A 1-ml portion of PBS was brought into the lung and sucked up. This was repeated twice. BALF cells were spun down, resuspended in PBS, counted by using a Coulter Counter, and visually differentiated after Giemsa staining.

![FIG. 1. Vaccination effects on total IgE. BALB/c (□) and C57BL/6 (■) mice were s.c. injected with DTaP or adjuvant 28 and 14 days before sacrifice. Blood was taken via the orbital plexus, and serial dilutions from test sera and normal mouse serum (NMS) were analyzed for total IgE by ELISA. The data are expressed as the mean level relative to NMS ± the SEM (n = 4). * P < 0.05; ***, P < 0.001 (ANOVA, followed by Bonferroni’s test).](http://clv.asm.org/Downloaded.fit/1cvi110110.pdf)
Statistics. Analysis of variance (ANOVA), followed by the Bonferroni or Tukey post-hoc test, was performed (SPSS, Chicago, IL). Significance levels were $P < 0.05$, $P < 0.01$, and $P < 0.001$ as indicated in the figures. Histological data were analyzed by using the nonparametric Wilcoxon test (SPSS). The $t$ test was used to analyze the effects of OVA sensitization, irrespective of vaccination (SPSS).

RESULTS

Strain-dependent increase in total serum IgE. We have previously shown that DTaP vaccination of BALB/c and C57BL/6 mice, followed by *B. pertussis* infection, resulted in a 66- and 7-fold increase, respectively, in total serum IgE. Here we measured the effects on total serum IgE after vaccination only.

In BALB/c mice, vaccination resulted in a 70-fold increase in total serum IgE compared to adjuvant-treated mice. In C57BL/6 mice, a similar vaccination resulted in an increase of only fourfold (not statistically significant). The total serum IgE was significantly lower in vaccinated C57BL/6 mice than in vaccinated BALB/c mice (Fig. 1).

LLNA response to the respiratory allergen TMA. It has been shown previously that the LLNA can detect altered immune function. Here we measured DTaP vaccination effects in the LLNA using the Th2-inducing allergen TMA and the Th1-inducing allergen DNCB.

In the adjuvant controls, TMA application resulted in a
dose-dependent increase in $[^{3}H]$thymidine incorporation and IL-4, IL-5, IL-10, IL-13, and IFN-γ production. At 5 and 10% TMA, the vaccinated mice showed a significantly decreased cell proliferation compared to the adjuvant controls (Fig. 2A). Next, at 10% TMA (only), these mice showed a significantly decreased production of IL-4 (Fig. 2B), IL-10 (Fig. 2D), IL-13 (Fig. 2E), and IFN-γ (Fig. 2F) compared to the adjuvant controls. IL-5 production was not significantly different between the vaccinated mice and adjuvant controls.

**LLNA response to the contact allergen DNCB.** In the adjuvant controls, DNCB application resulted in a dose-dependent increase in $[^{3}H]$thymidine incorporation and IL-4, IL-5, IL-10, IL-13, and IFN-γ production. At 0.5% DNCB, the vaccinated mice showed a significantly increased production of IL-4 (Fig. 3B) and IL-5 (Fig. 3C) compared to the adjuvant controls. Next, at 1% DNCB they showed a significantly increased cell proliferation (Fig. 3A), as well as a significantly increased production of IL-5 (Fig. 3C) and IFN-γ (Fig. 3F), compared to the adjuvant controls. IL-10 (Fig. 3D) and IL-13 (Fig. 3E) production was not significantly different between the vaccinated mice and adjuvant controls.

**TGF-β production in the LLNA response.** At 10% TMA, vaccination resulted in a strongly decreased LN cell proliferation and cytokine production (except IL-5), whereas a 1% DNCB vaccination resulted in an increased LN cell proliferation and IL-5 and IFN-γ production. To search for a possible mechanism underlying these effects, the production of TGF-β was measured.

In the adjuvant controls, TMA and DNCB application resulted in a dose-dependent increase in TGF-β production.
At 10% TMA, TGF-β production was significantly increased in the vaccinated animals compared to the adjuvant controls (Fig. 4A), whereas at 1% DNCB a trend toward the opposite was seen (Fig. 4B). Contribution of the individual vaccine components to the decreased LLNA response to TMA.

In order to possibly pinpoint the decreased LLNA response to TMA to individual vaccine components, the three pertussis vaccine components, as well as DT, were analyzed individually. The combined components were also analyzed, as was DTaP itself.

Mice were vaccinated and exposed to 10% TMA (only). LN cell proliferation, IFN-γ production, and IL-4 production were measured, with IFN-γ production giving the most clear-cut results (Fig. 5). PT, PRN, and DT, but not FHA, vaccination resulted in a decreased IFN-γ production compared to the adjuvant control, while also vaccination with the combined components as well as DTaP resulted in decreased production of this cytokine.

Vaccination effects on OVA-specific IgE. The intranasal OVA model has been used to detect the effects of particulate matter on OVA-induced lung allergy. Besides, it has been described as a model for human lung allergy. Here we measured DTaP vaccination effects in this model.

Mice that were vaccinated prior to sensitization showed a similar level of OVA-specific IgE compared to mice that were only sensitized. Mice that were only vaccinated, as well as saline-treated mice, did not show OVA-specific IgE (Fig. 6).

Vaccination effects on lung pathology. Sensitization resulted in an increased peribronchiolitis (Fig. 7A), perivasculitis (Fig. 7B), and hypertrophy of the bronchiolar mucus cells (Fig. 7C) compared to vaccination alone or saline treatment. Since a significant proportion of OVA-sensitized animals (irrespective of vaccination) did not show alveolitis or eosinophilia, the effects were not statistically significant. When the results of two replicate experiments were combined, sensitization resulted in increased ($P < 0.05$) alveolitis and eosinophilia compared to vaccination alone or saline treatment. This effect was irrespective of vaccination (data not shown).

**Vaccination effects on the number of BALF cells.** Sensitization resulted in an increased number of BALF neutrophils (Fig. 8A) and eosinophils (Fig. 8B) but did not affect the number of macrophages and lymphocytes or the total number of cells (data not shown). Vaccination prior to sensitization did not affect the total number of BALF cells or the number of any
of the individual cell types in the BALF (Fig. 8A and B and data not shown).

DISCUSSION

Here we have shown that vaccination with a DTaP vaccine strongly increased total serum IgE in BALB/c mice but not in C57BL/6 mice, decreased the LLNA response to TMA while increasing the response to DNCB, and did not modulate OVA-induced changes in IgE, lung pathology, and BALF cell numbers or composition.

It may be questioned whether the IgE that was measured is specific for the vaccine antigens or resulting from polyclonal B-cell activation. As we were unable to detect PT-specific IgE in the sera of vaccinated mice, it is unlikely that a major fraction of IgE is specific for the vaccine antigens. It has previously been shown that addition of PT during primary tetanus toxoid (TT) vaccination increased TT-IgE but also total IgE (32). Thus, most likely vaccination induced both antigen-specific and polyclonal IgE production. The clear difference in total IgE levels between BALB/c mice (more Th2 cytokine milieu) and C57BL/6 mice (more Th1 cytokine milieu) implicates a role for the cytokine milieu in total IgE production.

Previous findings on DTaP and its constituents PT, FHA, and PRN are equivocal in the type of immune response (Th1, Th2, or both) that is induced upon vaccination. PT has long been known for its capacity to induce IgE in mice and rats (24, 27). Moreover, injection of a combined D, T, and pertussis vaccine, with only PT as pertussis vaccine, increased the IgG1 titer, which in mice is a marker of Th2 responses, and IL-4 production by activated splenocytes (13). Other reports have shown, however, PT to induce Th1 responses through its action on human dendritic cells in vitro (3, 9) or mixed Th1/Th2 responses in mice (29). FHA was found to suppress T-cell responses in a human in vitro cell system and Th1 responses in mice (20, 21, 22). PRN was found to suppress Th1 responses in mice (22). Acellular pertussis vaccine (PT, FHA, and PRN combined) induced in mice Th2 responses (28) or mixed Th1/Th2 responses (37) and in humans Th1 responses (18, 40).
or mixed Th1/Th2 responses (2, 15, 30). In addition, acellular pertussis vaccine was found to induce PT-IgE in humans (11, 26).

It is generally accepted that Th1 responses result in higher IFN-γ production than do Th2 responses. It may therefore seem remarkable that the Th2-inducing allergen TMA and the Th1-inducing allergen DNCB induce a similar IFN-γ production (in unvaccinated animals). We have previously shown, however, that TMA and DNCB induce a similar IFN-γ production but largely different IL-4 production (35). More specifically, when corrected for differences in cell proliferation, the induction of IFN-γ by TMA compared to DNCB was 0.62 (the present study) and 0.46 (35), whereas the induction of IL-4 by TMA compared to DNCB was 4.92 (the present study) and 20.6 (35). It has to be noted, however, that the LLNA response is different from a bona fide Th1 response. First, the LLNA response is only an afferent response. Second, we have previously found that the LLNA response to DNCB is unaffected in mice that have a targeted mutation in IFN-γR, IL-12, IL-18, and T-bet, all of which are critical for a Th1 response (R. J. Vandebriel et al., unpublished data).

In response to the respiratory allergen TMA, vaccination resulted in a fourfold decrease in the proliferative response; a four- to sixfold decrease in the production of the Th2 cytokines IL-4, IL-10, and IL-13; and a fourfold decrease in the production of the Th1 cytokine IFN-γ. In contrast, in response to the contact allergen DNCB, vaccination resulted in a twofold increase in the proliferative response, a two- to fourfold increase in the production of IL-4 and IL-5; and a twofold increase in the production of IFN-γ. Collectively, these results suggest that DTaP vaccination affects the afferent phase of the immune response and does so differently for Th2-directed and Th1-directed responses. Immunosuppression due to measles virus infection (25) and the immunosuppressive drug cyclosporine (38) could be identified by using the LLNA, suggesting that this assay can detect altered immune function related to immunosuppression. In these two studies the proliferative response was measured to a contact allergen. Since DTaP vaccination resulted in a small increase in proliferation to the contact allergen DNCB, it may be suggested that this vaccination results in a small immunostimulation. The present study is the first to measure treatment effects in conjunction with a respiratory allergen. The fact that we measured a much larger vaccination effect in response to the respiratory allergen TMA compared to the contact allergen DNCB holds promise for using respiratory allergens (next to contact allergens) in the LLNA. Additional studies with immunomodulating agents (infections, vaccines, and drugs) in conjunction with respiratory allergens are required to better understand the relevance of the decreased response due to DTaP vaccination. In any case, the observed effects due to DTaP vaccination suggest an immunomodulatory effect.

To search for a possible mechanism underlying the reduced LN cell proliferation and cytokine production due to vaccination (in the case of TMA), TGF-β production was measured. This cytokine is produced by T cells and is associated with suppressive activities. The results showed a significantly increased TGF-β production due to vaccination at 10% TMA, in line with the decreased LN cell proliferation and cytokine production. The trend toward decreased TGF-β production at 1% DNCB fits a more modest increase in LN cell proliferation and cytokine production. In conclusion, TGF-β may be involved in affecting LN cell proliferation and cytokine production. Other factor(s) are, however, likely to also play a role.

To identify the DTaP vaccine component(s) that are responsible for reduced proliferation and cytokine production in response to TMA, mice were vaccinated with DT vaccine; the individual pertussis vaccine components PT, FHA, and PRN; the DT and pertussis vaccine components combined; and DTaP itself. The effects on IFN-γ production were mostly clearcut, with DT, PT, PRN, all components combined, and DTaP showing a significant decrease. Thus, these data suggest that, with the exception of FHA, all DTaP vaccine components decrease the LLNA response to TMA. It is tempting to speculate that this observation fits with the observed suppressive activity of FHA (6, 20, 21, 22), but clearly additional investigations are required to draw more definitive conclusions.

Sensitization and challenge by intranasal OVA administration induced OVA-specific IgE, lung pathology, and alterations in the BALF cell numbers, similar to previous observations (33). DTaP vaccination did not affect these responses, suggesting that this vaccine, at least using the protocol described here, does not modulate protein-induced lung allergy. Our data are in agreement with those of Ennis et al. (12), who observed that an acellular pertussis vaccine did not modulate OVA-specific IgE and lung pathology in a murine lung allergy model. These authors did observe, however, an increase in IL-10 and IL-13 production by OVA-stimulated spleen cells and in the BALF. We were unable to find treatment-related differences in IL-10 and IL-13 in the BALF (data not shown).

The lack of DTaP vaccination effects on OVA-specific IgE in mice fits with observations that IgE to unrelated antigens is not affected by pertussis vaccination in both adults (1) and children (19, 31). The present study used a lung allergy model that has been used predominantly to mimic an allergic reaction of the human airways (14), suggesting that our data do not support an effect of DTaP vaccination on lung allergy in humans. Pediatric diphtheria-tetanus-pertussis vaccination has in fact been associated with an increased risk of atopic disorders, although this was not confirmed by others (see references 4, 5, and 17 and references therein). It should be noted, however, that most or all of these studies relate to vaccination with WCV, at least during the first year of life. WCV induces a more Th1-directed response compared to acellular vaccine (2, 30), implying that acellular vaccines may be more prone to play a role in inducing atopic disorders. A recent study showed a different cytokine response in children at the age of 6 months that had been vaccinated with either WCV or ACV (19). Next to a similar Th1 cytokine response in WCV- and ACV-vaccinated children, a Th2 response not only to pertussis antigens but also to TT and the food allergen β-lactoglobulin was seen in ACV-vaccinated children. Also, the T-cell mitogen phytohemagglutinin induced Th2 cytokines in ACV- but not WCV-vaccinated children. This suggests that studies on the association between pertussis vaccination and atopic disorders should be repeated in children that received all pertussis vaccinations with acellular vaccine. Our findings in the lung allergy model do not support DTaP vaccination effects on lung allergy. Still, the increase in total serum IgE and altered LLNA responses found in the present study, as well as the induction of IL-10 and IL-13...
observed by others (12), may suggest that unwanted side effects do occur, again supporting further investigation in humans.

In conclusion, ACV vaccination resulted, next to increased total serum IgE in BALB/c but not C57BL/6 mice, in a decreased Th2 response and an increased Th1 response in the LLNA and showed a lack of effect in a lung allergy model. This latter observation underlines the notion that there is no positive evidence that pediatric vaccinations result in an increase in allergic disorders.

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