

Original article

Common vaccine antigens inhibit allergen-induced sensitization and airway hyperresponsiveness in a murine model

Background: Co-vaccination with cellular pertussis vaccine down-regulates allergic sensitization to diphtheria and tetanus antigens. Using a murine model, we investigated whether vaccination with diphtheria/tetanus toxoids, administered separately or simultaneously with the whole cell vaccine of *Bordetella pertussis*, inhibits subsequent allergen-induced immune and inflammatory responses.

Methods: BALB/c-mice were vaccinated intracutaneously with a combination of diphtheria and tetanus toxoids or a combination of diphtheria and tetanus toxoids with a whole cell vaccine of *B. pertussis* (three times, days –21 to –7) prior to systemic sensitization (days 1–14) and repeated airway challenges (days 28–30) with ovalbumin.

Results: Compared with negative controls, systemic sensitization and airway allergen challenges induced high serum levels of allergen-specific IgE, predominant Th2-type cytokine production, airway inflammation and development of *in vivo* airway hyperreactivity. Vaccination with diphtheria and tetanus toxoids prior to sensitization suppressed IgE formation and development of eosinophilic airway inflammation. Co-vaccination with a whole cell pertussis vaccine inhibited allergen sensitization, airway inflammation and development of *in vivo* airway hyperreactivity. Prevention was due to an allergen-specific and general shift from a predominant Th2 towards a predominant Th1 immune response.

Conclusion: Vaccination with diphtheria and tetanus toxoids alone or in combination with whole cell pertussis vaccine prior to allergen sensitization prevented allergen-induced Th2 immune responses. Vaccine antigens may down-regulate allergic responses to a range of common allergens.

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There is a long controversy regarding the impact of vaccination on the development of allergic immune reactions and disease. The suggestion of a positive correlation may be attributed to the coincidence of first vaccinations and the onset of first allergic symptoms around 3 months of life. The epidemiological evidence, however, is incoherent. Some retrospective studies indicated a lesser prevalence of atopy among children with lower vaccination coverage against diphtheria, tetanus, or pertussis (1–3). No association between diphtheria, tetanus, and pertussis vaccination and childhood asthma was found in a large database of health maintenance organizations (4). Similarly, pertussis vaccination had no effect on the atopy rate in an intervention study (5). By contrast, we found a transient inverse association between the total number of vaccination doses and the prevalence of allergic

sensitization or atopic disease in a longitudinally followed observational birth cohort (6). Consistent with these results, a reduced risk of asthma or atopic disease was found among adolescents from a population-based sample vaccinated against diphtheria, tetanus, and pertussis (7).

Vaccine antigens such as diphtheria or tetanus toxoid and pertussis commonly elicit in children a specific IgE response, which is exaggerated and prolonged in those individuals predisposed to atopy (8–10). While the occurrence of such a specific immune response among nonatopic children suggested that IgE formation against vaccine antigens was part of the regular immune response, these findings fuelled the apprehension that routine childhood vaccinations may promote IgE formation against unrelated environmental allergens and may thus contribute to the rise in the prevalence of atopic diseases currently observed in many industrialized countries (11).

Previously, we found that specific IgE and IgG4 production against diphtheria and tetanus toxoids was

Abbreviations: AR, airway reactivity; i.p., intraperitoneal; MNC, mononuclear cells; D, diphtheria toxoid; Pw, whole cell pertussis; T, tetanus toxoid.

down-regulated in a dose-dependent fashion when whole cell pertussis vaccine was co-administered (12). No effect, however, was observed with regard to Ig production against common environmental allergens, which of course were encountered at times distant from the vaccination. These findings invited the hypothesis that vaccinations may actually contribute to prevention of allergic sensitization if vaccine antigens and allergens are presented simultaneously.

The primary aim of the present study therefore was to further delineate the immunological effects of vaccination against diphtheria, tetanus and pertussis on the development of allergic sensitization and allergen-mediated airway disease, utilizing an established murine asthma model.

Materials and methods

Experimental protocol

Female BALB/c mice 6–8 weeks of age were purchased from Harlan Winkelmann (Borchen, Germany). Between day 0 and 14, mice were systemically sensitized by six intraperitoneal (i.p.) injections with ovalbumin (OVA, 10 µg/100 µl/mouse, Grade VI, Sigma, USA) without aluminiumhydroxide. Controls for effects of sensitization were accordingly sham-sensitized with phosphate-buffered saline (PBS) (100 µl/mouse, i.p.). To investigate the effects of vaccination prior to sensitization, mice were treated by three intracutaneous injections in the back on days –21, –14 and –7 with (A) a combination of diphtheria (D, 12IE/500 µl/injection/mouse; Aventis Pasteur MSD, Liemen, Germany) and tetanus toxoids (T, 16IE/500 µl/injection/mouse, Aventis Pasteur MSD); or (B) with a combination of D and T and a whole cell vaccine of *Bordetella pertussis* (Pw, 6×10^9 bacteria/500 µl/injection/mouse; Behring, Marburg, Germany). Applied vaccine antigens were adsorbed to aluminiumhydroxide. Single vaccine doses were calculated to 40% of absolute human vaccine doses in accordance with previously published data for immunization of mice with DT (13) directed to 'proof the concept' rather than to establish an immunization protocol. To investigate the effects of vaccination simultaneous to sensitization, mice were treated with a combination of D and T on days 3, 7 and 14. Controls for effects of vaccination were sham-vaccinated with PBS following the same protocols. Negative controls were sham-vaccinated and sham-sensitized with PBS. All groups were challenged with aerosolized OVA (100 mg/10 ml, Grade V; nebulizer by Medical Assistance System) for 20 min daily, on days 28, 29 and 30. All experimental procedures were approved by the institutional animal ethics committee.

In vitro culture conditions for isolated spleen mononuclear cells

On day 32, spleen mononuclear cells (MNC) were isolated and cultured with concanavalin A (ConA, 2.5 µg/ml; Sigma, Deisenhofer, Germany) or OVA (Grade VI, 50 µg/ml) or with D (5 LF/ml) or T (5 LF/ml) or the Pw (10^7 K/ml).

Proliferation assay with ³H-thymidine

Proliferative responses of cultured spleen MNC were determined by ³H-thymidine incorporation (0.5 µCi/200 µl; Amersham Buchler, Braunschweig, Germany) as previously reported (14).

Serum levels of total and OVA-specific immunoglobulins

On day 32, blood samples were taken out of tail veins and serum levels of total and OVA-specific Ig were measured by ELISA, as previously described (15). Levels of OVA-specific IgE and IgG2a were related to pooled standards, generated in our laboratory, and expressed as arbitrary units per ml (U/ml).

Broncho-alveolar lavage

On day 32, lungs were lavaged twice with 0.8 ml PBS. Cells of both aliquots were pooled; cytospin slides were stained with Diff Quik (Dade Behring AG, CH). Cells were differentiated due to morphological criteria by counting 200 cells under light microscopy.

In vivo airway reactivity

On day 31, *in vivo* lung function (Sigma) was performed by whole-body plethysmography (EMKA Technologies, F, similar to Buxco®-system), as previously reported (16). Animals were exposed to aerosolized PBS for baseline reading and then to increasing concentrations of methacholine (MCh) (6–50 mg/ml). Airway reactivity (AR) was expressed as the increase of enhanced pause (Penh) values for each concentration of MCh relative to baseline Penh values.

In vitro cytokine production

Levels of cytokines were assessed in cell culture supernatants of spleen MNC by ELISA, as previously described (14, 15). Detection limits were 32 pg/ml for IL-4 and IL-5 and 78 pg/ml for IFN-γ. Levels of IL-10 were assessed by Pharmingen optEia kits (PharMingen, Hamburg, Germany) according to the manufacturer's instructions.

Statistical analysis

Data of animals treated in the same way in up to four independent experiments were pooled. Values for all measurements are expressed as mean ± SEM. Pairs of groups were compared by Mann-Whitney U-test. Statistical significance was set at $P < 0.05$. Values of $P < 0.01$ were rated as statistically highly significant.

Results

Antigen-specific immune responses following vaccination and sensitization

In sham-vaccinated OVA-sensitized mice, proliferative responses of spleen MNC cultured with OVA were significantly enhanced compared with negative controls (Table 1). DT- or DTPw-vaccination induced antigen-specific proliferative responses of spleen MNC cultured with the respective antigen compared with negative controls (Table 1). Stimulation with pertussis antigen resulted also in mild unspecific proliferative response, as indicated by splenic MNC of mice with DT-vaccination during OVA-sensitization. DT-vaccination prior to or during OVA-sensitization or DTPw-vaccination prior to OVA-sensitization did not alter OVA-specific proliferative responses significantly (Table 1).

Table 1. Effects of DT- and DTPw-vaccination and ovalbumin (OVA)-sensitization on antigen- and allergen-specific immune responses

Vaccination	Sensitization	n	Diphtheria	Tetanus	Pertussis	OVA
PBS	PBS	6	5.3 ± 1.6	2.8 ± 0.8	1.1 ± 0.6	1.3 ± 0.4
PBS	OVA	12	15.6 ± 3.9	3.5 ± 0.5	1.8 ± 0.5	3.2 ± 0.8**
DT	OVA	19	40.2 ± 9.5**	22.9 ± 8.2**	5.3 ± 1.7	2.8 ± 0.4*
PBS	DT + OVA	10	40.2 ± 6.7**	11.6 ± 1.9**	2.5 ± 0.3**	2.6 ± 0.2*
DTPw	OVA	16	42.2 ± 9.2**	16.3 ± 3.5**	22.4 ± 5.3**	2.3 ± 0.3**

Splenic MNC of mice vaccinated intracutaneously with DT prior to or during OVA-sensitization period or with DTPw prior to OVA-sensitization were cultured with D, T, Pw or OVA.

Proliferative responses of splenic MNC were expressed as fold increase of ³H-thymidine incorporation relative to that of splenic MNC cultured with medium (**P* < 0.05, ***P* < 0.01 vs negative controls).

PBS, phosphate-buffered saline; MNC, mononuclear cells.

Effects of vaccination on vaccine-specific cytokine production

In DT- and DTPw-vaccinated mice, *in vitro* production of IL-5, IL-10 and IFN- γ by spleen MNC stimulated with the respective vaccine antigen was significantly higher than in sham-vaccinated animals (Table 2), demonstrating a significant and specific immune response elicited by the vaccination. Following DTPw-vaccination, *in vitro* IL-5 and IL-10 production by spleen MNC after stimulation with D (data not shown) or T was significantly decreased compared with DT-vaccination only (Table 2). Furthermore, DTPw-vaccination caused significantly higher IFN- γ production by spleen MNC in response to D (data not shown), T, or particularly Pw compared with DT-vaccination (Table 2). Concordant with proliferation responses, cultivation with pertussis antigen resulted in mild unspecific stimulation of IL-5 and/or IL-10 in mice with DT-vaccination prior or during OVA-sensitization.

Effects of vaccination on total and OVA-specific immunoglobulin production

OVA-sensitization led to significantly increased production of total and OVA-specific IgE as well as of OVA-specific IgG2a, but not total IgG2a compared with negative controls (Fig. 1). Serum levels of total IgE were

significantly enhanced after DT- and DTPw-vaccination, whereas levels of total IgG2a were strongly increased only after DTPw-vaccination, compared with sham-vaccinated sensitized mice (day 1, Fig. 1). A transient rise in total serum IgE was observed in response to vaccination and sensitization, most pronounced in individuals vaccinated prior to sensitization (Fig. 1). DT- or DTPw-vaccination prior to or DT-vaccination during OVA-sensitization significantly inhibited production of OVA-specific IgE. In contrast, levels of OVA-specific IgG2a were extensively augmented in DTPw-vaccinated mice compared with sham-vaccinated OVA-sensitized mice, whereas specific IgG2a levels were reduced in mice vaccinated with DT during OVA-sensitization period (Fig. 1).

Effects of vaccination on allergen-induced airway inflammation

Broncho-alveolar lavage (BAL) fluids of negative controls contained few cells, which were differentiated mainly as monocytes (Fig. 2). Cellular pattern of BAL fluids of DT- and DTPw-vaccinated sham-sensitized mice were very similar to that of negative controls (data not shown). OVA-sensitization caused allergen-induced airway inflammation, shown by significant influx of cells into the airways, mainly lymphocytes and eosinophils (Fig. 2). In contrast, DT- and DTPw-vaccination prior to or DT-

Table 2. Effects of DT- and DTPw-vaccination on antigen-specific cytokine production by splenic MNC *in vitro*

Vaccination	Sensitization	n	T <i>in vitro</i>			Pw <i>in vitro</i>		
			IL-5 (pg/ml)	IL-10 (pg/ml)	IFN- γ (pg/ml)	IL-5 (pg/ml)	IL-10 (pg/ml)	IFN- γ (pg/ml)
PBS	PBS	11	32 ± 0	38 ± 4	78 ± 0	32 ± 0	56 ± 18	78 ± 0
PBS	OVA	18	33 ± 2	32 ± 0	78 ± 0	36 ± 4	47 ± 9	79 ± 1
DT	OVA	6	1275 ± 294**	1017 ± 249**	120 ± 33*	37 ± 6	266 ± 51**	97 ± 19
PBS	DT+OVA	8	1929 ± 431**	1398 ± 334**	163 ± 44*	167 ± 45**	190 ± 28**	170 ± 59
DTPw	OVA	10	231 ± 78**‡	165 ± 44**‡	623 ± 246**†	444 ± 158**†	796 ± 272**‡	4505 ± 1942**‡

Mice were vaccinated intracutaneously with DT prior to or during OVA-sensitization period or with DTPw prior OVA-sensitization.

In vitro cytokine production by splenic MNC cultured with T or Pw was measured by ELISA.

PBS, phosphate-buffered saline; OVA, ovalbumin; MNC, mononuclear cells.

P* < 0.05, *P* < 0.01 vs negative controls; †*P* < 0.05, ‡*P* < 0.01 vs mice vaccinated with DT prior to or during OVA-sensitization period.

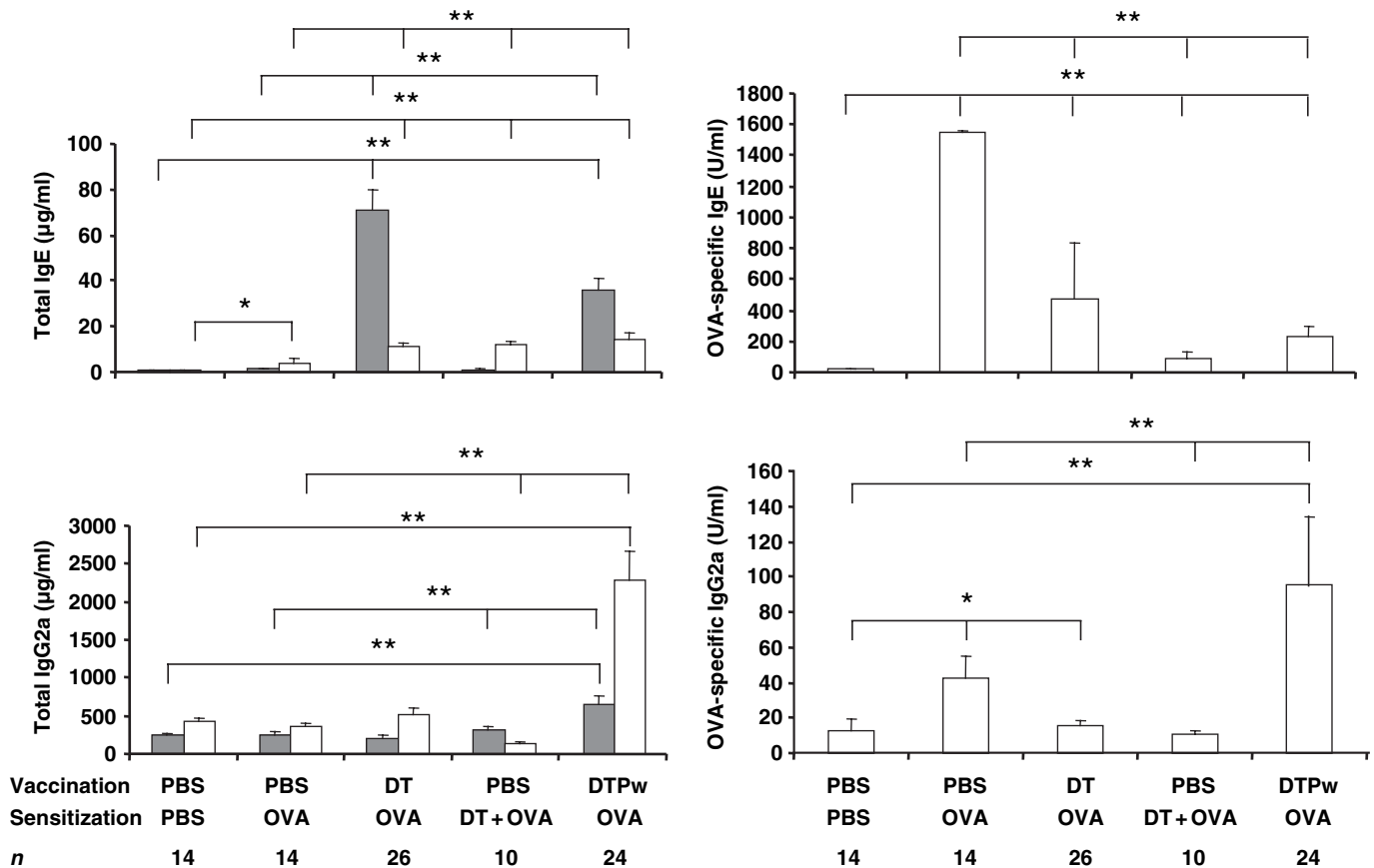


Figure 1. Effects of DT- and DTPw-vaccination on total and ovalbumin (OVA)-specific immunoglobulins. Mice were vaccinated intracutaneously with DT prior to or during OVA-sensitization period or with DTPw prior to OVA-sensitization. Serum levels of total and OVA-specific Ig were quantified on days 0 (gray columns) and 32 (white columns) by ELISA (* $P < 0.05$, ** $P < 0.01$).

vaccination during OVA-sensitization almost completely prevented allergen-induced development of eosinophilic airway inflammation (Fig. 2). However, in animals vaccinated with DTPw prior to OVA-sensitization, a significant infiltration of lymphocytes into the airways was observed (Fig. 2).

Effects of vaccination on *in vivo* AR

Ovalbumin-sensitization caused increased *in vivo* AR in response to unspecific airway provocation with MCh, shown by significantly increased Penh values compared with negative controls (Fig. 3), whereas DT- or DTPw-vaccination without allergen sensitization had no effect on AR (data not shown). Similar, DT-vaccination prior to or during OVA-sensitization did not significantly alter the development of increased *in vivo* AR after airway allergen challenge, compared to OVA-sensitized nonvaccinated animals (data not shown). In contrast, DTPw-vaccination prior to OVA-sensitization completely inhibited the development of increased *in vivo* AR as demonstrated by Penh values very similar to those of negative controls (Fig. 3).

Effects of vaccination on allergen-induced cytokine production

In sham-vaccinated OVA-sensitized mice, production of the Th2 cytokines IL-4, IL-5 and IL-10 by spleen MNC in response to specific allergen (OVA) or to mitogen (ConA) was strongly enhanced compared with negative controls (Table 3). DT- and particularly DTPw-vaccination prior to or DT-vaccination during OVA-sensitization strongly and significantly diminished the *in vitro* production of Th2 cytokines by spleen MNC in response to OVA, compared with sham-vaccinated OVA-sensitized mice (Table 3). In addition, in all groups of vaccinated OVA-sensitized mice, Th2 cytokine production in response to mitogen was decreased compared with sham-vaccinated OVA-sensitized mice, pointing towards an immune modulating phenomenon independent of allergic sensitization. In all groups of OVA-sensitized mice, *in vitro* production of IFN- γ by spleen MNC in response to OVA was not significantly altered compared with that of negative controls (Table 3). In contrast, production of IFN- γ by spleen MNC cultured with ConA was significantly increased in all groups of vaccinated and sensitized animals compared with sham-

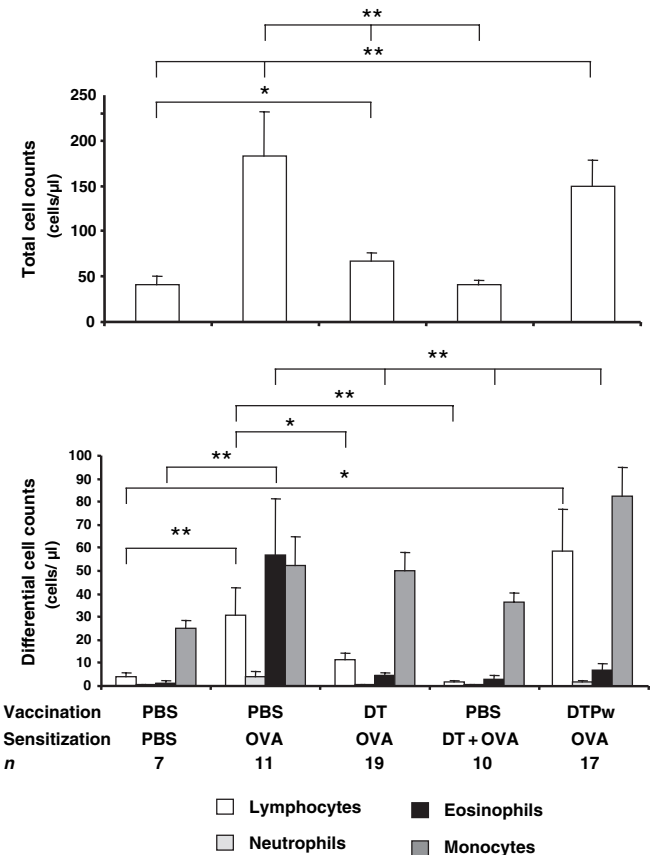


Figure 2. Effects of DT- and DTPw-vaccination on allergen-induced airway inflammation. Cells of broncho-alveolar lavage fluids obtained from mice vaccinated intracutaneously with DT prior to or during ovalbumin (OVA)-sensitization period or with DTPw prior to OVA-sensitization were differentiated to assess allergen-induced airway inflammation (* $P < 0.05$, ** $P < 0.01$).

vaccinated, sensitized mice, and was most pronounced in DTPw-vaccinated animals (Table 3). Therefore, DTPw-vaccination in particular induced a general shift from a predominant Th2 cytokine pattern induced by OVA-sensitization towards a predominant Th1 immune response in vaccinated animals.

Discussion

Previous studies in DT immunized children demonstrated that co-vaccination with Pw vaccine down-regulated D- and T-specific IgE production (12). The aim of the present study was to investigate whether this phenomenon could also apply to a common allergen and to delineate the immunologic effects of vaccination on subsequent allergen sensitization utilizing a murine model for allergen-induced sensitization and airway inflammation. This study demonstrated that vaccination of mice with DT prior to allergen sensitization with

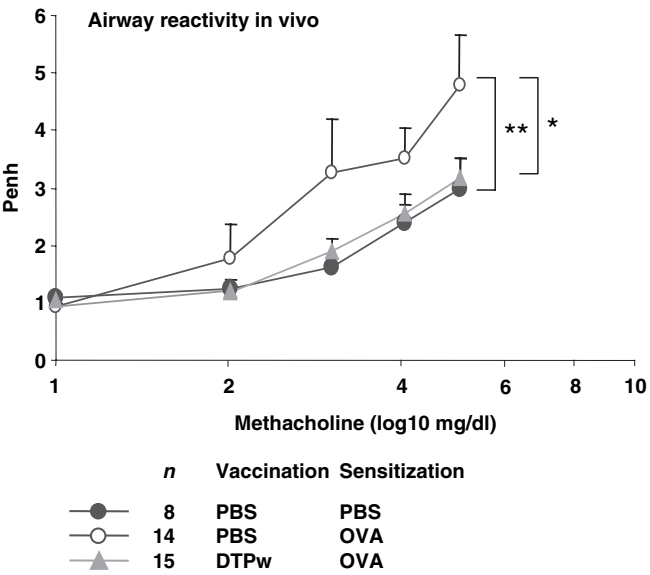


Figure 3. Effects of DTPw-vaccination on development of increased *in vivo* AR. Mice were vaccinated intracutaneously with DTPw prior to ovalbumin-sensitization. *In vivo* airway reactivity in response to increasing doses of aerosolized methacholine (MCh) was calculated by whole-body plethysmography. Data are expressed as Penh values for each concentration of MCh (* $P < 0.05$, ** $P < 0.01$).

OVA significantly down-regulated allergen-induced IgE formation and airway inflammation. While direct conclusions regarding effects of DT-vaccination on production of unrelated allergen-specific IgE production in children are impossible, these findings of the previous study support the concept of a preventive rather than an enhancing role of commonly used vaccine compounds for the development of allergic diseases.

To the best of our knowledge, down-regulation of IgE production against an allergen by D or T has not been studied before. Tolerance induction by other toxoids to unrelated allergens, however, has been demonstrated in experimental models, employing the B subunit of cholera toxin (17) or the heat-labile subunit of *Escherichia coli* enterotoxin directly coupled to OVA (18). Development of IgE formation and eosinophilic airway inflammation depend on allergen-induced Th2-type cytokine production (19) and can be inhibited either by Th1-cytokines such as IL-12, IFN- γ and IL-18 or by tolerance induction via regulatory T cells (20). In our study, DT-vaccination prior to OVA-sensitization was accompanied by an allergen-specific and systemic shift from a predominant Th2 towards a predominant Th1 immune response, even though used vaccines were adsorbed to aluminiumhydroxide which is known to induce allergen-specific IgE-production (21). Timing of vaccination and allergen exposure was a critical factor in our study. In contrast to the experimental study by Wiedermann et al. (17) employing allergen/cholera toxoid conjugates, simultaneous application of DT and OVA did not induce

Table 3. Effects of DT- and DTPw-vaccination on allergen-induced cytokine production by splenic MNC *in vitro*.

Vaccination	Sensitization	n	OVA <i>in vitro</i>				ConA <i>in vitro</i>	
			IL-4 (pg/ml)	IL-5 (pg/ml)	IL-10 (pg/ml)	IFN- γ (pg/ml)	IL-5 [pg/ml]	IFN- γ (ng/ml)
PBS	PBS	8	25 $\pm$ 3	39 $\pm$ 5	37 $\pm$ 11	89 $\pm$ 24	270 $\pm$ 45	7.9 $\pm$ 3.4
PBS	OVA	13	137 $\pm$ 37**	4075 $\pm$ 1075**	1128 $\pm$ 256**	78 $\pm$ 0	664 $\pm$ 117*	5.6 $\pm$ 1.8
DT	OVA	16	73 $\pm$ 14**	818 $\pm$ 179**†	469 $\pm$ 150**‡	326 $\pm$ 131†	325 $\pm$ 37‡	14.7 $\pm$ 2.6‡
PBS	DT + OVA	8	32 $\pm$ 1‡	590 $\pm$ 230**†	362 $\pm$ 125**†	78 $\pm$ 0	534 $\pm$ 62**†	33.8 $\pm$ 7.7*‡
DTPw	OVA	15	50 $\pm$ 4**†	311 $\pm$ 62**‡	107 $\pm$ 30‡	149 $\pm$ 33†	474 $\pm$ 59**†	36.6 $\pm$ 12.0**†

Mice were vaccinated intracutaneously with DT prior or during OVA-sensitization period or with DTPw prior to OVA-sensitization. *In vitro* cytokine production by splenic MNC cultured with OVA or ConA was measured by ELISA.

PBS, phosphate-buffered saline; OVA, ovalbumin; MNC, mononuclear cells.

* $P < 0.05$, ** $P < 0.01$ vs negative controls; † $P < 0.05$, ‡ $P < 0.01$ vs OVA-sensitized mice.

allergen-specific energy, as demonstrated by adequate allergen-specific proliferative responses of spleen MNC from vaccinated and sensitized animals. Further, we did not observe an increase in numbers of spleen T cells of the CD4⁺ CD25⁺ phenotype characterizing a certain subset of regulatory T cells (examined by FACS analysis, data not shown) nor increased *in vitro* production of the regulatory cytokine IL-10 by spleen MNC from mice vaccinated with DT prior to allergen sensitization. Instead, simultaneous application of DT and OVA or DT-vaccination prior to OVA-sensitization led to significantly suppressed allergen-specific Th2 cytokine production and induced a general and predominant Th1 immune response. The divergence in the outcome of the two studies may be explained by different antigen presentation of coupled antigen-allergen complexes (17) vs simultaneous application of uncoupled vaccines and allergen, as utilized in our study.

Co-administration of Pw vaccine with DT did not only suppress levels of total and OVA-specific IgE but also enhanced levels of total and OVA-specific IgG2a production. In accordance, DTPw-vaccination was accompanied by an allergen-specific as well as unspecific shift from a predominant Th2 towards a predominant Th1 cytokine pattern. Whereas DT-vaccination itself led to strongly enhanced levels of total IgE and *in vitro* Th2 cytokine production in response to the T vaccine, addition of Pw-vaccine induced strong Th1 shifted immune responses by spleen MNC stimulated with T- or Pw-vaccines, suggesting a strong Th1 immune modulation by Pw vaccine. Correspondingly, DTPw-vaccination not only abolished allergen-induced Th2 cytokine production and IgE formation following sensitization but also inhibited eosinophilic airway inflammation and development of airway hyperreactivity following allergen airway challenges of sensitized animals. This effect may be due to immunomodulatory cell wall components like lipopolysaccharides (LPS) or nuclear factors such as CpG that both were shown to down-regulate allergen-immune reactions: In agreement with our results, recently published data by Kim et al. (22) showed that CpG motifs of *B. pertussis* are able to suppress allergen-induced airway inflammation and

hyperreactivity by suppressing local Th2 cytokine secretion.

Previous studies in mice (23) and in children (24, 25) demonstrated that Pw vaccine induced a less Th2-skewed immune response than vaccination with the acellular pertussis vaccine. In children, Pw vaccination suppressed IgE formation to pertussis toxin (10). Likewise, formation of IgE and IgG4 to co-vaccinated DT was suppressed after Pw vaccination of children in comparison with vaccination with DT only (12) or DT plus the acellular pertussis vaccine (C. Grüber, S. Kakat, H. de Voieze, S. Stapel, U. Wahn, R. Aalbese, L. Nilson, unpublished data). Concordantly, increased Th1-skewed immune responses have been observed in mice and children after vaccination with Pw when compared with vaccination with acellular vaccines (23, 25). These differences may be attributed to the distinct molecular structure of the respective vaccines. Whereas the acellular vaccine includes the pertussis toxoid and few other antigens, Pw contains additionally compounds of the bacterial cell wall. Accordingly, Pw may exert additional immune regulatory effects through cell wall components, particularly LPS, that strongly induces production of IL-12 (26), the key cytokine initiating Th1- and antagonizing Th2-type immune responses (27, 28). In contrast, vaccination of mice with acellular pertussis vaccine did not influence IgE production to OVA, down-regulated enhanced IL-10 secretion, and enhanced allergen-induced airway hyperreactivity (29). The concept that bacterial cell wall components might contribute to the anti-allergic effect of Pw is supported by our studies showing that systemic or local application of LPS prior to sensitization of mice down-regulated allergic airway inflammation in an IL-12 dependent manner (14). Moreover, the association of increased LPS exposure in early life and subsequent lower prevalence rates of atopy and allergic airway disease in children growing up on farms provided indirect evidence of a potential role for LPS as anti-allergic immune response modifier (30, 31). Thus, exposure to bacterial products seems to be able to down-regulate, through stimulation of the innate immune system, inflammatory processes induced by the adaptive immune system.

Our study supports the biological plausibility of a preventive effect of vaccination with bacterial compounds on simultaneous or subsequent allergic sensitization and disease in response to the particular allergen. The allergen specific immune response in general, however, is a prerequisite for the production of protective IgG antibody titers and is not suppressed. Studies in children suggested that vaccines exerted inhibitory effects on the level of allergic sensitization to concomitantly administered vaccine antigens, but that this did not affect immune responses to other environmental allergens encountered at different time points (12). It is tempting to speculate that, similar to our experimental results, co-vaccination of Pw or other vaccine antigens with allergens pertinent in the development of atopy in infancy may inhibit Th2-immune immune responses to these allergens. If true, this would be a novel means to prevent allergic sensitization and airway disease in children at high risk for allergies. While the risk of such

an approach in regards of anaphylaxis to the vaccine or to the co-administered allergen seems small on the grounds of this and other studies, clinical evaluation in infants has yet to confirm this notion. Further, the exact type, dosing and timing of vaccination resulting in optimal inhibitory effects on allergic sensitization has to be delineated for the clinical situation before the first intervention studies may be initiated.

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