

Case report

Hypersensitivity to the diphtheria component in the Di-Te-Pol vaccine. A type I allergic reaction demonstrated by basophil histamine release

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We describe a two year-old multiallergic boy who developed generalized urticaria after the third Di-Te-Pol vaccination. A Type I reaction to the vaccine was demonstrated by performing basophil histamine release to the complete vaccine. Further, we found that the reaction could be exclusively ascribed to Diphtheria-Toxoid whereas no release was observed by the Polio and Tetanus component. The latter result was confirmed since no specific IgE to Tetanus Toxoid could be demonstrated.

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Introduction

We have examined a child in respect to basophil histamine release who developed urticaria after Di-Te-Pol vaccination.

There are several reports in the literature claiming that Di-Te-Pol vaccination can induce allergic reactions (2, 3). These have been ascribed to egg components (3, 4, 5) and the Tetanus toxoid (2). However, Aalberse and co-workers (1) demonstrated that no allergic reactions occurred even in two donors who received another booster injection at the time they had a high IgE titre to Tetanus, and they found that specific IgE to vaccines is frequently – but not only – associated with atopy-related factors.

At present there is a Tetanus specific IgE test (CAP-RAST, Pharmacia) available in clinical routine whereas this is not the case for Polio and Diphtheria toxoid. An alternative to IgE in the allergen specific diagnosis is to perform basophil histamine release to the suspected allergen(s) (6, 7). The specificity of histamine release can be verified by 1) removing IgE from the surface of the patient's basophils and 2) to transfer the reaction by passive sensitization using patient plasma and cord blood basophils (8, 9).

Verification of an IgE mediated reaction might be of clinical importance for a later booster vaccination.

Materials and methods

Case history

The patient was a two year-old boy with atopic dermatitis, asthma and hayfever. Both parents suffered from atopic diseases (mother: asthma bronchiale and father: hayfever).

The boy had a positive Skin Prick Test (>3 mm), specific IgE (> Class 2 by CAP-RAST, Pharmacia) and Histamine Release-Test (HR-Test, The Reference Laboratory, Denmark) to milk and egg.

The positive *in vitro* findings were clinically verified since the patient's skin symptoms disappeared on a diet free from milk and egg and recurred after open provocations to the two food items.

At the age of 5 and 6 months the boy received the two first vaccinations of Di-Te-Pol without any changes in the atopic symptoms. At the third vaccination at the age of 16 months, the boy developed urticaria localized to truncus and legs.

On suspicion of a type I allergic reaction histamine release was performed with Di-Te-Pol from

the same ampoule as used for the vaccination. The patient's response to the vaccine and further the laboratory investigations were subsequently performed in order to identify which of the components in the vaccine the patient reacted to.

Later the boy received oral polio immunization without any subsequent reactions.

Antigens and Anti-IgE used for *in vitro* histamine release

Vaccines used for *in vitro* histamine release were provided from The National Serum Institute, Copenhagen, Denmark: Di-Te-Pol vaccine (Aluminium adsorbed); Diphtheria vaccine (Aluminium adsorbed); Tetanus vaccine (Aluminium adsorbed); Polio vaccine, inactivated (Salk); Diphtheria Toxoid E and Q 50 Lf/ml without preservation and Aluminium adsorption.

For histamine release all vaccines were used in the following final concentrations: 1:10; 1:100; 1:1000 and 1:10.000 Vol/Vol.

Anti-IgE from Behringwerke, Germany was used as a positive control for histamine release in final concentrations of 400, 40 and 4 U/ml.

In vitro histamine release

Histamine release was performed as described elsewhere (6, 7). In brief 5 ml blood was drawn from the patient into a heparinized tube. The blood was centrifuged for 5 min at $400 \times G$ at room temperature. Plasma was removed and replaced by Pipes buffer. Removed plasma was later used for passive sensitization experiments.

Aliquots of 25 μ l of vaccines and Anti-IgE in the above mentioned concentrations, all in duplicates, were incubated with samples of 25 μ l of the washed blood in glass fibre coated microplates (The Reference Laboratory, Copenhagen, Denmark) for 60 min at 37°C. The plates were then rinsed with deionized water followed by another incubation with 300 μ l 0.4% SDS/well for 30 min at 37°C (to ensure a complete dissolution of interfering substances). Subsequently the plates were rinsed again with deionized water.

The histamine content in the wells were analyzed by the OPT-method (6). A histamine release > 15 ng/ml washed blood was considered positive.

In order to exclude unspecific histamine release by the different vaccine components identical release experiments were performed on blood from two healthy non-allergic individuals.

Further, the release experiments were performed on leukocytes where the IgE was removed from the surface of the patient's basophils according to the method described elsewhere (9).

Finally we performed identical release experiments on native and passive sensitized cord blood basophils using plasma from the patient and from a healthy non-allergic donor. These experiments were done in order to demonstrate the passive transfer of sensitization by patient plasma to non-sensitized basophils.

The method for performing passive sensitization has previously been described (8). In short 5 ml of cord blood was sampled in a heparinized tube and 3 ml of the washed cord blood was incubated with plasma for 120 min at 37°C. Thereafter the blood was washed free of plasma and incubated with the above mentioned vaccine antigens and Anti-IgE.

Specific IgE

Specific IgE to Tetanus was performed by CAP-RAST, Pharmacia Research Forum, Tetanus Toxoid (c 208).

Results

Table 1 shows the results of all histamine release experiments. As can be seen from this Table, there was a significant histamine release from the patient's basophils by the following vaccines: Di-Te-Pol, Diphtheria and Diphtheria Toxoid E and Q. No release was observed after incubation with the Polio and Tetanus components. Further, we found no release with any of the antigens after incubation with leukocytes from the two non-allergic individuals.

When IgE was removed from the patient's basophils, histamine release was abolished by any of the antigens.

Finally it appears from the Table that passive sensitization of cord blood basophils using plasma from the patient resulted in sensitization of the basophils demonstrated by histamine release after incubation with diphtheria containing vaccines and the purified diphtheria toxoids without preservation. This is in contrast to the results obtained from non-sensitized cord blood basophils and cells sensitized with plasma from non-allergic individuals where no release was observed.

Discussion

It has been claimed that Di-Te-Pol immunization of children may result in development of allergic reactions to this vaccine. Specific IgE to components in vaccine preparations has thus been demonstrated to Tetanus Toxoid (1) and egg residues from the Polio vaccine (3), but commercial IgE assays to Di-Te-Pol vaccine or Diphtheria Toxoid are not yet available for use in the clinic and it is therefore not possible to

Table 1. Histamine release by vaccines (Histamine ng/ml)

Dilutions of vaccines	DIRECT RELEASE (ng/ml)												RELEASE AFTER PASSIVE SENSITIZATION (ng/ml)											
	Di-Te-Pol				Di				Te				Pol				Di-E				Di-Q			
	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K	Pt	K
1:10	35	0	35	0	5	0	0	0	37	0	48	3	23	1	25	2	0	0	1	0	28	2	27	1
1:100	34	2	26	0	2	0	3	1	46	1	46	0	10	0	16	4	1	0	1	0	23	0	25	0
1:1000	14	4	12	4	2	3	0	0	45	0	45	1	6	0	10	1	2	0	1	1	16	1	10	0
1:10000	0	2	0	1	1	1	0	5	43	1	41	1	0	0	5	0	1	1	1	2	7	0	9	2

A histamine release > 15 ng/ml was considered positive.

Note: When IgE was removed from patient's basophils, no significant release was observed after challenge with the different vaccines. Results not shown.

Pt = Patient; K = Control; Di = Diphtheria; Te = Tetanus; Pol = Polio; Di-E = Diphtheria Toxoid E; Di-Q = Diphtheria Toxoid Q.

demonstrate presence or absence of specific IgE to these components. However, in order to provide evidence of a specific Type I allergic reaction it is also possible to perform allergen specific histamine release from the patient's basophils.

For these reasons we have applied histamine release to demonstrate a vaccine specific reaction in a two year-old boy who developed generalized urticaria after the third Di-Te-Pol vaccination. The case history strongly indicates that this clinical reaction was mediated by specific IgE against one or more of the components in the vaccine.

Vaccine induced histamine release was observed only from the patient's leukocytes and not from the cells of healthy individuals. When IgE was removed from the patient's basophils, the histamine release was abolished. Further, that sensitization of cord blood basophils only occurred using patient's plasma whereas plasma from non-allergics showed no Diphtheria specific sensitization.

The fact that cells are washed free of plasma components in both direct histamine release and passive sensitization experiments excludes the involvement of non-homocytotropic antibodies, the complement system or the coagulation system.

We therefore conclude from the histamine release results that the patient had a specific reaction restricted to Diphtheria and to the purified Diphtheria Toxoid in the vaccine and exclude a reaction to Tetanus and polio components of the mixed vaccine.

The present case report raises several questions: What is the frequency of Type I allergic reactions to vaccine components in allergic and non-allergic children? What is the natural course of the hypersensitivity? Sensitization to Diphtheria or Tetanus is probably not common but it would be of benefit to know the prevalence of risks of allergic reactions. The boy in this study had positive SPT, CAP and histamine release to egg and milk. Also open provocations showed probable skin manifestations to egg and milk. Possibly urticaria to Diphtheria could be a

reaction only found in multi-sensitized children? It would be of interest to determine if any particular food sensitization would predict allergic vaccine reactions.

It has important clinical implications to demonstrate or exclude IgE mediated vaccine reactions when a booster vaccination is considered in people with a history of adverse allergic reactions. This is especially the case when Tetanus vaccination is considered. Also vaccination against Diphtheria is of increasing importance due to the increasing frequency of Diphtheria in eastern Europe.

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