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Anaphylactic reaction to diphtheria–tetanus vaccine in a child: specific IgE/IgG determinations and cross-reactivity studies

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Abstract

The present study describes the occurrence of an anaphylactic reaction after the administration of the fifth booster dose of DT vaccine in a six-year-old child. Skin test, in vitro determinations of specific IgE antibodies and immunoblotting assays showed that the IgE response was directed against tetanus and diphtheria toxoids (Dtx). IgG antibodies were also detected by ELISA and immunoblotting. The RAST and immunoblotting inhibitions showed no cross-reactivity between the two toxoids, indicating the presence of co-existing but non-cross-reacting IgE and IgG antibodies. This was maintained in two subsequent determinations done 18 and 30 months after the episode. To our knowledge, this is the first study of cross-reactivity between tetanus and diphtheria antigens. We show that simultaneous IgE antibodies to two different toxoids may occur, indicating that after an immediate reaction to DT, a search for IgE antibodies to both tetanus and Dtx should be undertaken.

Keywords: Tetanus toxoid; Diphtheria toxoid; Anaphylaxis

1. Introduction

Adverse reactions to vaccines are common and those of an immunological nature, such as anaphylaxis and Arthus type reactions, are of more concern. The mechanisms proposed are the production of specific IgE antibodies to any of the vaccine components and the formation of immune complexes between IgG antibodies and the vaccine antigens [1]. IgE mediated anaphylactic and local reactions have been observed after immunisation with tetanus (Ttx) and diphtheria toxoids (Dtx) notwithstanding these are showing a very low frequency in the whole population [2–5]. Since vaccines are composed of several constituents, the identification of the responsible allergens or haptens requires a detailed analysis of all the vaccine components. Components and contaminants of toxoid vaccines, such as gelatine or peptones and preservatives such as thimerosal, have been reported as causal agents in these type of reactions [6–8].

The present report describes the occurrence of an anaphylactic reaction immediately after a diphtheria–tetanus (DT) booster dose in a child. A detailed immunochemical study was undertaken to identify the allergenic component of the vaccine and the possible occurrence of cross-reactivity between the toxoids.

2. Material and methods

2.1. Case

A six-year-old child suffered a severe adverse reaction after the fifth intra-muscular dose of a DT vaccine plus the simultaneous oral supply of polio vaccine. Thirty minutes after administration, the patient developed a large local inflammatory response at the injection site, followed by a generalized systemic reaction with erythema, pruritus, urticaria, palpebral oedema, discomfort and nausea. The systemic reaction improved 15–20 min after treatment with adrenaline and anti-histamines, but the palpebral and local oedema subsided four days later. The patient was evaluated 6 months after episode and sequential serum samples were obtained at different points: 6, 18 and 30 months after the allergic reaction, and stored at −80 °C until use.

2.2. Skin tests

Prick tests were carried out 6 months after the reaction as previously described [9], and an increase greater than

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3 mm × 3 mm in the wheal area was considered as positive. The same lot of the DT vaccine producing the reaction contained 25 Lf of tetanus toxoid (Ttx), 10 Lf of Dtx, 0.45 mg of aluminium phosphate and 0.05 mg of thimerosal (anatoxal diphtheria–tetanus, Berna, Madrid, Spain). Tetanus vaccine (Anatoxal Tetanus, Berna), diphtheria vaccine (anatoxal diphtheria, Berna), trivalent polio vaccine (Alcala Farma S.L., Madrid, Spain) and thimerosal (1% w/v in saline buffer) were also tested. The study included possible allergenic contaminants of the DT vaccine from the culture media such as peptone, papain, toxiproton, beef meat, and yeast extract, tested at 10 mg/ml in phosphate–saline buffer. All were negative in a control group of DT vaccine immunised subjects. Patch testing was performed with a standardised concentration of aluminium chlorhydroxide (10%, w/v in aqueous solution) and thimerosal (0.1%, w/v in vaseline) [10].

2.3. IgE antibody determinations

Specific IgE against Ttx and Dtx (both provided by Berna) were assessed by radioallergoabsorbent test (RAST). Each toxoid was coupled to CNBr-activated discs at an optimal concentration of 20 Lf/ml. The conjugated discs were incubated with each serum during 3 h. They were then washed three times with PBS containing Tween 20 at 0.5% (v/v) (Sigma), and finally 50 μl of ^{125}I-labelled anti-human IgE (Pharmacia, Uppsala, Sweden) were added and the mixture incubated overnight. RAST values (% of label uptake) were assessed using 12 negative control sera with total IgE values ranging between 8 and 1200 kU/l. A positive value was considered to be the mean ± 2 S.D. of these negative controls. This was 2% of label uptake. All samples were tested in duplicate and in the same assay. Total sera IgE were assessed by IMx-method (Abbott Diagnostics, Chicago, IL, USA).

2.4. IgG antibody determinations

Ninety-six well plates (Dynatech, Sussex, UK) were coupled with 100 μl per well of 160 Lf/ml of tetanus or Dtx. The sera were assessed diluted 1:100. Goat anti-human IgG-HRP (Southern Biotechnology Associates, Birmingham, AL, USA), diluted 1:2000, was used as a second antibody. The enzymatic reaction was carried out using *o*-phenylenediamine dihydrochloride (Sigma) and the absorbance was measured at 492 nm.

2.5. RAST inhibition

The IgE specificity and cross-reactivity between both toxoids was studied by direct and cross-competitive RAST-inhibition. Each serum (50 μl) was co-incubated overnight with 30 μl of inhibitor (tetanus or Dtx) at a final concentration of 20 Lf/ml, after which the toxoid conjugated discs were added. The rest of the procedure was the same as for the RAST assay.

2.6. SDS-PAGE, immunoblotting and immunoblotting-inhibition

SDS-PAGE was performed according to the method of Laemmli [11], at 10% of polyacrylamide concentration. The proteins were stained with Brilliant-blue R-250 or electrophoretically transferred to PVDF membranes (Immobilon, Millipore, Molsheim, France), essentially according to Towbin et al. [12]. After blocking, the blots were incubated overnight with 30 months serum diluted 1:100 and 1:10 for IgG and IgE detection, respectively. Finally, alkaline phosphatase-labelled mouse anti-human IgG or mouse anti-human IgE (Southern Biotechnology Associates, Birmingham, AL, USA), diluted 1:2000 and 1:500, respectively, were used as a second antibody and developed using the amplified alkaline phosphatase immuno-blot kit (Bio-Rad, Hercules, CA, USA).

The immunoblotting-inhibition assays were carried out as follows: three independent PVDF sheets were prepared, each containing two lanes corresponding to the transference of tetanus and diphtheria proteins from SDS-PAGE. One sheet was incubated with diluted serum alone (negative control of inhibition), another was co-incubated with diluted serum plus 40 Lf/ml of Ttx, and the third was co-incubated with diluted serum plus 40 Lf/ml of Dtx. The rest of the procedure was the same as for the immunoblotting assay.

3. Results

3.1. Skin tests

The skin prick tests were positive with DT, tetanus and diphtheria vaccines. The polio vaccine and the DT vaccine components and contaminants were negative. Patch testing with aluminium hydrochloride and thimerosal were also negative.

3.2. Serological studies

The RAST assays showed specific IgE antibodies to tetanus and Dtx, in the first serum and in the two subsequent samples. There was no variation in the total IgE levels over this period of time (Table 1A).

The direct and cross-competitive RAST-inhibition studies (Table 1B) showed that when Ttx antigen was compared in the solid and fluid phases there was complete inhibition. The same results were observed using Dtx antigens in the solid and fluid phases. However, no inhibition was observed using Ttx versus Dtx or Dtx versus Ttx, indicating non cross-reactivity at the IgE level between tetanus and Dtx in the patient's three sera.

In SDS-PAGE (Fig. 1A), two intense bands and one weak band corresponding to 250, 150 and 42 kDa, respectively, were observed for Ttx. For Dtx, there were at least three bands of intense staining, corresponding to 250, 150 and 48 kDa.

Table 1
Serological determinations of IgE and IgG antibodies against toxoids

	(A) Serum			(B) Serum			
	Total IgE	Ttx IgE	Dtx IgE	Ttx vs. Ttx	Ttx vs. Dtx	Dtx vs. Dtx	Dtx vs. Ttx
6 months	220 [635 ± 187][a]	8.2 [1.4 ± 0.8][a]	5.2 [1.7 ± 0.6][a]	100	0	100	3
18 months	200	8.6	5.7	100	2.3	100	1.3
30 months	220	7.3	4.6	100	0	100	0

(A) Levels of total IgE (in kU/l) and specific IgE against Ttx and Dtx in % of label uptake. The mean ± 2 S.D. values obtained from 12 control subjects are showed in square brackets. (B) Results of RAST-competition assays shown in % of inhibition. The first named antigens are in the solid phase and the second named antigens in the fluid phase (% inhibition).

[a] The age of these subjects was ranging between 10 and 50 years.

The corresponding analysis of the IgE antibodies binding to the different Ttx and Dtx bands is shown in Fig. 1B. Lanes 1 and 2 show that for Ttx the 250 and 150 kDa bands were able to fix IgE antibodies, and for Dtx the binding of IgE antibodies corresponded to the 150 and 48 kDa proteins, respectively.

The IgE immunoblotting-inhibition assays are also shown in Fig. 1B. When Ttx was in the fluid phase, full inhibition with absence of the corresponding bands was observed in the Ttx lane 3, but no inhibition was observed in the Dtx (lane 4), whereas, the inhibition using the Dtx in the fluid phase showed complete disappearance of the immunodetection of the diphtheria antigens but no inhibition of the tetanus antigens (lanes 5 and 6).

Specific IgG antibodies to Ttx and Dtx were detected in each of the patient's three samples by ELISA. No differences were observed in the IgG levels to both toxoids among these samples. The patterns of protein recognition and cross-reactivity of the IgG antibodies were studied by immunoblotting (Fig. 1C). The protein bands binding IgE antibodies were also recognized by IgG antibodies, but a new band was observed not detected in Ttx, corresponding to a MW of 42 kDa (Fig. 1C, lane 1). Inhibition of Ttx on membrane with itself in the fluid phase produced no immunodetection of Ttx antigen bands, though the Dtx bands were not inhibited. The same but opposite pattern was observed when the two antigens on membrane were inhibited with Dtx.

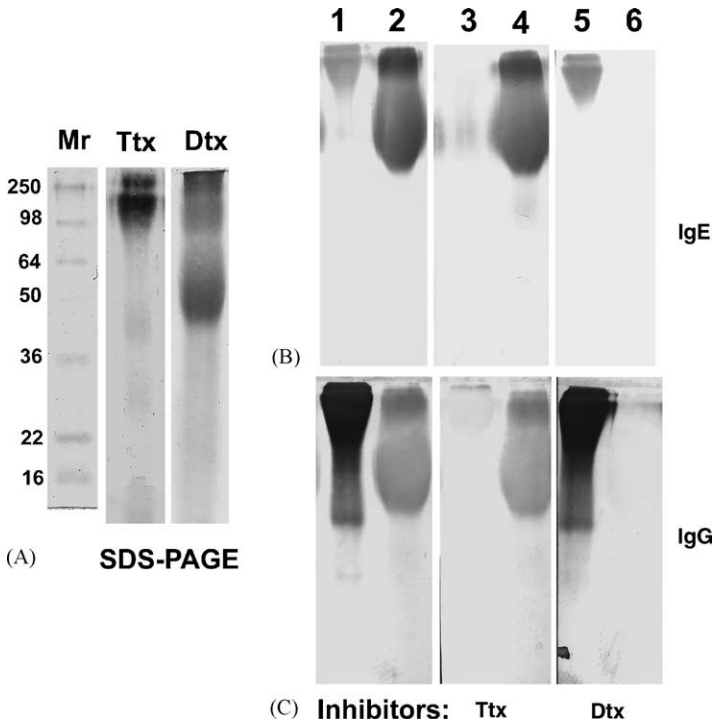

Fig. 1. SDS-PAGE, immunoblotting and immunoblotting inhibition assays of Ttx and Dtx. (A) SDS-PAGE with Ttx and Dtx stained with Brilliant blue. The standard molecular weights are in the left lane. (B) IgE immunoblotting using the patient's last serum against Ttx (lane 1) and Dtx (lane 2). Lanes 3 and 4 correspond to immunoblotting-inhibition with Ttx in the fluid phase, and lanes 5 and 6 correspond to inhibition with Dtx. (C) IgG immunoblotting and immunoblotting-inhibition with the same distribution of lanes and conditions as in (B).

4. Discussion

Although anaphylactic reactions are extremely rare cases have been reported in individuals immunized with toxoids [13]. Most of these cases provide just a clinical description and in only a few was the presence of IgE antibodies studied [2,4,6,13]. The present report describes the occurrence of an anaphylaxis in a six-year-old child after the simultaneous administration of the fifth booster dose of DT vaccine and oral polio vaccine. The skin tests and serological studies showed the presence of specific IgE antibodies to tetanus and Dtx, but not to other components or possible contaminants of the two vaccines. SDS-PAGE and immunoblotting showed that the immunogenic/allergenic proteins corresponded to the more abundant bands in the tetanus and Dtx. The competition RAST and immunoblotting-IgE results demonstrated that no cross-reactivity existed between the tetanus and Dtx. We have found no study examining cross-reactivity between toxoids in immunized individuals with IgE antibodies. In our case, the presence of specific IgE to both toxoids without cross-reactivity suggested that the anaphylactic reaction could have been induced by either or by both toxoids simultaneously. To our knowledge, no similar case have been reported.

We found protective IgG levels to both toxoids, but again without cross-reactivity. These IgG and the IgE antibodies were still detectable 30 months after the reaction. Thus, the child remained immunized to both toxins because the IgG levels to the toxoids remained high in the last serum sample. If the antitoxoid titre in these cases (IgG levels over 0.01 IU/ml [14]) shows the subject to be immune, further vaccination should not be administered, in order to avoid another anaphylactic reaction. However, if the child proves not to be immune, we propose the use of a desensitization protocol [15,16].

Our study demonstrates that when a reaction occurs with a vaccine, one or more components can be the allergens. Thus, to avoid further exposure, a detailed study should be undertaken in order to establish the causal agent or agents.

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