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.

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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 (ana-
toxal diphtheria–tetanus, Berna, Madrid, Spain). Tetanus vaccine (Anatoxal Tetanus, Berna), diphtheria vaccine (ana-
toxal 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 cul-
ture 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 Trx and Dtx (both provided by Berna)
were assessed by radioallergosorbent test (RAST). Each
toxoid was coupled to CNBr-activated discs at an optimal
concentration of 20 LF/ml. The conjugated discs were incu-
bated with each serum during 3 h. They were then washed
twice with PBS containing Tween 20 at 0.5% (v/v)
(Sigma), and finally 30 μL of 125I-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 con-
sidered to be the mean ± 2 S.D. of these negative controls.
This was 2% of label uptake. All samples were tested in du-
plicate 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 cou-
pled 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, Birm-
tingham, AL, USA), diluted 1:2000, was used as a second
antibody. The enzymatic reaction was carried out using
o-phenylenediamine dihydrochloride (Sigma) and the ab-
sorbance 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 conju-
gated 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 (Immo-
blon, Millipore, Molsheim, France), essentially according
to Towbin et al. [12]. After blocking, the blots were incu-
bated overnight with 30 months serum diluted 1:100 and
1:10 for IgG and IgE detection, respectively. Finally, alka-
line phosphatase-labelled mouse anti-human IgG or mouse
anti-human IgE (Southern Biotechnology Associates, Birm-
tingham, 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 transfere 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 Trx, 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 neg-
ative.

3.2. Serological studies

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

The direct and cross-competitive RAST-inhibition stud-
ies (Table 1B) showed that when Trx antigen was compared
in the solid and fluid phases there was complete inhibi-
tion. The same results were observed using Dtx antigens
in the solid and fluid phases. However, no inhibition was ob-
served using Trx versus Dtx or Dtx versus Trx, 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 48 kDa, respectively,
were observed for Trx. 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

<table>
<thead>
<tr>
<th>(A) Serum</th>
<th>(B) Serum</th>
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<tbody>
<tr>
<td>Total IgE</td>
<td>IgE Ttx vs. Ttx</td>
</tr>
<tr>
<td>6 months</td>
<td>220 [635 ± 187]</td>
</tr>
<tr>
<td>18 months</td>
<td>200</td>
</tr>
<tr>
<td>30 months</td>
<td>220</td>
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(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).

* 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 remained high in the last serum sample. If the antitoxin 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.

References