
Immediate allergy to tetanus toxoid vaccine: determination of immunoglobulin E and immunoglobulin G antibodies to allergenic proteins

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Background: Adverse reactions to tetanus toxoid (TT) vaccine are mostly mild and limited to the injection site. However, immunoglobulin (Ig)E-mediated reactions may occur, and the incidence of anaphylactic responses to TT immunization is 0.001%. When TT induces an allergic reaction, the potential causative agents can be TT antigens, thimerosal or aluminum phosphate.

Objective: We studied four children who developed immediate urticaria after TT vaccine, soon after the reaction and 5 years later.

Methods: Skin tests were performed separately with TT vaccine and two vaccine components, thimerosal and aluminum phosphate, and the diagnosis was confirmed by provocation test. IgE and IgG antibodies to TT and their specificities were determined. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting were performed to characterize the antigenic proteins.

Results: All four children were immediate skin test-positive to TT, but negative to thimerosal and aluminum phosphate; 3 developed a reaction after intramuscular provocation using increasing doses of TT vaccine; and 1 refused to be tested. All these tests were negative in five controls, all of whom received TT vaccine and developed only local swelling at the site of application 24 hours after vaccine administration. After 5 years the IgG antibodies were still high in all cases and the IgE antibody values fell by 50%. Patients allergic to TT vaccine produced IgE and IgG antibodies, which decreased at different rates but remained for at least 5 years. The pattern of antibody decrease was confirmed by radioallergosorbent test, enzyme-linked immunosorbent assay, or immunoblotting assay. IgE and IgG antibodies recognized two proteins derived from TT, of 150 and 50 kDa, corresponding to the intracellular form and to a chain of the extracellular form of the tetanus neurotoxin.

Conclusions: In children with immediate allergic reactions to TT vaccine, antibodies may persist for at least 5 years, requiring evaluation by skin and/or in vitro tests before subsequent treatment.

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INTRODUCTION

Tetanus toxoid (TT) antigen is administered alone or together with diphtheria toxoid (DT) and pertussis antigens, the DPT polyvalent vaccine. This combination has proven to be safe and to induce an immunologic response.¹ Although this response, produced mainly by immunoglobulin (Ig)G antibodies, is generally protective, adverse reactions have nevertheless been reported,² which in most instances are mild and limited to the site of vaccine application.³ There is evidence that urticaria reactions to diphtheria toxoid are more frequent in subjects who have been repeatedly immunized than in subjects who have received only one dose.⁴ However, these reactions do not usually contraindicate the subsequent admin-

istration of the vaccine because they are not generally life-threatening³ and induced by the pertussis component of the DPT vaccine.⁵

However, TT is often administered alone for prophylactic treatment of tetanus infection, and when it does induce an allergic reaction, the potential causative agents can be TT antigens, thimerosal or aluminum phosphate. Although TT has been used widely, immediate reactions are relatively rare,⁶ and the incidence of anaphylactic responses to TT immunization has been established as 0.001%.⁷ In these cases, if tetanus immunization is recommended two possibilities exist: if the antitoxoid titer shows the subject to be immune, further vaccination should not be administered, or if the subject proves not to be immune, a desensitization protocol may be performed.⁸

Although systemic reactions to TT vaccine have been reported,^{9,10} few studies have been carried out to determine the presence of IgE antibodies^{8,11–13}; and further, to our knowledge, no detailed information is available about the

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allergenic component of the TT vaccine. We studied the immunologic response of four children who developed symptoms compatible with an immediate allergic reaction to TT vaccine, showing that the TT vaccine induces an IgE and IgG response to TT antigen, and not to the other vaccine components.

METHODS

Patients and Controls

Patients. Four children who developed an immediate reaction after a single full-dose injection of TT vaccine (Anatoxal te Berna, Instituto Berna, Madrid, Spain) were included in the study (group A). In all cases this vaccine was administered because of some type of wound. Case 1 was a 10-year-old boy, with no history of atopy, who developed an urticarial reaction on the legs and abdomen 30 minutes after the injection of TT vaccine. Case 2 was a 13-year-old boy with allergic rhinitis who developed an immediate urticarial reaction on the legs, left arm, and neck 20 minutes after TT vaccine administration. Three years before, he had developed a similar reaction after administration of the same TT vaccine. Case 3 was a 12-year-old boy with allergic bronchial asthma, who developed generalized urticaria on the legs, abdomen, chest, and face 14 minutes after TT vaccine administration. Case 4 was a 12-year-old boy with no history of atopy, who developed a generalized urticaria 13 minutes after TT vaccine application.

Skin and immunologic tests were performed twice. The first evaluation (T1) was performed 4, 1, 4, and 1 months after the reaction, for cases 1 to 4, respectively, and the second time (T2) 5 years after T1. Between T1 and T2 the children had not received any TT vaccination.

Control group. Five age- and sex-matched children who received TT vaccine because of some type of wound, and who developed only local swelling (from 10 to 15 cm) at the site of application 24 hours after vaccine administration were included in group B. They were evaluated from 1 to 4 months after immunization (T1) and 5 years later (T2).

The study was approved by the institutional review board, and informed consent for all the diagnostic procedures was obtained from the families for both patients and controls.

Skin Tests

These were made by prick using TT vaccine (Anatoxal te Berna) without diluting and, if negative, intradermal tests were carried out at 1:1,000 and 1:100 dilutions. Intradermal tests were also performed with thimerosal at a concentration of 0.01% and aluminum phosphate at 0.05 mg/mL. These were carried out as described.¹⁴ In brief, 0.05 mL solution was injected into the forearm skin and the wheal area was marked. The final area was marked and an increase $> 3 \times 3$ mm was considered positive. Control histamine was used as a positive control and saline as negative in both prick and intradermal testing.

Provocation Tests

To confirm the diagnosis at T1, patients were readministered the TT vaccine in a single-blind, placebo-controlled way at increasing doses (0.05, 0.15, and 0.3 mL) at 1-hour intervals.

TT Protein Purification

Vaccine (25 mL; Anatoxal te Berna), containing 10 limit flocculation (Lf) TT, 0.4425 mg aluminum phosphate and 0.05 mg thimerosal in each 0.5-mL vial, were ultracentrifuged (Beckman L8-70 Ultracentrifuge, San Ramon, CA) for 1 hour at 30,000 rpm. The supernatant included the soluble TT proteins (sTT) and thimerosal, and the latter were separated by Sephadex G-10 filtration (Pharmacia, Uppsala, Sweden) and membrane dialysis with a cut-off point of 20 kDa (Medicell International, London, UK). Later, the sTT were concentrated by ultrafiltration using Microcom centrifugal filter devices (Millipore SA, Molsheim, France) with a 10 kDa cut-off, up to a final protein concentration of 5 mg/mL measured by a modified Bradford assay (Pierce Chemical, Rockford, IL). The precipitate phase contained TT proteins absorbed to aluminum phosphate (aTT).

IgE and IgG Antibody Determination

IgE determination was carried out by radioallergosorbent test (RAST) as described,¹⁵ using cyanogen bromide-activated discs conjugated to sTT. The optimal protein concentration conjugated to discs for determining IgE antibodies, calculated by concentration-effect curves, was 10 μ g/mL sTT. The cut-off between positive and negative was established as the mean plus two standard deviations of a group of 10 subjects who had not received a TT vaccine for at least 10 years. The results showed that a value of 1.5% of label uptake could be considered as positive.

IgG was measured by enzyme-linked immunoadsorbent assay (ELISA) as described,¹⁶ using 1 μ g/mL sTT coupled to the plates. A human Ig to TT (Grifols SA, Barcelona, Spain), the antitoxin content of which had been determined in vivo in terms of the International Standard for Tetanus Antitoxin, was used as the reference preparation. The results were expressed in IU of IgG antibodies to sTT and an antibody level >0.02 IU/mL was considered protective.

Specificity of IgE Antibody Study

This was carried out by RAST inhibition assay as previously described.¹⁷ In the solid phase, discs conjugated to sTT were used and sTT, aTT, thimerosal, and purified TT antigen (provided by SB Schw.serum-U Impfinstitut, Germany) were used as inhibitors in the fluid phase, at the following concentrations: 100, 10, 1, and 0.1 μ g/mL. To avoid precipitation of the aTT, the incubation with the sera was shaken for 3 hours.

Determination of Molecular Weight of sTT and Capacity of Fixing IgE and IgG Antibodies

The molecular weight was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli.¹⁸ In brief, 50 μ g sTT were mixed 3:1 with dissociation buffer heated at 100° C for 5

minutes and electrophoresed in 8% analytical SDS-polyacrylamide gel. Sigma high range standards were used as molecular weight markers. The separated proteins were stained with brilliant blue R-250 (Sigma, St. Louis, MO) or transferred to nitrocellulose sheets (Millipore) to determine which sTT proteins were able to fix IgE or IgG antibodies by immunoblotting assay. This was performed according to Towbin et al¹⁹ using a Trans-blot apparatus (Mini Protean III, Bio-Rad, Hercules, CA). After blocking, with 5% wt/vol fat-free milk powder, membranes were incubated with serum diluted 1:200 for IgG detection, or 1:10 for IgE detection. After washing, blots were incubated with horseradish-peroxidase-labeled goat anti-human IgG or anti-human IgE antibodies (Sigma) diluted 1:10,000 and 1:400, respectively. Finally, detection was made using an enhanced chemiluminescence method (ECL, Amersham Pharmacia Biotech, Uppsala, Sweden) or with 4-chloro-1-naphthol.

RESULTS

In Vivo Studies

Skin tests were positive in all children from group A in the first evaluation (T1): case 3 was positive by prick at 1:1 (4 mm diameter), cases 2 (3 mm increase in diameter) and 4 (3 mm increase in diameter) both intradermally at 1:1,000, and case 1 (4 mm increase in diameter) intradermally at 1:100. In cases 1 and 4, skin tests remained positive, at the same dilutions used in T1, 5 years after the first evaluation (T2), case 3 was negative by prick and intradermally positive at 1:100, and case 2 was intradermally positive at 1:100-fold dilution. These concentrations gave a negative skin response in the control group (group B) at both T1 and T2. Skin tests with thimerosal and aluminum phosphate were negative in all cases and controls.

A provocation test was performed to confirm the diagnosis at T1. Case 1 developed an urticaria 40 minutes after the administration of 0.3 mL TT vaccine. Case 2 developed generalized pruritus and light urticaria 30 minutes after administration of 0.15 mL. Case 4 developed urticaria on the trunk 40 minutes after the administration of 0.3 mL. Case 3 refused to be tested.

IgG Antibody Determination

The levels of IgG antibodies at T1 and T2 in groups A and B are shown in Figure 1. At T1 the levels of IgG antibodies to sTT were high in all children from both groups but higher in those with immediate allergic reactions to TT. In both groups the samples obtained at T2 showed a slight decrease in the IgG levels. Five years after the reaction, 80% of the patients and 60% of the controls still had IgG antibodies to TT vaccine and were therefore still immunized.

IgE Antibody Determination

The results of IgE antibody levels at T1 and T2 can be seen in Figure 2. In group A, at T1, all cases had positive RAST values ($>1.5\%$) ranging from 53.7 to 2.6%. Five years later (T2) the four children remained positive with RAST values

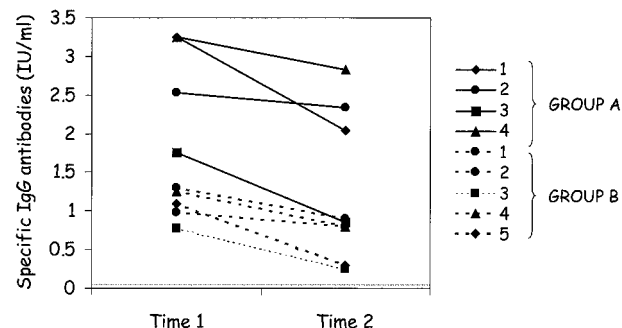


Figure 1. IgG antibody levels determined by ELISA in sera of four children with an immediate allergic reaction to TT vaccine (group A) and five children with non-immediate local reactions (group B). Blood samples were obtained at the time of the evaluation, which was during the first year after the reaction (T1), and 5 years later (T2).

ranging from 23.9 to 1.9%. Comparison of T1 and T2 IgE antibody levels showed a decrease of 50% for all cases. In contrast, in all children from group B, the RAST results were negative ($<1.5\%$) at both T1 and T2.

Specificity of IgE Antibody

RAST inhibition assay was made to study the specificity of the IgE antibodies to the different vaccine components. In all cases, sTT and purified TT were the most potent inhibitors, with a mean inhibition of 80% at 100 $\mu\text{g/mL}$, and 60% at 10 $\mu\text{g/mL}$. Although to a lesser degree, aTT protein also showed inhibition with a mean value of 60% at the maximum concentration and 30% at 10 $\mu\text{g/mL}$. Thimerosal showed no inhibition at any of the concentrations tested. Figure 3 presents the mean inhibition results.

Determination of the Molecular Weight of the sTT Proteins and Their Capacity to Fix IgE and IgG Antibodies

Immunoblotting tests to detect IgG and IgE antibodies to TT were carried out with each patient's sera obtained at T1 and T2. In all patients both IgG and IgE antibodies recognized two different molecular weight bands corresponding to 150 and 50 kDa. Although the pattern was similar at both times for all patients, the blot band intensity decreased in parallel with the decrease of both IgG and IgE antibodies, determined by ELISA and RAST, respectively. Representative results of case 1 are shown in Figure 4. The left lane shows the SDS-PAGE of the molecular markers, lane 1 is the SDS-PAGE of sTT, and lanes 2 and 3 correspond to the immunoblotting of sTT with the patient's serum, determining IgG and IgE antibodies, respectively.

DISCUSSION

There is evidence that TT vaccines may contain antigenic components able to induce an immediate or delayed allergic response.²⁰⁻²² These include TT proteins,¹¹ component traces of proteins from the culture media,^{9,10} thimerosal,²⁰ and aluminum phosphate.²³ The allergenic properties of thimerosal have been studied and can induce experimental sensitization

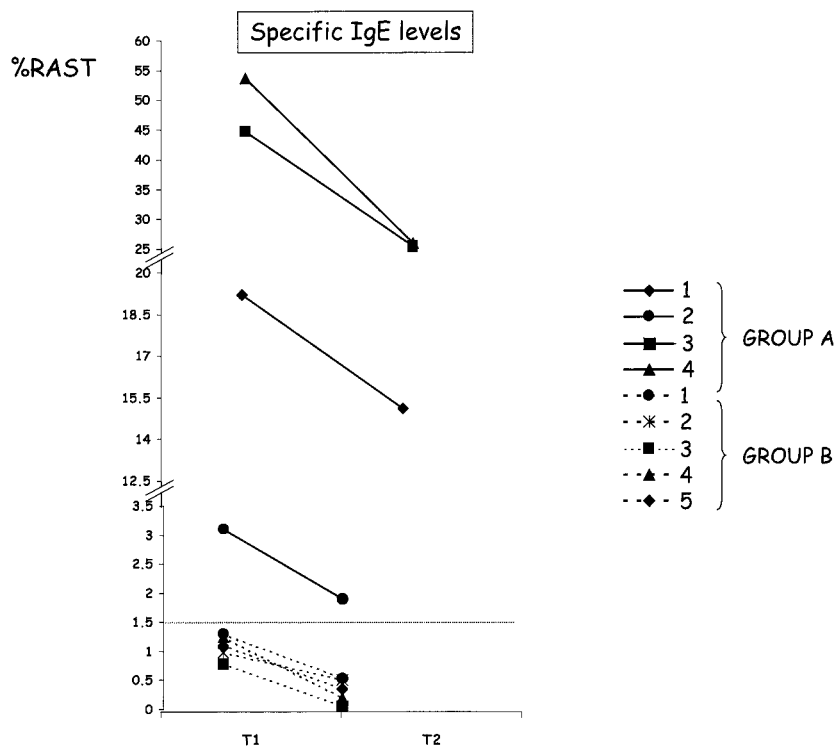


Figure 2. Levels of IgE antibodies determined by RAST in sera of four children with immediate reactions to TT vaccine (group A) and five children with non-immediate local reactions (group B). Blood samples were obtained at the time of the evaluation, which was during the first year after the reaction (T1), and 5 years later (T2).

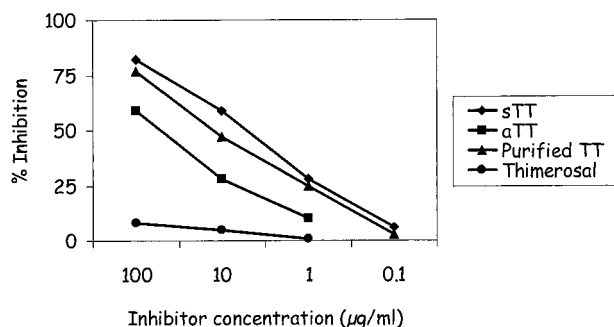


Figure 3. Mean inhibition results of the RAST inhibition assay. Different concentrations of sTT (◆), (aTT) (■), purified TT (▲), and thimerosal (●) were used as inhibitors in the fluid phase. In the solid phase discs conjugated to sTT were used.

in guinea pigs.²⁴ Likewise, thimerosal can result in positive delayed-hypersensitive patch tests in 1 to 18% of subjects.²⁵ In our cases an immediate or delayed allergy response to thimerosal was excluded because the children had no positive history of allergy to mercury compounds, their skin tests were negative in both the immediate and delayed readings, and in the RAST inhibition assay thimerosal was unable to inhibit the binding of IgE antibodies to TT proteins. Aluminum adjuvants, used for improving the immune response of poorly immunogenic toxoids, may contribute to or increase the production of IgE antibodies,²⁶ and sensitization to aluminum has been reported in children.²³

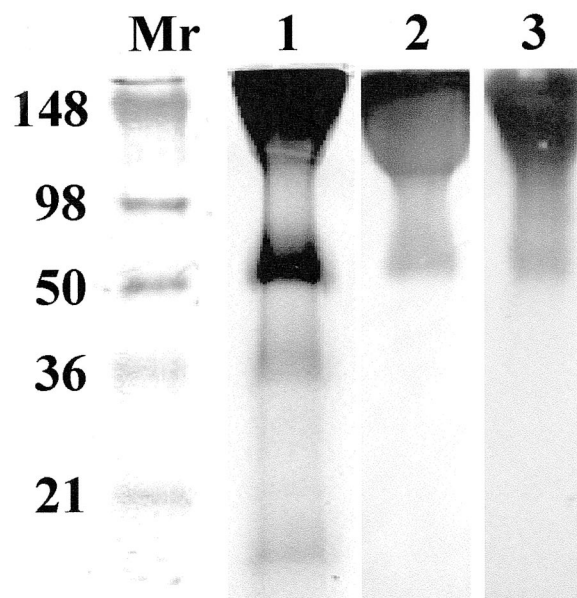


Figure 4. Representative results of SDS-PAGE and immunoblotting assays with sTT protein, in case 1. Lane 1 shows the SDS-PAGE of sTT (50 μg of total protein); lane 2 the immunoblotting of sTT developed with anti-human IgG; and lane 3 the immunoblotting of sTT developed with anti-human IgE.

However, in our cases, the possibility of an immediate or delayed reaction to this compound was also discarded because immediate and delayed intradermal tests to this com-

pound were negative. IgE and IgG antibodies to TT have been found in 25% of subjects receiving a tetanus immunization without any reaction,¹¹ and this is not restricted to subjects with an atopic background.^{2,12} However, in our study, in the group of children with local non-immediate reactions (group B), no IgE antibodies could be detected at either of the two determinations, but in children with immediate allergic reactions (group A), all cases had circulating IgE antibodies. Although we have studied a limited number of cases, the difference in IgE response between both groups is clear, and is in agreement with the patient's reactions and with the allergologic study results. Of note was the fact that IgG antibodies were detected at T1 in all cases of both groups, although they were higher in those with immediate reactions, which probably only indicates immunization, as has been described with subjects with no allergic reactions to β -lactams after penicillin treatment.²⁷ Evidence shows that although IgG antibodies persist, IgE antibodies may not be detectable after 2 years in one-third of all cases initially found positive.¹³ In our study, the IgG response after a longer period, 5 years, still showed similar IgG values and the IgE response was still detectable, although the RAST values of these latter decreased approximately 50%. This may imply that patients are still at risk of developing an allergic response if they are vaccinated 5 years or later after having had a reaction. The persistence of IgG antibodies in these children shows that they are immune, indicating that further vaccination may not be needed.⁸

It is important to mention that although there is evidence of the IgE response in subjects immunized with TT vaccine,^{3,11,28} no studies have been carried out to identify the responsible allergenic proteins. The results of SDS-PAGE and immunoblotting showed that, in the sTT, the antigens able to fix both IgG and IgE antibodies were two proteins of approximately 150 and 50 kDa, the molecular weights of which correspond to the intracellular form and to a chain of the extracellular form, respectively, of the tetanus neurotoxin.²⁹ This indicates that in these patients neurotoxin is probably the main immunogenic protein in the TT vaccine. We demonstrated by RAST inhibition that these proteins were also present in the precipitate and that none of the other TT vaccine components was able to fix IgE antibodies from the patients' sera. The protein pattern of recognition in the immunoblotting assay was identical for both IgG and IgE isotypes for all children studied, with the only difference being in the intensity of the bands.

CONCLUSION

Although severe reactions to TT vaccine are not common, possibly because of the presence of blocking IgG antibodies in subjects with IgE antibodies,^{11,28} immediate systemic reactions do occur.⁶ This side effect of the vaccination limits compliance even after a long period of immunization. Thus, searching for the presence of IgE and IgG antibodies to these antigens would help in identifying 1) those who are still immunized and do not need more vaccine administration and 2) those who are at risk and need to be desensitized. Further,

the correct identification of the allergenic proteins would help improve the current immunoassays to detect both antibodies.

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