



Technical note

Usefulness of basophil activation test for the diagnosis of IgE mediated hypersensitivity to tetanus toxoid vaccine

Blanca Herreros^a, Yesica Méndez^b, Francisco Feo-Brito^{b,c}, José Miguel Urra^{a,c,*}^a Immunology, Hospital General Universitario de Ciudad Real, Ciudad Real, Spain^b Allergy, Hospital General Universitario de Ciudad Real, Ciudad Real, Spain^c Medicine School, Universidad de Castilla la Mancha (UCLM), Ciudad Real, Spain

ARTICLE INFO

Keywords:

Tetanus toxoid

Basophil activation test

IgE

CD63

ABSTRACT

A great number of vaccinated patients develop specific anti-tetanus toxoid IgE, but usually do not undergo any adverse effect. Most of the allergic reactions to tetanus toxoid vaccine usually present with unspecific symptoms of local inflammation. In the presence of severe reactions, and in a special way if the vaccine is provided together with other drugs, it is difficult to establish which is the harmful drug responsible for IgE-mediated adverse reaction. A patient with an anaphylactic reaction after the administration of Toxoid Tetanic (TT) along with several drugs is described. All skin test were negative. The basophils activation test (BAT) in a clear way, identified TT as the allergen that triggered anaphylaxis. The results achieved demonstrates the usefulness of BAT to clarify patients with hypersensitivity to tetanus toxoide when the clinic is severe and the vaccine has been administered together with other drugs.

1. Introduction

Tetanus toxoid (TT) is administered as a vaccine in the prophylaxis of tetanus infection. The vaccine also contains other agents that may be potentially antigenic, such as thimerosal and aluminum phosphate. Most of the allergic reactions to TT vaccine usually present with unspecific symptoms of local inflammation. Only rarely (1/10⁶) have been described anaphylactic reactions after TT vaccine (Bellioni et al., 2001). These reactions are caused by IgE mediated reactions, and the specific IgE presence is evidenced by skin tests or serological studies. An elevated specific IgE indicates antigen sensitization and a propensity to suffer allergy symptoms, but does not indicate irrefutably the appearance of clinical symptoms after a contact with the allergen. The clinical significance of specific IgE to TT is unclear, since tetanus vaccine may induce specific IgE antibodies even in subjects who do not experience any adverse effects after immunization. Fifty percent of 24-month-old children developed detectable TT-specific IgE antibodies in response to immunization, but no major systemic or large local clinical reactions were reported after immunization with TT (Dannemann et al., 1996).

To complete the study in certain allergy diseases, cellular test as they are the lymphoblastic transformation test or, the most frequently used, basophil activation test (BAT) are assayed in vitro. The BAT allows the accurate diagnosis of immediate hypersensitivity type 1 reactions, which involved IgE antibodies (Leysen et al., 2011).

2. Patient and methods

Patient: A 80 year old man to which after a stab wound the following drugs were administered: Naproxen tablet orally, intravenous 2 g of amoxicillin and intramuscular tetanus toxoid. The patient immediately began with blurred vision, generalized rash, and cardiac arrest. The patient required intubation and entered the intensive care unit. One month after the adverse reaction, and after their clinical recovery, the study of the potential allergic response was carried out to beta-lactams and tetanus toxoid. Initially specific IgE antibodies and skin test were performed, and subsequently BAT were tested.

BAT was performed from heparinized blood sample. The cells are previously stimulated for ten minutes at 37 °C with an IL3-containing buffer at a concentration of 2 ng/mL (HEPES 20 mM, NaCl 133 mM, KCl 5 mM, CaCl 7 mM, MgCl2 3,5 mM, Albumin 1 mg/mL, pH 7,4). After a specific activation of basophils at doses of 1 and 0.1 µg of the drug tested for 20 min at 37 °C, the blood sample was incubate with two monoclonal antibodies, anti-IgE monoclonal antibody PE conjugated (AB_10847917) to identify basophils, and anti-CD63 monoclonal antibody FITC conjugated (AB_976622). Anti-CD63 recognizes the gp53 glycoprotein (CD63) expressed only on surface of the degranulated basophils. The sample is analyzed by flow cytometry. The selection of basophils was performed using the lin Side Scatter/log FL2 (Anti-IgE) plot as shown in Fig. 1, and results were obtained on the percentage of

* Corresponding author at: Immunology, Hospital General Universitario de Ciudad Real, Avda Obispo Torija s/n 13005, Ciudad Real, Spain.
E-mail address: jmurra@sescam.jccm.es (J.M. Urra).

<https://doi.org/10.1016/j.jim.2017.11.009>

Received 26 June 2017; Received in revised form 2 October 2017; Accepted 17 November 2017
0022-1759/ © 2017 Elsevier B.V. All rights reserved.

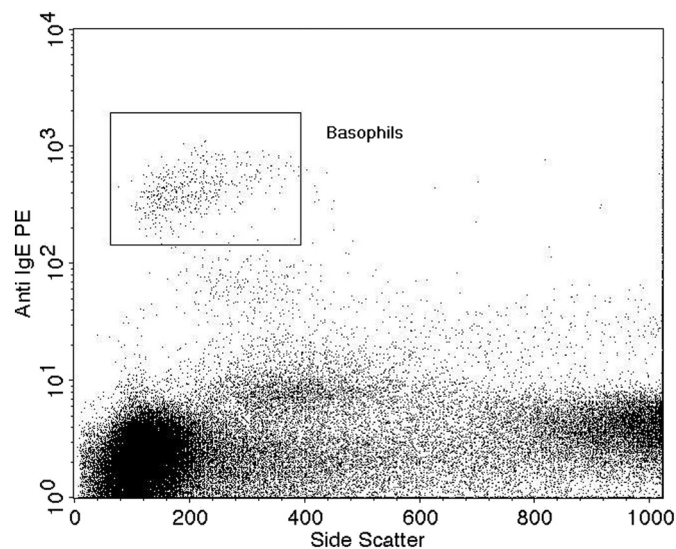


Fig. 1. Selection of blood basophils performed in the flow cytometry using the plot lin SSC/logFL2 (Anti-IgE).

activated basophils induced for each one of the drugs tested. Non-specific activation was used with the N-formyl-Met-Leu-Phe- (fMLP) peptide as a positive control and culture medium only as negative control. The test is considered positive when more than 15% of basophils expressed on surface the CD63 molecule.

3. Results

Skin prick tests were negative for all allergens analyzed in the patient. Results for specific IgE were negative for amoxicillin, penicillin V and penicillin G, and were positive for TT (1.45 IU/mL). The levels of serum total IgE were 683 IU/mL, and at the time of the study the tryptase values were in normal range, 2.3 µg/L.

Subsequently BAT test for amoxicillin, tetanus toxoid and naproxen were performed. The results were negative for naproxen and amoxicillin for both doses of drugs assayed with values of CD63 + basophils less than 2,5% in all cases. The negative control had a value of 1.9%, and positive control with nonspecific activation with the N-formyl-Met-Leu-Phe- (fMLP) peptide of 74.3%. Conversely the test was positive for both doses of TT (75.9% CD63 + at dose of 1 µg/µL and 24.6% at 0.01 µg/µL of TT) as show in Fig. 2. As a test control, four patients vaccinated with TT who developed an IgG protective level and who did not present any adverse reaction to the vaccine were studied. In none of them BAT values were greater than 5% of basophil degranulation (TT 1 µg/µL, 3.7% ± SE 0.3; TT 0.1 µg/µL, 3.1% ± SE 0.2). With these

results the patient was diagnosed of TT hypersensitivity IgE-mediated. The production in the patient of a protective level for IgG specific to TT was verified, and the vaccination schedule discontinued.

4. Discussion

The diagnosis of the causative agent of an allergic reaction is usually based on the determination of total and specific IgE in addition to performing skin tests. Rarely, including drug allergies, these tests yield results inadequate because previous studies suggest that only a 5% of patients with positive history of hypersensitivity and skin test negative show a positive provocation test. Adverse reactions to TT ranged from redness limited in the application site to fever, urticaria, and rarely there have been reports of severe anaphylactic reactions. It is also important stand out that specific IgE antibodies may be detect in more than half of the immunized individuals, however, this finding does not correlate with the development of hypersensitivity reactions (Dannemann et al., 1996). Aluminum-containing adjuvants as in TT vaccine are known to stimulate a Th2 type response and increase the production of specific IgE antibodies (Bortolatto et al., 2015). Transient formation of IgE against the vaccinated antigens is commonly detected and seems to be part of the control of the immune response (Ownby et al., 2009). The biological function of this phenomenon has not been elucidated fully, but it may be the expression of balanced Th1-Th2 type response that optimizes host defense. In the patient described in the present study the skin tests along with the specific IgE did not identify the harmful drug. The usage of BAT clarified who is the causal agent of the anaphylactic reaction when they were administered several drugs simultaneously. There is little published literature on the use of BAT in hypersensitivity to TT because this test is used preferentially in severe immediate hypersensitivity reactions, which are more common to antibiotics, anticonvulsants or NSAIDs. The results achieved in the patient demonstrates the usefulness of an in vitro test as BAT to identify patients with hypersensitivity to TT when the clinic is severe. BAT is a functional test, reports directly about the ability of one antigen to cause degranulation of blood basophils and consequently its capacity of developing anaphylaxis. Specific IgE measurements and skin tests indicate sensitization and do not prove clinical relevance on their own. Recent studies have shown that BAT diagnoses allergy with high accuracy and can be particularly useful in cases with unclear clinical history or equivocal results of other diagnostic tests. In a wide variety of food allergies (peanut, hazelnut, milk, egg, wheat, apple, celery, carrot) BAT has a sensitivity varying between 85 and 100% and a specificity between 77 and 100% (Santos and Lack, 2016). Moreover BAT was found to be an objective marker of airway allergen sensitivity in stable allergic asthmatics, that may be used to predict airway responsiveness when bronchial challenge tests cannot be performed (Dahlén et al., 2011). In our situation BAT was able to highlight about the IgE induced by the

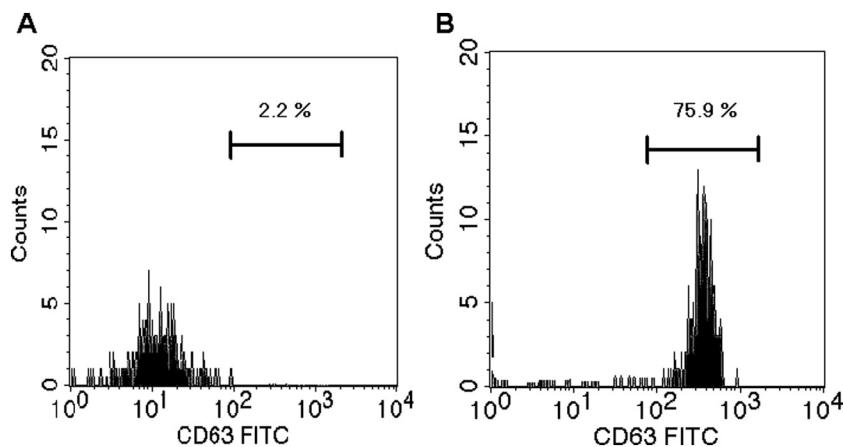


Fig. 2. The figure shows the number of basophils with a surface expression of the CD63 molecule (degranulated). A) BAT after stimulation of cells with Amoxicillin at 1 µg/mL. B) BAT after stimulation of cells with tetanus toxoid at 1 µg/mL. The figure shows a high proportion of degranulated basophils after stimulation with tetanus toxoid.

vaccine, its ability to induce hypersensitivity.

In the presence of severe TT vaccine reactions it is essential to assess the title of anti-toxoid IgG antibodies produced by patient. If the patient has been immunized subsequent vaccinations must be suspended, and whether vaccination remains to need, desensitization protocols should be assessed. In patients with immediate hypersensitivity reactions to TT vaccine, antibodies may persist for at least 5 years, requiring evaluation by skin and/or in vitro tests before subsequent treatment (Mayorga et al., 2003). Reaction to TT vaccine correlates with serum antitoxin IgG levels. Among 104 adults with adverse reactions to TT vaccine, 97% had at least four times the minimal protective level of antibodies (i.e., 0.01 IU/mL), and 58% had levels higher than 5 IU/mL (Levine and Edsall, 1981). The possibility that the reaction to TT is mediated by IgG can be ruled out since, in the cytometric assay basophils were identified according to the presence of IgE on its surface using a monoclonal antibody anti-IgE, and the presence of IgG has been reported to inhibit IgE-mediated anaphylaxis (Strait et al., 2006). In fact, no IgG-associated anaphylaxis has been reported in humans, although alternative mechanisms have been proposed based in mouse models (Finkelman, 2007). Because of the severity of the symptoms, and also by ethics reasons, the in vivo provocation test was not performed, so there is still not conclusively could affirmed that the adverse reaction presented in the study was caused by TT. Drug provocation tests remain the gold standard for the identification of an eliciting drug when allergologic tests are negative, not available, or inconclusive. BAT is a functional test resembling an ex vivo provocation, is an additional tool in the diagnosis of drug allergy that is safer, and gentler, than a provocation test and, in some instances, is the only available diagnostic tool (Hoffmann et al., 2015).

Given the few cases described up to the present it will be required further study about the specificity and sensitivity of BAT in

hypersensitivity to TT vaccine.

References

- Bellioni, B., Paganelli, R., Bruno, G., et al., 2001. Allergy to tetanus toxoid vaccine. *Allergy* 56, 701–702.
- Bortolatto, J., Mirotti, L., Rodriguez, D., Gomes, E., Russo, M., 2015. Adsorption of toll-like receptor 4 agonist to alum-based tetanus toxoid vaccine dampens pro-T helper 2 activities and enhances antibody responses. *J. Immunol. Res.* 2015, 280238. <http://dx.doi.org/10.1155/2015/280238>. (9 pp.).
- Dahlén, B., Nopp, A., Johansson, S.G., et al., 2011. Basophil allergen threshold sensitivity, CD-sens, is a measure of allergen sensitivity in asthma. *Clin. Exp. Allergy* 41, 1091–1097.
- Dannemann, A., Van Ree, R., Kulig, M., et al., 1996. Specific IgE and IgG4 immune responses to tetanus and diphtheria toxoid in atopic and nonatopic children during the first two years of life. *Int. Arch. Allergy Immunol.* 111, 262–267.
- Finkelman, F.D., 2007. Anaphylaxis: lessons from mouse models. *J. Allergy Clin. Immunol.* 120, 506–515.
- Hoffmann, H.J., Santos, A.F., Mayorga, C., et al., 2015. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy* 70, 1393–1405.
- Levine, L., Edsall, G., 1981. Tetanus toxoid: what determines reaction proneness? *J. Infect. Dis.* 144, 376.
- Leysen, J., Sabato, V., Verweij, M., et al., 2011. The basophil activation test in the diagnosis of immediate drug hypersensitivity. *Expert. Rev. Clin. Immunol.* 7, 349–355.
- Mayorga, C., Torres, M.J., Corzo, J.L., et al., 2003. Immediate allergy to tetanus toxoid vaccine: determination of immunoglobulin E and immunoglobulin G antibodies to allergenic proteins. *Ann Allergy Asthma Immunol* 90, 238–243.
- Ownby, D.R., Partridge, M.E., Wegienka, G.R., et al., 2009. Influence of dose and frequency of antigen injection on IgE development in young children: a comparison of fire ant stings and tetanus immunizations. *Ann Allergy Asthma Immunol* 103, 337–341.
- Santos, A.F., Lack, G., 2016. Basophil activation test: food challenge in a test tube or specialist research tool? *Clin. Trans. Allergy* 6, 10.
- Strait, R.T., Morris, S.C., Finkelman, F.D., 2006. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and FcγRIIb cross-linking. *J. Clin. Invest.* 116, 833–841.