



LETTER TO THE EDITOR

Allergenic vaccines administration and inhibitor development in haemophilia

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Currently, the development of antifactor VIII (FVIII) inhibitors is the most serious treatment-related complication for haemophilia A patients. Inhibitor development is still considered a poorly predictable multifactorial event, including both genetic and environmental risk factors [1], the later including certain vaccines [2]. In this sense, it has been postulated that simultaneous vaccine administration and FVIII infusion could be perceived by the immune system as 'danger signals' and subsequently, it would promote the development of inhibitors [3]; nevertheless, there are no available evidences in the literature showing a link between the formation of inhibitors and the desensitizing therapy commonly used in allergic patients.

Here, we report a teenager patient suffering severe haemophilia and a history of high-response inhibitor rituximab eradicated years ago. Late recurrence inhibitor is manifested simultaneously with the beginning of desensitizing therapy using allergenic vaccines against grass pollen.

The patient was diagnosed of haemophilia A caused by the inversion of intron 22 at 8-months old, after presenting a large haematoma secondary to left inguinal hernia surgery. He was treated with rFVIII during 10 days. At 30-months old, the patient developed a high-titer inhibitor (max. 466 BU) after second exposition to FVIII, consisting in 4 days of intensive treatment with rFVIII for right knee haemarthrosis. The immune tolerance induction (ITI) therapy, extended for 30 months according to the Bonn protocol, did not result in removal of the inhibitor; from that moment, prophylaxis and treatment of bleeding episodes were performed with recombinant factor VIIa concentrate (rFVIIa). The patient had severe clinical course with recurrent spontaneous bleeding episodes, both muscular and large joints, although it managed to preserve joint function due to the good haemostatic response obtained with rFVIIa. At the age of six, rituximab was administered to ITI rescue, according to the usual pattern of a weekly dose over a month (375 mg kg^{-1} body weight), together with cyclophosphamide (3 mg kg^{-1} body weight), high dose immunoglobulin (IVIG) and steroids. One month later the inhibitor title was undetectable. From that moment, the patient received high dose of rFVIII (100 IU kg^{-1} body weight per day) in combination with cyclophosphamide and IVIG, weekly. Three months later, pharmacokinetics of FVIII has been normalized and standard prophylaxis with rFVIII for 3 days non-consecutive per week was provided. Infectious prophylaxis with sulfamethoxazole and IVIG held back over 6 months after immunomodulatory therapy. Neither during this period nor within subsequent years there were infections or other treatment-related complication, unless the patient developed allergic rhinitis to grass pollen. Six years later, the patient began a desensitization programme against pollen consisting in subcutaneous administration of different dilutions of the allergen to coincide with the infusion of FVIII of his

routine prophylaxis. Five weeks after initiation of immunotherapy, high-titer inhibitor (peak maximum 10 UB) was detected in routine monitoring. Before starting immunotherapy with allergenic vaccines, quarterly screening of inhibitors had been negative. Prophylaxis was maintained during the following weeks, but the frequency and severity of spontaneous muscle and joint bleeding episodes that occurred, initially treated with high doses of rFVIII and later with rFVIIa, recommended to try a new ITI course. Three months later, Rituximab was administered weekly with high doses of rFVIII (100 IU kg^{-1} body weight per day) concomitantly during 4 weeks. At the end of the first month, the inhibitor titer was achieved undetectable. The patient continued with high-dose rFVIII for 3 months and, following confirmation of complete response, standard prophylaxis with rFVIII was restarted. Nine months later the patient maintains complete response and has not presented significant bleeding episodes. Infectious prophylaxis with sulfamethoxazole held back over 6 months after immunomodulatory therapy. Neither infectious complications nor other treatment-related adverse events have been observed.

To our knowledge, we describe the first known association between inhibitor development in haemophilia and allergen immunotherapy. The reappearance of the inhibitor coinciding with the simultaneous administration of rFVIII and allergen vaccines, suggests the existence of a causal relationship between immunotherapy and development of the inhibitor. To date, there are no evidences to support that administration of vaccines in conjunction with the infusion of FVIII concentrates might favour the formation of inhibitors in haemophilia, although the presence of any inflammatory process in coexistence with the administration of FVIII has been considered as a factor risk for inhibitor formation. In animal models, it has been shown that infections and inflammatory processes are associated with a higher frequency of inhibitor development [4], thus it could be speculated that inflammatory reactions trigger the production of lymphokines that enhances the immune response. Some experiences in haemophilic patients who agreed vaccinations, infections, bleeding or surgery to the time of the first exposure to FVIII, indicated that this association may influence the risk of inhibitor development [2]. To this respect, the 'theory of danger' developed by Matzinger [5] suggests that the immune system is more committed to the 'danger signals' from damaged tissue than to the recognition of foreign or exogenous proteins. This model may explain the development of inhibitors in relation to 'inflammatory' processes, as surgery, tissue damage, infection or vaccination, regardless of other risk factors. Based on this theory, Kurnik *et al.* [6] published the results of a case-control study in patients with haemophilia, where authors tried to minimized exposure to immune 'danger signals' by early treatment of bleeding, which was done by preventing the first treatment FVIII occur in a situation of bleeding, or during an infectious process, by deferring the surgery after the first 20 days of exposure to factor, and preventing vaccinations coincided with the administration of FVIII. Only 1 of the 26 patients developed inhibitor, suggesting that the minimization of the 'danger signals' during the first 20 days of exposure to FVIII may reduce the risk of inhibitor formation. The mechanism is not completely understood, but recent experimental data suggest that proinflammatory or immunological 'danger signals' may support inhibitor development. Although exposure to

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FVIII by itself may not be enough to trigger an immune response [7], an intensive exposure to FVIII in the presence of such 'danger signals' can activate antigen-presenting cells (APCs), up regulating costimulatory signals for T lymphocytes and ultimately enhancing antibody production [8]. Thus, vaccination in conjunction with exposure to FVIII is one of the non-genetic risk factors that, in theory, is capable of generating immune 'danger signals'. Only one case-control study has investigated the vaccination as a factor involved in the development of inhibitors [9], evaluating the coexistence of infection or vaccination match during the administration of FVIII in 60 patients and 48 controls. There was infection or vaccines that were administered during the active treatment of FVIII in 12 patients and 11 controls. Although no significant association was found between vaccination or infection and the development of inhibitors, hence no conclusions can be drawn because it is a single study in which selection bias cannot be ruled out.

Due to humoral response that occurs against FVIII is T lymphocyte dependent, other possible explanation of inhibitor development after administration of allergen vaccines is the modification in T cell response induced by this therapy. The cellular immune response against an extracellular peptide antigen, such as FVIII occurs through several interactions between APC, the CD4+ T lymphocytes and B lymphocytes. CD4 T lymphocytes can be divided into two subgroups, which differ in function and cytokine secretion. The first group, Th1 lymphocytes, secrete cytokines proinflammatory and promote the synthesis of IgG subclasses capable of fixing complement. The other group of T lymphocytes is called Th2, which secrete anti-inflammatory cytokines and promote the synthesis of immunoglobulin which are not capable of fix complement. Th2 cells are able to perform effector functions by secreting IL-4, which is a growth factor for B lymphocytes and is necessary for the production of IgE and other

complement-fixing antibodies; on the other hand, Th2 cells suppress the immune response by secreting cytokines anti-inflammatory producing an inhibition of both the APCs as Th1 effector lymphocytes [9]. Vaccines are used in medicine as immune response modifiers, including in this definition allergenic vaccines [10]. Allergy shots or allergen immunotherapy, comprising administering to a subject of gradually increasing amounts of an allergen, usually subcutaneously, with the aim of improving the symptoms caused by further exposure to the causative allergen. Although the mechanisms by which immunotherapy is able to modify the immune response is probably very heterogeneous, recent studies suggest that the main effect induced by immunotherapy in the response of the T cells to the allergen is the change in Th2 responses to Th1 responses. It should be speculated that parenteral administration of a peptide antigen, as exogenous FVIII, coinciding with increased Th1 immune response favoured by immunotherapy with allergenic vaccines, could lead to an immunological 'danger signal', culminating in the alloantibody development.

Both explanations support the hypothesis that allergenic vaccines administration coinciding with the infusion of FVIII should be avoided, as it could be perceived by the immune system as 'danger signals', and its coincidence with increased Th1 immune response favoured by allergic immunotherapy could facilitate to the development of inhibitors.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

References

- Gouw SC, van der Berg HM. The multifactorial etiology of inhibitor development in hemophilia: genetics and environment. *Semin Thromb Hemost* 2009; 35: 723–34.
- Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood* 2007; 109: 4648–54.
- Mancuso ME, Graca L, Auerswald G, Santagostino E. Haemophilia care in children – benefits of early prophylaxis for inhibitor prevention. *Haemophilia* 2009; 15(Suppl. 1): 8–14.
- Kaufman RJ. Advances toward gene therapy for hemophilia at the millennium. *Hum Gen Ther* 1999; 19: 2091–107.
- Matzinger P. The danger model: a renewed sense of self. *Science* 2002; 296: 301–5.
- Kurnik K. New early prophylaxis regimen that avoids immunological danger signals can reduce FVIII inhibitor development. *Haemophilia* 2010; 16: 256–62.
- Pfistershammer K, Stöckl J, Sieckmann J *et al.* Recombinant FVIII and FVIII/VWF complex do not present danger signals for human dendritic cells. *Thromb Haemost* 2006; 96: 309–16.
- Reipert BM, van Helden PM, Schwarz HP *et al.* Mechanisms of action of immune tolerance induction against factor VIII in patients with congenital haemophilia A and factor VIII inhibitors. *Br J Haematol* 2007; 136: 12–25.
- Reding MT, Wu H, Krampf M *et al.* CD4+ T cell response to factor VIII in haemophilia A, acquired hemophilia and healthy subjects. *Thromb Haemost* 1999; 82: 509–15.
- Bousquet J, Lockey RF, Malling H-J. WHO Position Paper: allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1998; 53: 1–49.