

Editorial

Specific immunotherapy – the induction of new IgE-specificities?

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Allergic diseases affect more than 25% of the population in the Western world and hence represent a major health threat (1). For the past nine decades specific immunotherapy has been used to treat allergic diseases caused by inhalant allergens (e.g. pollen, mite, animal dander allergens) and *Hymenoptera* venoms (2). Allergen-specific immunotherapy, the administration of increasing doses of allergen extracts, is the only specific and curative approach towards the treatment (3) of IgE-mediated allergy (4). The aim of specific immunotherapy is to induce immunological tolerance against the applied allergen and thus is indicated for patients who have IgE antibodies against clinically relevant allergens (3). It does not only affect the natural course of allergic disease, but also seems to prevent the development of asthma in patients with allergic rhinitis. Since allergen-specific immunotherapy can be associated with anaphylactic side effects, the indication for treatment has to be based on careful diagnosis and requires experience (3). The clinical efficacy of specific immunotherapy has been documented in a considerable number of placebo-controlled trials (3). It is also established that immunotherapy induces prolonged clinical remission above all when the treatment is continued for several years (5), which has raised the question of whether it should be considered earlier in the management of allergic disease. The mechanisms of immunotherapy are complex and still under investigation (3). Induction of allergen-specific IgG antibodies,

so-called “blocking antibodies” (6, 7), reduction in numbers of effector and inflammatory cells and release of mediators (8), induction of suppressor cells (9), decrease in the production of Th2-like cytokines and an up-regulation of Th1-like cytokines by T cells (10–12) are all considered to be immunological events associated with successful immunotherapy. Furthermore, IL-10 induced anergy in peripheral T cells and reactivation by microenvironmental cytokines seem also to be of importance (13).

An issue that recently gained attention is the question of whether the administration of allergen extracts in the course of immunotherapy can induce IgE reactivities and clinically relevant sensitizations to new allergens. In the present issue of *Allergy*, Movérare et al. address this question and report the development of new IgE reactivities to allergenic components in pollen extracts during specific immunotherapy (14). The authors monitored 24 children and adults who were treated with birch pollen rush immunotherapy for up to three years. The sera, which were collected at regular intervals, were analyzed by immunoblotting and the Pharmacia CAP System[®] (Uppsala, Sweden) using recombinant birch-pollen allergens (rBet v 1, rBet v 2 and rBet v 4). At the beginning of the study all patients had IgE to the major birch pollen allergen Bet v 1, but only three had detectable IgE to rBet v 2 and/or rBet v 4. However, IgE reactivities to new allergen components were observed by immunoblotting in 65%

of the patients after one to three years in addition to the original IgE-reactivities. Detailed analysis with the Pharmacia CAP System[®] disclosed that new reactivities to rBet v 2 and rBet v 4 had been induced at low levels in 29% of the treated patients. Interestingly, in one patient *de novo* reactivity to rBet v 4 had already started after two months of treatment. Among two control patients, new IgE reactivities became detectable during the birch pollen season of the first year, but these IgE levels were back to below the cut-off value outside the pollen season. These data are in keeping with the recent study of Ball et al. showing that patients receiving one year of grass pollen immunotherapy developed *de novo* immune responses not only to new epitopes but also to new allergens in the administered extract (15). The results of Movérare and Ball raise the question of whether sensitization to new allergens will have any clinical impact on the patient. In this context, it should be noted that many forms of plant food allergy are caused by primary sensitization to cross-reactive pollen allergens (16). With respect to Bet v 2 (birch profilin) (17) and Bet v 4 (18), these molecules are highly cross-reactive with fruits and vegetables (19, 20) and with tree, grass and weed pollens (21), respectively. Patients may, if sensitized to Bet v 2 and Bet v 4, due to cross-reactivity experience allergic symptoms on contact with a great variety of unrelated allergen sources (18, 21). In fact, the possible induction of food allergy during specific immunotherapy has been illustrated by van Ree et al. (22). This study showed that patients receiving house dust mite immunotherapy reported strongly aggravated clinical symptoms when ingesting shrimp, probably due to the induction of new cross-reacting IgE antibodies to tropomyosin. Although the serological study conducted by Movérare et al. had no indications that the development of new IgE reactivities to cross-reactive allergens resulted in any clinical symptoms (14) this issue warrants further clinical investigations.

The WHO position paper on allergen immunotherapy (3) highlights that the quality of allergens is critical for both diagnosis and treatment. To overcome the problems inherent in the use of crude allergen extracts, recombinant DNA technology has been used by several research groups during the last decade to produce defined recombinant allergen molecules for diagnostic and therapeutic purposes. Recombinant allergens from the most common allergen sources are now available and can be produced identically without biological or batch-to-batch variation of the protein (23). A great advantage of recombinant allergen components is that they can be used for component-resolved diagnostics, which allow establishment of the patient's individual IgE reactivity profile before selection of therapy (24). This has a major impact on therapy, since optimal forms of treatment can be selected on the basis of the reactivity profile. Furthermore, recombinant allergens

allow that patients can be treated according to their sensitivity profiles, which excludes the risk of therapy-induced sensitization against new allergens. Since recombinant allergens equal their natural counterparts, with respect to their biological and immunological properties, they can induce similar side-effects as natural allergens if used for immunotherapy. Therefore new strategies have been developed to reduce the allergenic potential of recombinant allergens. Peptide chemistry and recombinant DNA technology have allowed production of T cell epitope- and B cell epitope-containing peptides, recombinant allergen isoforms, mutants, fragments as well as recombinant allergen oligomers with reduced allergenic activity (25–28).

So far only the genetically modified hypoallergenic allergen derivatives of the major birch pollen allergen Bet v 1 (rBet v 1 fragments and trimer) (29, 30) have been well characterized *in vivo*. They have a >100-fold reduced potential to induce immediate skin type reactions (31, 32) and a significantly lower capacity to induce release of histamine, ECP, GM-CSF (33) in birch pollen allergic patients than rBet v 1 wild-type. In addition, the hypoallergenic rBet v 1 derivatives have a significantly lower allergenic and inflammatory activity at the nasal mucosa of birch pollen allergic patients compared to rBet v 1 (34). Since immunization with rBet v 1 fragments and trimer gives rise to IgG antibodies against wild-type Bet v 1- and Bet v 1-homologous allergens present in pollen of related trees and plant food (29, 30), one might anticipate that they have a positive effect on birch pollen-associated pollen and food allergies.

The mechanisms behind the beneficial effect of modified allergens are expected to be at the antigen presentation level, where they by employing phagocytic or pinocytic antigen uptake, bypass IgE-mediated effects, induce a balanced Th0- or Th1-like cytokine response, accompanied by the induction of a protective IgG response (35). A rationale for using hypoallergenic recombinant allergens is that higher doses can be administered with fewer injections and thus make immunotherapy comparable with vaccination. The favorable properties of hypoallergenic allergen derivatives also open new avenues for alternative routes of immunotherapy (e.g. sublingual or nasal). Furthermore many possibilities exist for conducting immunotherapy with single or with a mixture of recombinant allergen(s) and to co-administer immunomodulatory adjuvants. Thus, recombinant allergens represent promising tools for therapy of IgE-mediated allergy that meet the standards of regular vaccines (3). Clinical studies will now evaluate not only their safety and efficacy but also allow the study of mechanisms of immunotherapy at a molecular level.

References

1. KAY AB. Allergy and Allergic Diseases. Blackwell Science, Oxford, UK 1997.
2. NOON L. Prophylactic inoculation against hayfever. *Lancet* 1911;1:1572–1573.
3. BOUSQUET J, LOCKEY RF, MALLING HJ, WHO PANEL MEMBERS. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *Allergy* 1998;Suppl. 44:1–42.
4. JOHANSSON SGO, HOURIHANE JO, BOUSQUET J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;56:813–824.
5. DURHAM SR, WALKER SM, VARGA EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;341:468–475.
6. LEYNADIER F, ABUAF N, HALPERN GM, MURRIETA M, GARCIA-DUARTE C, DRY J. Blocking antibodies after rush immunotherapy with mites. *Ann Allergy* 1986;57:325–329.
7. VAN NEERVEN RJJ, WIKBORG T, LUND G, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4⁺ T cells by inhibiting serum IgE-facilitated allergen presentation. *J Immunol* 1999;163:2944–2952.
8. FURIN MJ, NORMA PS, CRETICOS PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J Allergy Clin Immunol* 1991;88:27–32.
9. ROCKLIN RE, SCHEFFER AL, GREINER DK, MELMON KI. Generation of antigen-specific suppressor cells during allergy desensitization. *N Eng J Med* 1980;302:1213–1219.
10. JUTEL M, PICHLER WJ, SKRBIC D, URWYLER A, DAHINDEN C, MÜLLER UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;154:4187–4194.
11. EBNER C, SIEMANN U, BOHLE B, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from Th2 to Th1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997;27:1007–1015.
12. DURHAM SR, TILL SJ. Immunological changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157–164.
13. AKDIS CA, BLASER K. IL-10 induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB* 1999;13:603–609.
14. MOVÉRARE R, ELFMAN L, VESTERINEN E, METSO T, HAAHTELA T. Development of new IgE reactivities to allergenic components in pollen extracts during specific immunotherapy studied with immunoblotting and the Pharmacia CAP System. *Allergy* 2002;57:423–430.
15. BALL T, SPERR WR, VALENT P, et al. Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999;29:2026–2036.
16. KAZEMI-SHIRAZI L, PAULI G, PUROHIT A, et al. Quantative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. *J Allergy Clin Immunol* 2000;105:116–125.
17. VALENTA R, DUCHÈNE M, PETTENBURGER K, et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;253:557–560.
18. TWARDOSZ A, HAYEK B, SEIBERLER S, et al. Molecular characterization, expression in *Escherichia coli* and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem Biophys Res Commun* 1997;239:197–204.
19. VAN REE R, FERNANDEZ-RIVAS M, CUEVAS M, VAN WIJNGAARDEN M, AALBERSE RC. Pollen-related allergy to peach and apple: an important role for profilin. *J Allergy Clin Immunol* 1995;95:726–734.
20. EBNER C, HIRSCHWEHR R, BAUER L, et al. Identification of allergens in fruits and vegetables. IgE cross-reactivities with the important birch pollen allergen Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962–969.
21. VALENTA R, DUCHÈNE M, EBNER C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377–385.
22. VAN REE R, ANTONICELLI L, AKKERDAAS JH, GARRITANI MS, AALBERSE RC, BONIFAZI F. Possible induction of food allergy during mite immunotherapy. *Allergy* 1996;51:108–113.
23. VALENTA R, VRTALA S, LAFFER S, SPITZAUER S, KRAFT D. Recombinant allergens. *Allergy* 1998;53:552–561.
24. VALENTA R, LIDHOLM J, NIEDERBERGER V, HAYEK B, KRAFT D, GRÖNLUND. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
25. OLDFIELD WL, KAY AB, LARCHÉ M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 2001;167:1734–1739.
26. FOCKE M, MAHLER V, BALL T, et al. Nonanaphylactic synthetic peptides derived from B cell epitopes of the major grass pollen allergen, Phl p 1, for allergy vaccination. *FASEB* 2001;15:2042–2044.
27. OLSSON S, VAN HAGE-HAMSTEN M, WHITLEY P. Contribution of disulphide bonds to antigenicity of Lep d 2, the major allergen from the dust mite *Lepidoglyphus destructor*. *Mol Immunol* 1998;35:1017–1023.
28. VALENTA R, VRTALA S, FOCKE-TEJKE M, et al. Inflammatory mechanisms in allergic diseases. In: ZWEIMAN, B, SCHWARTZ, LB, , editors. *Synthetic and Genetically Engineered Allergen Derivatives for Specific Immunotherapy of Type 1 Allergy*. New York, Basel: Marcel Dekker Inc, 2000, 495–517.
29. VRTALA S, AKDIS CA, BUDAK F, et al. T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. *J Immunol* 2000;165:6653–6659.
30. VRTALA S, HIRTENLEHNER K, SUSANI M, et al. Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. *FASEB* 2001;15:2045–2047.
31. VAN HAGE-HAMSTEN M, KRONQVIST M, ZETTERSTRÖM O, et al. Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1: Results obtained with a mix of two recombinant Bet v 1 fragments and recombinant Bet v 1 trimer in a Swedish population before the birch pollen season. *J Allergy Clin Immunol* 1999;104:969–977.
32. PAULI G, PUROHIT A, ØSTER JP, et al. Comparison of genetically engineered hypoallergenic rBet v 1 derivatives with rBet v 1 wild-type by skin prick and intradermal testing: results obtained in a French population. *Clin Exp Allergy* 2000;30:1076–1084.
33. NOPP A, HALLDÉN G, LUNDAHL J, et al. Comparison of inflammatory responses to genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, and to recombinant Bet v 1 wild type in skin chamber fluids collected from birch pollen allergic patients. *J Allergy Clin Immunol* 2000;106:101–109.

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34. VAN HAGE-HAMSTEN M, JOHANSSON E, ROQUET A, et al., Nasal challenges with recombinant derivatives of the major birch pollen allergen Bet v 1 induce fewer symptoms and lower mediator release than rBet v 1 wild-type in patients with allergic rhinitis. Submitted.
35. AKDIS CA, BLASER K. Regulation of specific immune responses by chemical and structural modifications of allergens. *International Arch Allergy Immunol* 2000;**121**:261–269.