

EDITORIAL

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Could T cell component-resolved diagnosis differentiate between food sensitization and food allergy?

This editorial discusses the findings of the paper by Flinterman et al. [14] pp. 590–597.

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The underlying mechanisms of IgE-mediated food allergies are type I hypersensitivity reactions. Indeed, at least in animal models of allergy, IgE transfer is both necessary and sufficient to trigger allergic responses such as passive cutaneous anaphylaxis [1]. Conversely, IgE blockade (e.g. with a high affinity soluble IgE receptor), abrogated allergic symptoms triggered by oral allergen exposure of mice in which a hybridoma that secreted allergen-specific IgE had been implanted [2]. The link between food allergy and the presence of food allergen-specific IgE is thus well established. Paradoxically however, large numbers of individuals who have food allergen-specific IgE are still able to consume the respective foods without any symptom of allergy.

Such individuals are considered to be sensitized but not allergic to the respective food. They pose significant problems in terms of diagnosis because it is not easy to distinguish between sensitized but tolerant individuals and allergic patients because they all have significant levels of allergen-specific IgE.

The correlation between the level of food-specific IgE and the risk of allergic reactions to the respective food (as defined by double-blind, placebo-controlled food challenges, DBPCFC, which represent the golden standard in this respect) is well characterized [3–5]. It was found that allergen-specific IgE had good negative predictive accuracy but relatively poor positive predictive accuracy. Indeed, in the United States, relatively high specific IgE levels (of 15 kU(A)/L for peanut, 32 kU(A)/L for cow's milk or 6 kU(A)/L for egg) were required to reach 95% predictive value for the respective food allergy [3]. These levels were similar for a large UK paediatric population: peanut-specific levels of 15 kU(A)/L and above had a 92%

predictive value for peanut allergy, with a 74–99% confidence interval [6]. Conversely, a study in France concluded that higher peanut-specific IgE levels, of 57 kU(A)/L and above were associated with a positive predictive value of 100% [7], so that DBPCFC were required for unequivocal diagnosis of peanut allergy in children with lower specific IgE values.

Unfortunately DBPCFC are time-consuming, resource-intensive and may sometimes lead to severe allergic reactions. Moreover, especially in very young children, subjective allergic reactions that may occur during positive DBPCFC are not easy to discern. So, unsurprisingly, significant efforts were devoted towards investigating allergen-specific IgE in depth (and also developing other diagnostic tools, such as epicutaneous/skin prick tests and basophil activation/degranulation assays).

Two recent approaches for improving the diagnostic value of allergen-specific IgE are (i) epitope mapping using overlapping peptide microarrays [8, 9] and (ii) component-resolved diagnosis (CRD) [10–13].

In the case of peanut allergy, 419 overlapping 15-aminoacid peptides corresponding to the sequences of the major allergens Ara h1, Ara h2 and Ara h3 were placed on microarrays. Sera from peanut allergic and sensitized individuals were then incubated with the microarrays in order to determine the epitope specificity of the peanut-specific IgE and IgG4 [8]. It was found that IgE and IgG4 antibodies from the allergic ones recognized a larger number of epitopes than antibodies from sensitized but non-allergic individuals. However, no specific epitope could be correlated with peanut allergy or its severity. Finally, antibody epitope specificities from all individuals remained relatively stable over at least the 20 months studied. Similar approaches led to comparable findings in the case of cow's milk allergy [9].

CRD of allergies is based upon detecting the binding of IgE to intact molecules of purified or recombinant allergen rather than overlapping peptides. CRD allows for the detection of the conformational epitopes from allergens (which

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may be missed when using linear 25-mer peptides on microarrays). Furthermore, CRD is less cumbersome than peptide microarrays because only a few main allergen molecules need to be tested for CRD [10–11]. Nevertheless, it is unclear whether purified or rather recombinant allergens should be used for CRD. After determining the individual patient's sensitization patterns by CRD, it would be possible to undertake component-based immunotherapy, so that each patient would receive the personalized allergenic preparation to which he/she is sensitized rather than a general-purpose immunotherapy mixture of allergens [12].

However, despite that microarrays and CRD provide much more information because they allow for the identification of many allergens that are recognized by patients' IgE, the use of this information is limited by the lack of knowledge regarding the significance of these characteristics [13].

Flinterman and colleagues further develop their previous findings [8, 11] in the present issue of *Clinical & Experimental Allergy* and use the CRD approach to characterize the peanut-specific T cell responses of peanut-allergic, peanut-sensitized and tolerant, non-sensitized children [14]. For this purpose, they used well-established *in vitro* techniques to study primary peripheral blood mononuclear cells (PBMC) responses and also to develop short-term T cell lines specific to peanut antigens. In these systems they measure the proliferation and cytokine secretion induced by crude peanut extract and by purified peanut antigens (Ara h1, Ara h2, Ara h3, Ara h6). It was found that T cells from peanut-allergic individuals proliferate higher than those from sensitized and tolerant ones. Interestingly, this difference is apparent in the T cell lines rather than in the primary PBMC response. Higher levels of cytokine production by PBMC and T cell lines from peanut-allergic patients were also observed. Flinterman and colleagues also compared the responses of children who are peanut allergic with the response of peanut-sensitized ones (who have equally high levels of peanut-specific IgE but tolerate peanuts). In this respect, the major difference observed is that T cells from sensitized individuals have similar proliferation patterns as non-allergic controls, both being significantly lower than peanut-specific proliferation of T cells from peanut-allergic individuals. Notably, there is considerable overlap between peanut-allergic and peanut-sensitized individuals with regard to their component-specific T cell responses (peanut-specific proliferation and cytokine production), as described previously for T cell responses to whole peanut extracts [15, 16]. Therefore, the use of the CRD approach by Flinterman and colleagues confirms that peanut-allergic, sensitized and tolerant individuals have mixed T cell responses involving multiple subsets (Th1, Th2, possibly also regulatory T cells, given the levels of IL-10 production, etc.).

Since, in the frame of allergic responses, T cells are placed at the crossroads between dendritic cell presentation of allergens and B cell production of pathogenic IgE antio-

dies, these findings open new avenues of exploration of the immunological mechanisms that underlie allergy.

Thus, the presence (or the dominance) of certain peanut-specific T cell subsets in the mixed T cell responses from peanut-sensitized individuals could induce B cells to secrete blocking IgG antibodies, possibly similar to those induced by immunotherapy [17]. Such blocking antibodies could then act by modulating the strength of the signal leading to mast cell or basophil activation and thus prevent their degranulation [18].

Conversely, various T cell subsets must themselves be differentially selected and activated by dendritic cells that have captured allergens in particular mucosal microenvironments that are influenced by epithelia and other local factors [19]. In this respect, component-resolved characterization of distinct food allergen-specific T cell subsets could also be used as a research tool for studying the role of dendritic cell presentation and immunomodulation. Therefore, even though T cell subset CRD is unlikely to displace DBPCFC as the gold standard for diagnosis, this approach and the findings reported by Flinterman and colleagues should open the way for further mechanistic investigations of the complex immune interactions whose outcome is allergy or tolerance to foods.

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