

EDITORIAL

Clinical & Experimental Allergy

Modulation of Peanut-specific humoral and cellular responses pre- and post-oral immunotherapy

This editorial discusses the findings of the paper in this issue by Wisniewski et al. [3], pp. 1201–1213.

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Peanut allergy is common, particularly in Western countries. Clinical manifestations range from oral pruritus to fatal anaphylactic reaction and there is no cure. Clinical trials with either antigen-specific sublingual immunotherapy (SLIT) or oral immunotherapy (OIT) have shown promise; trials with epicutaneous immunotherapy (EPIT) are also moving forward [1]. However, the risk associated with immunotherapy (IT) has precluded its use in common clinical practice [2]. In food allergy, it is important to distinguish between the outcomes of desensitization vs. induction of sustained unresponsiveness after immunotherapy. Desensitization is defined as non-responsiveness to allergen challenge while on immunotherapy, or immediately after therapy. Induction of sustained unresponsiveness is usually defined as non-responsiveness to food challenge 4–6 weeks after the end of therapy. Current clinical trials in food allergy show that IT can lead to desensitization and sustained unresponsiveness. However, the finding of sustained unresponsiveness post-IT cannot be equated to induction of long-term immune tolerance without further studies. Additionally, it remains unclear whether different IT approaches in peanut allergy are equally effective in leading to transient desensitization, sustained unresponsiveness or the induction of long-lasting tolerance. Improved understanding of the mechanisms that lead to desensitization and tolerance would facilitate the refinement of clinical protocols to achieve optimal clinical efficacy,

while minimizing adverse side effects. To provide answers, studies have been undertaken to characterize peanut allergen-specific humoral and cellular responses in peanut-allergic (PA) subjects pre- and post-immunotherapy.

In this issue of Clinical and Experimental Allergy, Wisniewski et al. examined the modulation of humoral and cellular responses towards Ara h 1 and Ara h 2, two of the most studied peanut allergens, in PA subjects pre- and post-OIT [3]. In their patient cohort, the majority of subjects were sensitized to both Ara h 1 and Ara h 2 with Ara h 2 IgE levels exceeding those of Ara h 1. Mono-sensitized subjects were also observed, but these subjects generally had lower whole peanut IgE levels than the dual-sensitized subjects. The observed rarity of Ara h 1 mono-sensitized subjects could be taken to suggest that unique Ara h 1-specific IgE epitopes are uncommon. Correspondingly, the presence of Ara h 2 dual-sensitized subjects and mono-sensitized subjects might suggest Ara h 2 IgE epitopes could be subdivided into both unique and Ara h 1 cross-reactive epitopes as described earlier [4].

Peanut-specific T cell responses in PA were next functionally characterized by Wisniewski et al. For these experiments, PBMC were stimulated with either Ara h 1 or Ara h 2, cultured for 7 days and examined by flow cytometry. CD3+ T cells that up-regulated CD25 were considered to be peanut-specific T cells. Using this criteria, they observed activation of Ara h 1- and Ara h 2-specific cells in PA subjects, but not in non-allergic subjects. Notably, subjects that were mono-sensitized to Ara h 2 also had Ara h 1-specific T cell responses. By examining IL-4 and IFN- γ production within the CD25+ T cells, they observed a Th2 skewing amongst the Ara h 1- and Ara h 2-activated T cells.

Wisniewski et al. also examined humoral responses towards Ara h 1 and Ara h 2 in a group of 17 subjects following 12–24 months of OIT. These subjects

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were treated with a relatively low dose of peanut, that is less than 600 mg/day in this study, compared to 4000 mg/day of peanut or higher in some of the current trials [5]. All of these subjects were clinically desensitized (according to open food challenge), but 13 of 17 maintained a value of peanut-specific IgE of greater than 15 kU_A/L. PA subjects had significantly elevated peanut-specific IgG4 (in particular Ara h 2-specific IgG4) after OIT. Increases in peanut-specific IgG4 after OIT had been reported earlier [5–7]. However, increased IgG4 may simply be a consequence of repeated allergen exposure and is not necessarily linked to clinical efficacy. For example, a study by Vickery et al. showed that increases in peanut-specific IgG4 alone could not account for the induction of sustained unresponsiveness. However, the ratio of peanut-specific IgG4/IgE has been shown to effectively segregate the sustained unresponsiveness group from the desensitized group [5].

Peanut-specific T cell responses were similarly characterized in seven desensitized subjects while they were on maintenance therapy. Importantly, while peanut-specific Th2 cells were still detectable in these subjects, the ratio of IL-4- and IFN- γ -producing cells changed, such that the overall response was diverted from a Th2 profile towards a Th1 profile with more Th1 cells and less Th2 cells after OIT. Yet the proportion of peanut-specific cells that co-produced IL-4 and IFN- γ remained the same before and after OIT. These data appear to imply that (1) Th2 cells are not being converted to Th1 cells during OIT, which suggests a preferential reduction of Th2 cells and/or preferential expansion of Th1 cells) and (2) the residual PA-specific T cells, which produced Th2 cytokines as detected after OIT, are poised to be reactivated and undermine the durability of the OIT after treatment.

Induction of allergen-specific regulatory T cells is believed to play a key role in the induction of desensitization and tolerance through allergen-specific immunotherapy [8]. Yet, conclusive data from human studies that validate this mechanism are still lacking. The study by Wisniewski et al. addressed the question of whether antigen-specific IL-10-producing regulatory T cells (Type 1 regulatory T cells or Tr1) are induced as a consequence of OIT. Using PBMC samples stimulated *in vitro* with peanut allergens, they observed that the supernatants collected from the post-OIT samples had higher levels of IL-10 compared to pre-OIT sample from the same subject. Similar frequencies of IL-10-producing cells were observed in subjects before and after OIT. The authors speculated that most of IL-10 came from non-T cells in these cultures. Such non-T cells could include IL-10-secreting antigen-presenting cells and B cells [9, 10]. Alternatively, it is possible that activation of IL-10-secreting cells by peanut antigens during OIT elicits higher amounts of IL-10 on a per cell basis.

Induction of antigen-specific Foxp3⁺ regulatory T cells (Tregs) has likewise been explored as a major mechanism for successful immunotherapy [11]. Others have shown that both successful SLIT and OIT led to hypomethylation of promoter region of Foxp3 as well as expression of Foxp3 [12, 13]. Wisniewski et al. documented the presence of Foxp3⁺ allergen-specific T cells in post-OIT samples. They also discovered that when, peanut-specific cells expressed higher levels of Foxp3, they produced higher amounts of Th2 cytokines; similarly, IL-4⁺ T cells expressed higher levels of Foxp3 compared to IL-13⁺, γ -IFN⁺ and IL-17⁺ T cells. Therefore, it would appear that the Foxp3⁺ T cells observed in this study were activated Th2 cells and not regulatory T cells. Interestingly, the cytokine profiles of Foxp3⁺ peanut-specific T cells were similar before and after OIT. It is known that activated human CD4⁺ T cells transiently express Foxp3 and correspondingly acquire some suppressive properties [14]. The current data further support that expression of Foxp3 can be a marker of activated Th2 cells. Transient expression of Foxp3 in activated CD4⁺ T cells may be an intrinsic cellular mechanism that limits cell division. Cumulatively, these observations uncovered factors that must be considered when using Foxp3 as a marker to distinguish bona fide Treg from activated T cells in human studies.

Overall, Wisniewski et al. observed an increase in allergen-specific Th1 cells and a concomitant decrease in Th2 cells after OIT. Yet peanut-specific T cells that produced Th2 cytokines were still detected in desensitized subjects. The data did not reveal any clear induction of IL-10 Tr1 or Foxp3⁺ Tregs after OIT. It should be noted that there is no evidence that the subjects selected for this study had attained sustained unresponsiveness or long-term tolerance; this may account for the absence of Treg induction. In addition, LAP⁺CD4⁺Foxp3⁺ regulatory T cells that are capable of producing TGF- β [15] were not investigated in this study. Data interpretation was also confounded to some extent by the use of a 7-day *in vitro* expansion for detection of antigen-specific T cells, as this assay format would not be expected to favour the expansion of regulatory T cells. It is possible that a more direct approach, such as detecting allergen-specific T cells *ex vivo* using HLA class II tetramer staining or CD154 up-regulation assay, could provide a more conclusive evaluation of antigen-specific Treg induction during OIT [16, 17].

Despite the interesting observations of this new study, some aspects of the mechanisms that lead to the induction of desensitization and tolerance after antigen-specific IT remain unclear, although deletion of Th2 cells upon repeated antigen stimulation did appear to be a crucial part of the process. A similar mechanism for induction of tolerance in subcutaneous

IP for aeroallergens has been observed [18]. In addition to the deletion process, induction of regulatory T cells should be essential for maintenance of long-term tolerance. At present, it is still uncertain which subtypes of Tregs, such as Foxp3⁺ Treg, Foxp3⁺ IL-10-producing Tr1 or LAP1-1-associated Treg, are the key players in successful IT. Firm conclusions have been hampered by the difficulty in detecting these cells, which is probably caused by their short lifespan and inherently low frequencies [7]. Lastly, there remains the question whether OIT, SLIT and EPIT operate under the same mechanistic pathway that induces desensitization versus tolerance. OIT, SLIT and EPIT use different dosages of allergens (ranging from 4000 mg/day in OIT to 2 mg/day in SLIT, and less than 0.5 mg/day in EPIT), and the route of administration would dictate that allergens are encountered by immune cells in different anatomical location with different subsets of dendritic cells. Activation of T cells by different subsets of dendritic cells with different dosages of antigen could plausibly lead to very different pathways of immune modulation. As such, it is important to distinguish the cellular immune status of subjects that are utilized in mechanistic studies, differentiating between desensitization and tolerance induction. For example, a recent study showed that weekly

consumption of peanut by infants at high risk of developing peanut allergy for 3 years significantly reduced the incidence of peanut allergy by age 5 compared to the placebo arm. It is still under investigation whether subjects in the active arm of this study will develop peanut allergy once they stop consuming peanut [19]. Current technological advances, such as RNAseq, Fluidigm array, multicolour flow cytometry and mass cytometry, on allergen-specific T cells should help to more clearly delineate desensitization versus tolerance.

In spite of the remaining unanswered questions, this study by Wisniewski *et al.* and other mechanistic clinical studies of food allergy clearly emphasize the feasibility of identifying the aspects of cellular immune status that distinguishes between desensitization and tolerance induction. These pursuits are important not only to facilitate refinement of antigen-specific immunotherapy in food allergy, seasonal allergy and perennial allergy, but also to provide guidance for induction of tolerance in other disease settings, including the treatment of autoimmune diseases such as multiple sclerosis and type 1 diabetes [20, 21].

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