

## EDITORIAL

# Clinical & Experimental Allergy

## Monitoring the effect of allergen immunotherapy: a clinician's dream comes true?

This editorial discusses the findings of the paper by Campbell et al. [11] pp. 1025–1035.

D. M. A. Bullens

Laboratory of Experimental Immunology, Department of Woman and Child, Division Child, Leuven, Belgium

In 1911, Noon described the amelioration of complaints in patients with pollen 'toxin'-induced hayfever symptoms, by a subcutaneous injection of small doses of pollen extract. To explain this amelioration of symptoms, Noon hypothesized the induction of 'anti-toxins' by the treatment [1]. Since then, the preparation of allergen extracts to 'desensitize' allergic patients has undergone several modification steps. Moreover, during the last decade, the use of extracts that can be administered sublingually seriously competes with the use of modified subcutaneous extracts. The mechanisms by which these desensitizing extracts exert their ameliorative functions remain incompletely understood; however, a century later, the induction of 'anti-toxins' could perhaps be called the induction of 'blocking antibodies' (reviewed in [2]). Because of the heterogeneity of the allergen extracts used, the different routes of administration and the different clinical outcome parameters that are studied, it remains difficult to deduce the common routes of action in allergen-specific immunotherapy. However, multiple studies have shown that allergen-specific immunotherapy modifies the responses of monocytes, B cells and T cells as well as the number and function of basophils, eosinophils and mast cells that mediate the allergic response (reviewed in [3]). Monocytes, B cells and T cells from allergic patients who received active immunotherapy in comparison with placebo show increased IL-10 production [3]. At the B cell level, allergen-specific IgE decreases or shows less seasonal increase and IgG1, IgG4 and IgA antibodies increase after immunotherapy [3]. Whereas at the T cell level, allergen-specific *in vitro* T-cell proliferation is reduced, tissue T-helper type 2 (Th2)

cytokine production is decreased while tissue Th1 cytokine production is increased, and functional regulatory T cells are induced [3]. Upon active immunotherapy, the seasonal increase in basophils and eosinophils in the mucosae is reduced, as well as the *in vitro* allergen-induced basophil histamine release [3].

Assessment of the clinical efficacy of active immunotherapy in patients with seasonal allergy is complicated by the fact that pollen seasons are never alike. If, for example, patients participating in a randomized-controlled clinical trial designed to compare the effects of an allergen extract to placebo were recruited during a heavy pollen season, they could all show a significant decrease of symptoms in the next year's faint pollen season. Moreover, in real life, due to differences in pollen exposure, the clinician who offers immunotherapy to a patient might be confronted with seriously increased symptoms in that patient during the next year's heavy pollen season, even if the treatment might be efficacious. Challenging patients before and during or after immunotherapy treatment in a pollen challenge chamber with controlled exposure might offer possibilities to circumvent these difficulties [4]. However, it is very unlikely that all clinicians would have access to similar chambers in the near future. It would therefore be very helpful if they could rely on biochemical tests that would indicate whether a patient is responsive to immunotherapy or not. Clinicians first attempted to evaluate the efficacy of immunotherapy by measuring the reduction in patients' specific IgE antibody levels. However, during efficient immunotherapy, allergen-specific IgE levels initially increase and later revert to pre-treatment levels, and do not necessarily decline below pre-treatment levels [5]. Measurement of the increase in allergen-specific IgG antibody levels upon treatment with immunotherapy could also be easily performed by clinicians. However, although the induction of allergen-specific IgG4 and IgG1 antibodies by active immunotherapy has been documented in several studies, it is less clear whether any

### Correspondence:

D. M. A. Bullens, Laboratory of Experimental Immunology, Department of Woman and Child, division Child, 8th floor CDG, pb 811, Herestraat 49, B-3000 Leuven, Belgium.

E-mail: Dominique.Bullens@med.kuleuven.be

Cite this as: D. M. A. Bullens, *Clinical & Experimental Allergy*, 2010 (40) 958–961.

correlation exists between this increase and clinical responsiveness to the treatment [6]. Recently, we could demonstrate that allergen-induced basophil CD63 expression in the presence of a patient's serum obtained after active immunotherapy was significantly decreased in comparison with its expression levels in the presence of serum obtained from the same patient before immunotherapy [7]. However, the relation of this decrease in basophil CD63 expression to the clinical efficacy of immunotherapy still has to be defined. Detection of alterations in the number or the nature of allergen-specific T cells might also offer options for clinical evaluation of the treatment by immunotherapy. Based on Bet v 1 tetramer staining, the levels and characteristics of circulating birch-pollen-specific CD4<sup>+</sup> T cells could be, for example, compared before and after immunotherapy [8]. However, its relevance in clinical settings will remain limited as MHC-tetramer reagents are not available for most antigens and are specific for individual MHC alleles. Interestingly, in the current issue of *Clinical &*

*Experimental Allergy*, Campbell and colleagues take advantage of a recently described technique for detecting antigen-activated CD4<sup>+</sup> T cells based on transient CD154 (CD40 ligand) expression [9, 10] to quantify and follow the evolution in ragweed-specific T cell numbers post-ragweed exposure in patients before and after active immunotherapy [11]. Moreover, ragweed exposure was controlled in a ragweed challenge chamber outside the ragweed season. However, the number of circulating ragweed-specific CD154<sup>+</sup> T cells after experimental ragweed exposure was not influenced by active immunotherapy. Adding ragweed to the short-term (6 h) *in vitro* peripheral blood mononuclear cell (PBMC) cultures increased the pre- as well as the post-treatment levels of ragweed-specific CD154<sup>+</sup> T cells in both actively and placebo-treated patients similarly, resulting in comparable levels of allergen-specific CD154<sup>+</sup> T cells pre- and post-active immunotherapy or placebo treatment [11]. It is therefore not guaranteed that evaluating the number of circulating allergen-specific T cells upon immunotherapy

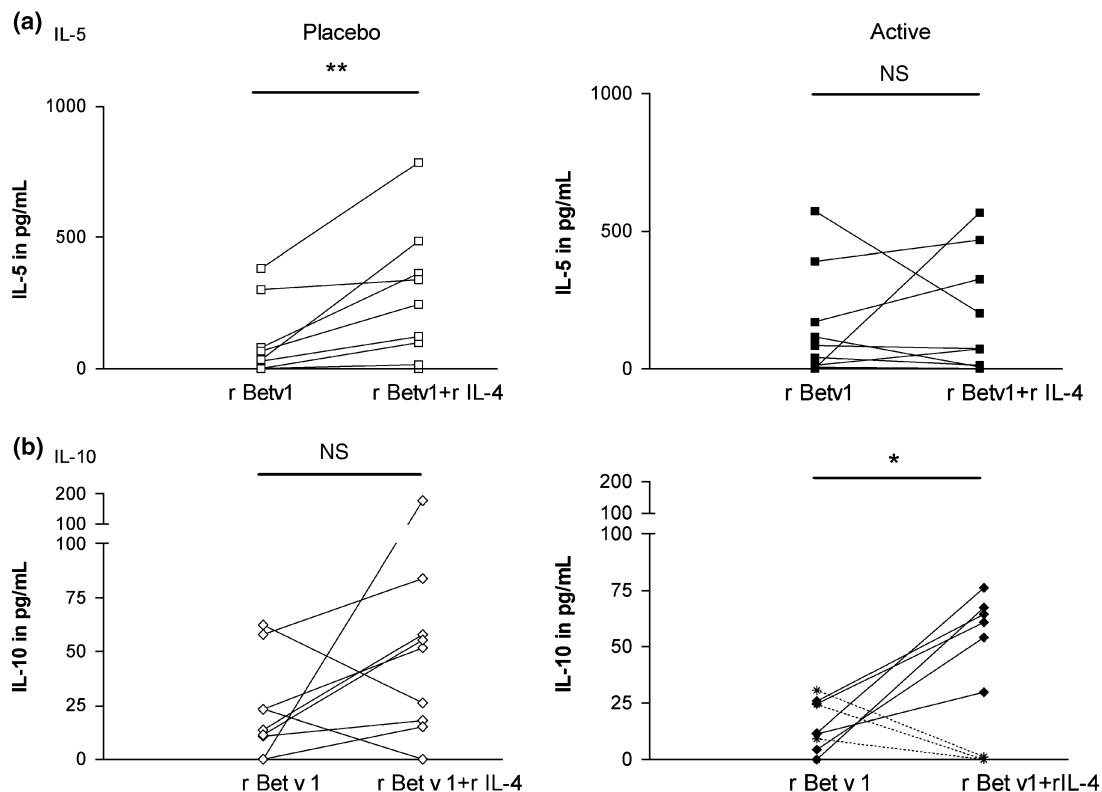


Fig. 1. Effect of IL-4 on rBet v 1-stimulated T cell cytokine production. In a subgroup of patients enrolled in the Purethal birch Efficacy Study Group [7] (those recruited at one particular centre), the effect of IL-4 on rBet v 1-stimulated T cell IL-5 and IL-10 production was studied. Peripheral blood mononuclear cells ( $1 \times 10^6$  in 1 mL) were stimulated with rBet v 1 (Biomay, Linz, Austria, 1  $\mu$ g/mL) in the absence or presence of rIL-4 (Shering-Plough, Kenilworth, NJ, USA, 5 ng/mL). Supernatants were collected after 7 days, and the concentrations of IL-5 (a) and IL-10 (b) in the supernatants were detected by ELISA. R Bet v 1-induced production of IL-5 and IL-10 was calculated by subtracting the value obtained under medium conditions from its value in medium + rBet v 1. The paired results without and with the addition of rIL-4 (tested using Wilcoxon's paired test) of the experiments from nine placebo-treated and 10 actively treated birch-pollen-allergic patients are shown; *in vitro* non-IL-4-responders are represented by a dotted line (b). \* $P < 0.05$ , \*\* $P < 0.01$  and NS, not significant.

would be helpful in predicting the clinical efficacy of immunotherapy. The authors were, however, able to study the changes in the intracellular cytokine pattern from allergen-specific CD154<sup>+</sup> T cells after a short-term *in vitro* culture in patients treated with active immunotherapy. They were able to demonstrate that the percentage of Th2 cytokine (IL-5 and IL-13)-producing CD154<sup>+</sup> T cells, upon six ragweed challenges, increased significantly in placebo-treated patients, whereas this increase was not observed in patients treated with active immunotherapy. Moreover, the authors observed a weak, although significant, correlation between nasal symptom scores and the proportion of IL-5-expressing CD154<sup>+</sup> T cells in all patients before treatment [11]. The increase in IL-5<sup>+</sup> CD154<sup>+</sup> T cells upon ragweed challenge could indicate that symptoms would tend to increase during ragweed exposure in the placebo-treated group, but not any more in the active immunotherapy group. However, the correlation between IL-5-expressing CD154<sup>+</sup> T cells and post-treatment symptom scores or the clinical efficacy of immunotherapy was not studied. Future studies are needed to address whether following the proportion of circulating IL-5<sup>+</sup> allergen-specific CD154<sup>+</sup> T cells longitudinally might be helpful to predict clinical responsiveness to immunotherapy. In accordance with these findings, using a long-term (7 days) *in vitro* stimulation approach, we were able to demonstrate that the levels of birch-pollen-induced IL-5 increased significantly upon addition of recombinant IL-4 in cultures from 1-year placebo-treated birch pollen-allergic patients, but not from patients actively treated with an aluminium hydroxide-absorbed allergoid preparation of birch pollen allergens [7] (Fig. 1a). The fact that, in actively treated individuals, the proportion of IL-5-producing allergen-specific T cells is less sensitive to IL-4 upon *in vitro* addition might reflect their resistance to *in vivo* allergen exposure, as observed by Campbell et al. [11]. Whether this resistance could be explained by alterations in the IL-4 receptor of Th2-secreting allergen-specific T cells after active immunotherapy has to be studied. We observed, on the other hand, significant increase in birch-pollen-induced IL-10 production upon addition of r IL-4 to *in vitro* PBMC cultures from actively treated individuals, but not from placebo-treated subjects (Fig. 1b). IL-4-induced allergen-specific IL-10 production was also observed in healthy subjects and this regulatory mechanism might be defective in non-treated allergic subjects [12]. Although several authors elegantly demonstrated the induction of a population of IL-10-producing T cells by active immunotherapy [13–18], it is not clear whether this parameter could be used to study clinical vaccine efficacy. Although in our study we could clearly indicate a group of actively treated patients who did not respond with increased IL-10 production upon addition of IL-4 (non-responders) (Fig. 1b), the actively treated patient group was too small

to evaluate vaccine efficacy between responders and non-responders.

In conclusion, although a century has passed since Noon's discovery that therapeutic benefit can be expected from a subcutaneous injection of small doses of pollen extract in pollen-allergic subjects, and decades of randomized placebo-controlled studies provided an insight into the immunological mechanisms behind this, clinicians are still looking for biochemical tests that could indicate whether a patient responds to immunotherapy or not. It would be of great help if, in the next decade, researchers would extend the current knowledge and use it to develop biomarkers of clinically effective immunotherapy to make a clinician's dream come true ...

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