

CORRESPONDENCE

Subcutaneous and sublingual immunotherapy and T regulatory cells: there is clinical relevance

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We read the paper of Eifan et al. [1] with great interest. These authors conducted an elegant study for comparing subcutaneous and sublingual immunotherapy (SLIT) in allergic children, also evaluating clinical, functional, and immunological parameters. This study confirmed that SLIT was able to increase IL-10 production induced by the causal allergen, thus confirming previous studies reviewed recently [2]. However, they failed to demonstrate changes concerning Th1- and Th2-dependent cytokines. This fact might depend on several factors, such as the timing of observation after starting SLIT, the time of recovering supernatants after cell incubation, the dose of the stimulating allergen, the dose of the vaccine, and of course, the case-study characteristics. In fact, the study population investigated in this study seems to show singular features. Only 19 out of 41 asthmatic children had bronchial hyperreactivity: this is quite strange as BHR is a *condicio sine qua non* for asthma diagnosis in untreated patients. Moreover, only 11 out of 38 rhinitic

children showed a positive response to an allergen-specific nasal challenge (ASNC) with the causal allergen. For definition, allergic rhinitis and asthma are characterized by the symptom occurrence after the exposure to the causal allergen; in other words, a positive relationship between history and sensitization should be demonstrated. This finding could suggest that the children with negative ASNC were sensitized alone or having a low allergy grade.

Another possible explanation for the failure of significant cytokine changes might be the assay method. In fact, we reported recently that the ELISA method may be inadequate to precisely measure cytokines, mainly IFN- γ , in the supernatants of stimulated T cells [3].

The study by Eifan et al. provided another interesting issue: the SLIT effect on TGF- β production, even though induced by a 'control' allergen, such as birch. This fact is firstly not surprising as it has been reported recently that SLIT is able to exert a 'carryover' effect on the response to

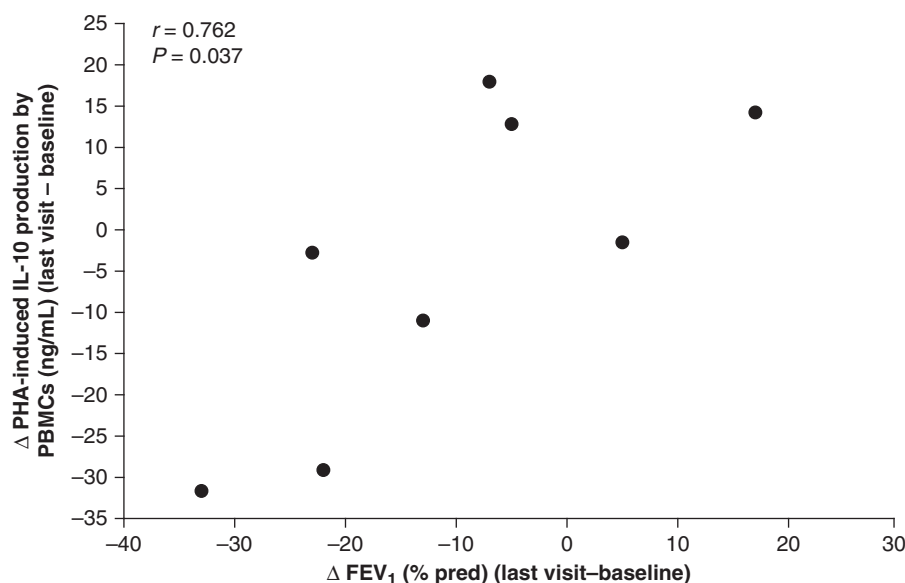


Fig. 1. Correlation between changes in forced expiratory volume in 1 s (FEV₁) and changes in interleukin (IL)-10 production by peripheral blood mononuclear cells (PBMCs) stimulated with phytohaemoagglutinin (PHA) in eight children with allergic asthma evaluated at baseline and 2 years later. Changes in FEV₁ (%pred) were expressed as delta, i.e. the difference between FEV₁ measured 2 years later and at baseline and reported on the ordinate, whereas changes in PHA-induced IL-10 production by PBMCs were expressed as delta, i.e. the difference between IL-10 production (ng/ml) measured 2 years later and at baseline and reported on the abscissa.

non-causal allergens [4]. In other words, SLIT may also induce a non-specific immunological response as well as anti-infective vaccination. The interpretation provided by the authors is most interesting: the increase in Bet v 1-induced TGF- β may possibly be considered as a mechanism for suppressing new sensitization [1]. In this regard, we recently provided evidence that IL-10 and IFN- γ production could be defective in allergic children prone to developing polysensitization [5]. Thus, SLIT could induce a T regulatory response capable of increasing the production of IL-10, TGF- β , and IFN- γ that may inhibit the development of new sensitizations.

Finally, we would like to remark that two previous studies provided evidence that SLIT was able to reduce both nasal eosinophils and BHR [6] and increase FEF₂₅₋₇₅ values [7], and overall, these changes were significantly related to increased PHA-induced IL-10 production. These data therefore confirm the clinical relevance of Treg restoration after SLIT. In this regard, we observed that there was a FEV₁ impairment in eight children with allergic asthma and monosensitized to mites (mean age 7.2 years) followed up for 2 years, which was strongly related ($r=0.76$, $P=0.03$) to reduced PHA-induced IL-10 production (unpublished data), as shown in Fig. 1.

References

- 1 Eifan AO, Akkoc T, Yildiz A *et al*. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy* 2010; **40**:922–32.
- 2 Calderón MA, Penagos M, Durham SR. Sublingual immunotherapy for allergic Rhinoconjunctivitis, allergic asthma, and prevention of allergic diseases. *Clin Allergy Immunol* 2008; **21**:359–75.
- 3 Ciprandi G, Fenoglio D, De Amici M, Marseglia G. ELISPOT and ELISA assessment of interferon-gamma after sublingual immunotherapy. *Eur J Inflam* 2010; **8**:29–33.
- 4 Ciprandi G, Sormani MP, Filaci G, Fenoglio D. Carry-over effect on IFN-gamma production induced by allergen-specific immunotherapy. *Int Immunopharmacol* 2008; **8**:1622–5.
- 5 Prigione I, Morandi F, Tosca MA *et al*. Interferon-gamma and IL-10 may protect from allergic polysensitization in children: preliminary evidence. *Allergy* 2010; **65**:740–2.
- 6 Ciprandi G, Fenoglio D, Cirillo I, Milanese M, Minuti P. Sublingual immunotherapy and regulatory T cells. *Allergy* 2006; **61**:511–3.
- 7 Ciprandi G, Cirillo I, Fenoglio D, Marseglia G, Tosca MA. Sublingual immunotherapy induces spirometric improvement associated with IL-10 production: preliminary reports. *Internat Immunopharmacol* 2006; **6**:1370–3.

Response by A. O. Eifan, N. N. Bahceciler and I. B. Barlan

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We thank Ciprandi and colleagues for their comments on our article [1], which raised several important issues.

We agree with the authors as our findings regarding Th1–Th2 cytokine profile may be affected by several factors like the timing of observation after starting SLIT, which was limited to only two time points, duration of SLIT, the dose of stimulating allergen and type of the allergen that has already been discussed [1], were factors contributing to have different results of cytokine profile.

As for the study population, patients had a median age of 7 years and those who were followed for 2 years receiving pharmacotherapy with persistent asthma and/or rhinitis symptoms according to GINA guidelines were included. We agree that bronchial hyperreactivity is considered as one of the key features of asthma, but it is difficult to assess specific/non-specific bronchial hyperresponsiveness tests in children due to a need of serial measurements and compliance. In our unit, children at this age group are encouraged to perform lung function test manoeuvres with the help of a computer program

adapted to the spirometer device. We used allergen-specific nasal provocation test with three different doses and evaluated the response by using clinical symptom score without other objective measurement like rhinomanometer, which is difficult for children to perform. We relayed more on history and symptom scores including severity, frequency and duration of nasal blockage, rhinorrhea, sneezing and response to treatment, as well as physical findings on diagnosing allergic rhinitis. In line with this our primary outcomes were to evaluate the early response after allergen challenge.

Regarding the T-regulatory effects of SLIT, an increase in Bet-V1-induced TGF- β in patients who were monosensitized to house dust mite (HDM) and were receiving HDM SLIT was an unexpected finding. Ciprandi and colleagues found that there is a carry over effect on Th1 cytokine production induced by specific immunotherapy [2], while in our results we highlighted the possible SLIT effect on mucosal immunity as allergen-specific TGF- β is being induced to probably block a new sensitization. We