

EDITORIAL

Clinical & Experimental Allergy

Adjuvants for immunotherapy: lost in translation?

This editorial discusses the findings of the paper by Majak, et al. [7] pp. 1830–41.

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Allergen-specific immunotherapy (SIT) has been used for the treatment of allergic diseases since it was first applied by Noon in 1911 [1]. Ever since, not much has changed in the original treatment protocol of subcutaneous injections with increasing doses of crude allergen extracts. This seems rather surprising because it is currently the only disease modifying treatment that offers long-term protection against allergic manifestations. Moreover, the efficacy of SIT is rather variable and appears to differ from patient to patient depending on the type of allergen, the type of allergic disease and on as yet unknown factors, including genetics. However, there is light at the end of the tunnel. Novel strategies are on their way to improve the burden of multiple subcutaneous injections by sublingual or intralymphatic administration, to improve its efficacy by using an adjuvant and to improve the standardization by using recombinant allergens [2, 3].

Currently, regulatory T (Treg) cells are considered to be largely responsible for clinically successful SIT and the underlying immunological responses, e.g. increased IgG4 levels [3, 4]. Clinical improvement after SIT has been associated with CD4⁺ Treg cells that produce the immunosuppressive cytokines IL-10 and TGF- β and with CD4⁺CD25⁺FOXP3⁺ Treg cells. Cytokine producing CD4⁺ Treg cells are induced early after starting SIT injections and may be responsible for the increased serum levels of specific IgG4 and IgA [3, 4]. Literature data on FOXP3⁺ Treg cells in SIT are still limited but increased numbers of CD4⁺FOXP3⁺ T cells have been observed after venom SIT [5]. Moreover, CD4⁺FOXP3⁺ cells are increased in the nasal mucosa after grass-pollen SIT and

were occasionally associated with IL-10 production by these T cells [6]. Considering the emerging role of Treg cells in SIT, strategies to facilitate their induction using adjuvants in combination with SIT may be promising to improve the efficacy of SIT.

Adjuvants that are currently being considered for SIT are mainly immunological in nature, e.g. immunostimulatory oligodeoxynucleotides and monophosphoryl lipid A [2, 3]. These adjuvants act on toll-like receptors (TLR), respectively TLR-9 and -4, expressed by antigen-presenting cells (APCs) and were initially aimed at promoting allergen-specific Th1 responses [2]. In this issue of the journal, Majak et al. [7] use, for the first time, pharmacological agents, glucocorticosteroids (GCS) alone or in combination with vitamin D3 (VitD3), as adjuvants for SIT. After uptake in the intestine, VitD3 is hydroxylated in the liver into calcidiol and subsequently calcitriol is formed in the kidney as well as in APCs by 25-hydroxylation of calcidiol. Calcitriol or 1,25-dihydroxyvitamin D3 (1,25VitD3) is the physiologically active form of VitD3 and binds to the vitamin D receptor, a nuclear hormone receptor, to exert its biological effects. The rationale to use GCS or GCS/VitD3 as adjuvant is the reported induction of IL-10 production by CD4⁺ T cells and up-regulation of CD4⁺FOXP3⁺ T cells by *in vivo* or *in vitro* treatment with this anti-inflammatory drug [8, 9]. Barrat et al. [10] were the first to demonstrate that human and mouse T cell activation in the presence of both GCS and 1,25VitD3, induces the generation of stable IL-10 producing CD4⁺ Treg cells *in vitro* which are able to suppress cytokine secretion by allergen-specific Th2 cells [11]. In contrast, 1,25VitD3 enhances Th2 differentiation and cytokine production when present during T cell activation [12]. The latter may preclude VitD3 as a single adjuvant for SIT. However, 1,25VitD3 has been shown to inhibit the maturation of dendritic cells (DCs) thereby facilitating the generation of adaptive Treg cells [13–15]. Interestingly, 1,25VitD3 and GCS have a synergistic suppressive effect on DC maturation and consequently display enhanced IL-10 production [16]. Van Overtvelt et al. [17] studied the

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combination of VitD3 and GCS as adjuvant for SLIT in an ovalbumin-allergic mouse model and showed enhanced suppression of airway hyperreactivity (AHR) associated with peripheral expansion of FoxP3⁺ Treg cells. Unfortunately, allergic inflammation was not studied and no effect on antigen-specific Ig levels was observed. Altogether, the rationale to use a combination of GCS and VitD3 as adjuvant for SIT appears better than using GCS alone.

In a randomized, double-blind, placebo-controlled trial with children aged 6–12 years with IgE-mediated asthma, Majak et al. [7] examined prednisone alone or in combination with VitD3 as adjuvants for SIT. Surprisingly, the group that received house-dust mite SIT with GCS as adjuvant, showed significantly less clinical improvement compared with the SIT control group. On the other hand, VitD3 seemed to neutralize this negative effect, as the group that received SIT with GCS/VitD3 adjuvant displayed similar improvement as the SIT control group. Clinical improvement was associated with increased FOXP3 and IL-10 expression at 3 months and FOXP3 expression at 12 months after SIT alone or with GCS/VitD3 adjuvant. Interestingly, 1,25VitD3 has been shown to be effective as adjuvant in an allergic mouse model of immunotherapy, potentiating the suppression of AHR, eosinophilic airway inflammation and serum IgE levels [18]. Moreover, these suppressive effects were mediated by IL-10 and TGF- β , pointing to a role of Treg cells. These mouse data together with the data from Majak and colleagues warrant further studies into the use of VitD3 as adjuvant for SIT.

There are several limitations in the clinical trial of Majak and colleagues that preclude a definite conclusion regarding GCS and GCS/VitD3 as adjuvant for SIT. First, no dose-finding study was performed with GCS or its optimal combination with VitD3. Dosing may be critical as both drugs have multiple effects not only on T cells but also on other immune and non-immune cells. Majak et al. [7] used a regular starting dose of prednisone (0.5–1.0 mg/kg). This dose of prednisone is suitable to exert anti-inflammatory effects, but these may be dissociated from a potential adjuvant effect. For VitD3 supplementation, Majak and colleagues used a single weekly dose of 25 μ g (1000 IU), the maximal daily dose for children. This oral dose of VitD3 reversed the unfavourable effect of GCS on SIT indicating that it was used within the effective range. Although this dosing is difficult to compare, daily oral administration of 0.5 μ g 1,25VitD3 has been shown to selectively increase IL-10 mRNA expression by CD4⁺ T cells when given for at least 3 days [19]. This direct effect of 1,25VitD3 on T cell IL-10 production was not studied by Majak and colleagues. However, it is tempting to speculate that it may have stimulated tolerance induction by SIT because IL-10 propagates induction of IL-10 producing Treg cells [20]. The precise dosing of both GCS and VitD3 to obtain the induction of IL-10 producing Treg cells as

previously demonstrated *in vitro* [10, 11] may even be more complicated. Secondly, the drugs were only given during the build-up phase of SIT and discontinued thereafter. Although clinical improvement by SIT was already observed after three months, at the end of the build-up phase, it remains possible that it takes longer for GCS to become effective as adjuvant. Last but not least, the timing and route of administration of both drugs may be critical. In the study of Majak et al. [7], GCS and VitD3 were given orally 4 h before subcutaneous SIT injections. In the already discussed mouse models of immunotherapy adjuvant activity of GCS and VitD3 was shown when the drugs were given at the same time and route as allergen SIT [17, 18]. Considering that DCs or T cells are potential direct targets, it cannot be excluded that local high concentrations of GCS and 1,25VitD3 are required that are not achieved by oral administration.

It can be concluded that despite strong *in vitro* and *in vivo* evidence in favour of a putative adjuvant effect for GCS and VitD3 in SIT, this first translational study did not observe beneficial effects using these adjuvants. Given all the limitations, more clinical studies are needed to determine whether these immunosuppressive drugs can be useful as adjuvant for SIT.

References

- Noon L, Cantab BC. Prophylactic inoculation against hay fever. *Lancet* 1911; **177**:1572–4.
- Thomas WR. Innovation in immunotherapy. *Clin Exp Allergy* 2009; **39**:450–4.
- Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov* 2009; **8**:645–60.
- James LK, Durham SR. Update on mechanisms of allergen injection immunotherapy. *Clin Exp Allergy* 2008; **38**:1074–88.
- Pereira-Santos MC, Baptista AP, Melo A *et al.* Expansion of circulating foxp3⁺CD25bright CD4⁺ T cells during specific venom immunotherapy. *Clin Exp Allergy* 2008; **38**:291–7.
- Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces foxp3-expressing CD4⁺CD25⁺ cells in the nasal mucosa. *J Allergy Clin Immunol* 2008; **121**:1467–72.
- Majak P, Rychlik B, Stelmach I. The effect of oral steroids with and without vitamin D₃ on early efficacy of immunotherapy in asthmatic children. *Clin Exp Allergy* 2009; **39**:1830–41.
- Karagiannidis C, Akdis M, Holopainen P *et al.* Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004; **114**:1425–33.
- Peek EJ, Richards DF, Faith A *et al.* Interleukin 10 secreting 'regulatory' T cells induced by glucocorticoids and beta2-agonists. *Am J Respir Cell Mol Biol* 2005; **33**:105–11.
- Barrat FJ, Cua DJ, Boonstra A *et al.* In vitro generation of interleukin 10-producing regulatory CD4⁺ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J Exp Med* 2002; **195**:603–16.
- Xystrakis E, Kusumakar S, Boswell S *et al.* Reversing the defective induction of IL-10-secreting regulatory T cells in

- glucocorticoid-resistant asthma patients. *J Clin Invest* 2005; **116**:146–55.
- 12 Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A. 1alpha, 25-dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol* 2001; **167**:4974–80.
- 13 Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1alpha, 25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. *Proc Natl Acad Sci USA* 2001; **98**:6800–5.
- 14 Penna G, Adorini L. 1 Alpha, 25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* 2000; **164**:2405–11.
- 15 Adorini L, Giarratana N, Penna G. Pharmacological induction of tolerogenic dendritic cells and regulatory T cells. *Semin Immunol* 2004; **16**:127–34.
- 16 Pedersen AE, Gad M, Walter MR, Claesson MH. Induction of regulatory dendritic cells by dexamethasone and 1alpha, 25-dihydroxyvitamin D(3). *Immunol Lett* 2004; **91**:63–9.
- 17 Van Overtvelt L, Lombardi V, Razafindratsita A *et al.* IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. *Int Arch Allergy Immunol* 2007; **145**: 152–62.
- 18 Taher YA, van Esch BC, Hofman GA, Henricks PA, van Oosterhout AJ. 1 α , 25-dihydroxyvitamin D3 potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF-beta. *J Immunol* 2008; **180**:5211–21.
- 19 Urry Z, Xystrakis E, Richards DF *et al.* Ligation of TLR9 induced on human IL-10-secreting tregs by 1alpha, 25-dihydroxyvitamin D3 abrogates regulatory function. *J Clin Invest* 2009; **119**: 387–98.
- 20 Groux H, O'Garra A, Bigler M *et al.* A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; **389**:737–42.