

SLIT Prevents the Development of Eczema in Percutaneous Allergen-Sensitized Mice

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TO THE EDITOR

Atopic dermatitis (AD) is a T-cell-mediated chronic inflammatory skin disease associated with cutaneous hyperreactivity to environmental antigens, such as house dust mite *Dermatophagoides farinae* (DF), which are innocuous to normal nonatopic individuals (Bieber, 2008). The hallmarks of AD are skin barrier dysfunction and T-cell infiltration, which are intimately linked. Current approaches to treating AD are directed at both the restoration of barrier function and the inhibition of inflammation. Development of new targeted therapeutic approaches is a constant preoccupation. Sublingual immunotherapy (SLIT) is an efficacious treatment for type I (IgE-mediated) respiratory allergies (Canonica and Passalacqua, 2006; Didier *et al.*, 2007) aimed at inducing allergen-specific tolerance. Until now, SLIT is not indicated for AD, and few data are available for either animal models or humans (Pajno *et al.*, 2007). Here, we tested the efficacy of SLIT against house dust mite DF in a mouse model of AD, as a proof of concept of using allergen-specific immunotherapy in type IV (T-cell-mediated) skin allergy. In this DF-induced AD model, we have previously shown that CD8⁺ T cells producing IFN- γ are essential for the development of AD skin inflammation (Hennino *et al.*, 2007). The importance of CD8⁺ T cells has been previously described in the physiopathology of the human disease (Akdis *et al.*, 1999; Seneviratne *et al.*, 2002; Hennino *et al.*, 2011). Upon DF skin sensitization, CD8⁺ T cells are induced in draining lymph nodes and recruited in

the challenged skin where they initiate the AD-like skin inflammatory reaction.

Mice were sensitized on the ear once a week for 4 weeks by application of a solution of DF (250 μ g per application), as previously described (Hennino *et al.*, 2007). Mice developed a specific ear inflammation 24–48 hours after the fourth DF skin challenge (day 21), but also at distant challenge times performed 4 (day 49) or 8 weeks (day 91) later (Supplementary Figure S1A, B online). Sensitized animals displayed an average frequency of IFN- γ spot-forming cells (SFC) of 45 cells per 10^6 spleen cells (data not shown), as detected by the ELISPOT technique. SLIT treatment was performed on unanesthetized mice using chitosan-formulated DF (CHI-DF) or the placebo control CHI-empty particules (CHI- Φ) twice a week for 8 consecutive weeks (from D28 to D88) in previously sensitized animals (D0–D21; Figure 1a). Concentration of DF in the particulated CHI was 8.3 times less than the immunization dose (i.e., 30 μ g ml⁻¹). CHI, a polysaccharide derived from chitin by deacetylation, had been previously identified to enhance allergen-specific tolerance when co-administrated sublingually with the antigen in ovalbumin-sensitized asthmatic mice (Razafindratsita *et al.*, 2007; Saint-Lu *et al.*, 2009). As shown in Figure 1b, mice treated with CHI-DF did not develop DF-induced skin inflammation 48 hours upon challenge, in contrast to placebo CHI- Φ -treated mice, suggesting that SLIT induced allergen-specific tolerance. An important finding was also that CHI-DF treatment of unsensitized mice did not lead to ear inflammation upon

challenge, demonstrating that repeated sublingual allergen exposure does not induce sensitization. Next, we analyzed the immune response in the spleen of CHI-DF- or CHI- Φ -treated mice. Mice were killed at day 91 and spleens were recovered and restimulated *in vitro* with DF antigen for 48 hours, and the frequency of IFN- γ -producing cells was evaluated by ELISPOT assay. Spleen cells were dispensed in the plates (5×10^5 per well) and incubated with 500 μ g ml⁻¹ Der f protein or Der f peptide at 2 μ M (data not shown) for 36 hours at 37 °C, 5% CO₂ in the presence of irradiated splenocytes as antigen-presenting cells. To determine Der f, class I peptide was also used for restimulation at a final concentration of 2 μ M. The peptide 113–122 sequence (YGISNYCQI) was determined by prediction using the Bioinformatics and Molecular Analysis Section and SYF-PEITHI epitope prediction databases.

Mice treated with placebo CHI- Φ showed a high frequency of allergen-specific IFN- γ -producing cells in the spleen (Figure 1b). In contrast, mice treated with CHI-DF showed a significant reduction of IFN- γ SFC, which was comparable with the frequency found in unsensitized mice (Figure 1b). These data suggest that allergen-specific SLIT is associated with a drastic reduction in the induction of DF-specific effector T cells.

As specific immunotherapy was shown to promote the production of IL-10 (Piconi *et al.*, 2010), we have further analyzed the immune response in the lymph nodes draining the SLIT mucosal sites (i.e., the sublingual lymph nodes). SLIT-draining lymph nodes were recovered at 91 days and restimulated *in vitro* with DF antigen for 48 hours; the production of IL-10 was evaluated by ELISA. As shown in Figure 2a,

Abbreviations: AD, atopic dermatitis; CHI, chitosan; DF, *Dermatophagoides farinae*; SFC, spot-forming cells; SLIT, sublingual immunotherapy

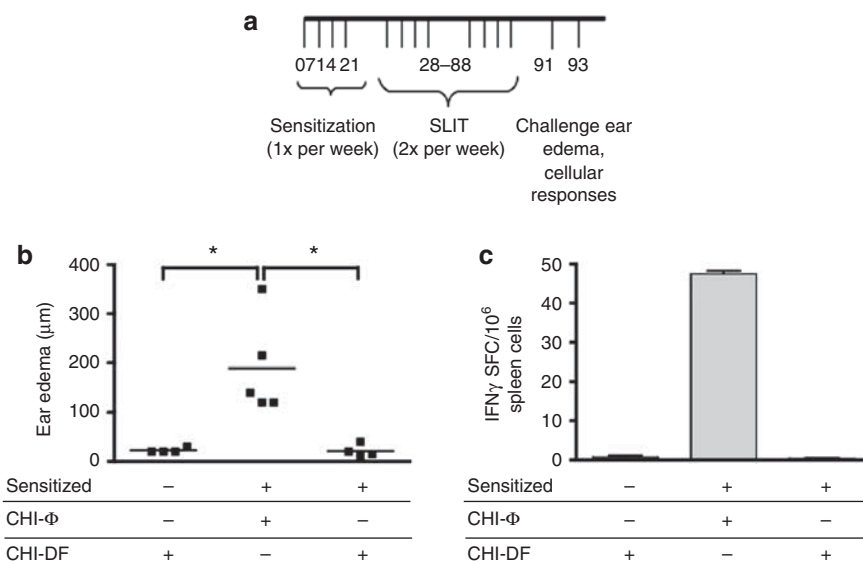


Figure 1. Sublingual immunotherapy (SLIT) prevents development of atopic dermatitis in sensitized animals. (a) Schematic protocol used for the study. (b) *Dermatophagoides farinae* (DF)-induced skin inflammation was analyzed at day 93 (48 hours post challenge). Results are expressed as the mean ear swelling (i.e., ear thickness of the DF-challenged right ear minus the ear thickness of the vehicle (DMSO 70%)-challenged right ear) and are representative of three independent experiments, using five mice per group. (c) DF-specific IFN- γ -producing cells were determined in total spleen cells at day 91. Results are expressed as the number of spot-forming cells (SFC) per 10 ^{6} spleen cells. Results are representative of three independent experiments. * $P < 0.05$ using unpaired t -test. CHI-DF, chitosan-formulated DF.

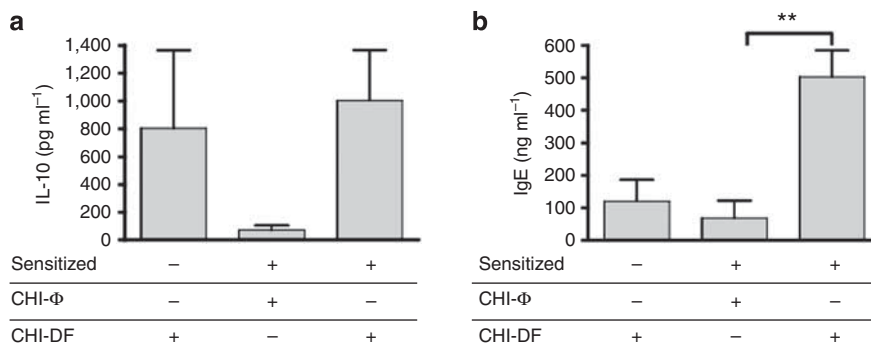


Figure 2. Sublingual immunotherapy (SLIT) induces IL-10 and IgE production. (a) *Dermatophagoides farinae* (DF)-specific production of IL-10 by SLIT-draining lymph node cells (day 91) after *in vitro* restimulation with DF (ELISA). (b) Total serum IgE levels (in ng ml $^{-1}$) at day 93. Results are representative of three independent experiments. ** $P < 0.05$ using two-tailed unpaired t -test. CHI-DF, chitosan-formulated DF.

DF-sensitized mice treated with CHI-DF displayed an enhanced production of IL-10 compared with CHI- Φ -treated mice. It is noteworthy that IL-10 was specifically induced by SLIT treatment and was independent of the sensitization as CHI-DF-treated unsensitized mice also showed increased IL-10 production. Finally, we analyzed the production of serum IgE in the different groups of mice. Consistent with our previous study (Hennino *et al.*, 2007), DF-sensitized mice treated with the CHI- Φ placebo SLIT developed increased IgE levels (mean 120 ± 60 ng ml $^{-1}$). CHI-DF immunotherapy increased the levels of

serum IgE in both DF-sensitized (mean 500 ± 80 ng ml $^{-1}$) and unsensitized (mean 68 ± 30 ng ml $^{-1}$) mice, demonstrating that mucosal exposure to DF is able to induce IgE production in naive mice and boost IgE production in mice already sensitized through the skin. This observation indicates that there is no correlation between SLIT-induced improvement of AD skin inflammation and IgE serum levels. Along this line, we have previously shown that the development of AD skin lesions was not influenced by serum IgE levels, as MHC II $^{-/-}$ mice, lacking CD4 $^{+}$ T cells and therefore unable to produce IgE, developed even

more severe AD skin lesions than wild-type mice (Hennino *et al.*, 2007).

In summary, we provide evidence that SLIT is efficient in a mouse model of AD, in which sensitized animals display high numbers of allergen-specific T cells. Systematic studies on the effectiveness of SLIT in patients with AD are rare. Two clinical studies, one in adults and one in children with specific IgE to house dust mite, showed that SLIT or subcutaneous immunotherapy improved mild-moderate atopic dermatitis (Werfel *et al.*, 2006; Pajno *et al.*, 2007). DF SLIT treatment led to decreased numbers of specific T cells,

increased production of IL-10, and enhanced protection from DF-induced skin inflammation upon challenge. We therefore postulate that SLIT could be considered as an attractive treatment for AD in well-defined patients, without polysensitization relying on the presence of antigen-specific T cells for a defined Ag (i.e., Der f or Der p) in peripheral blood or on positive atopy patch test to the allergen (Nosbaum *et al.*, 2010).

Approval of animal experiments

All experimental procedures were in accordance with the Comité régional d'éthique pour l'expérimentation animale guidelines on animal welfare.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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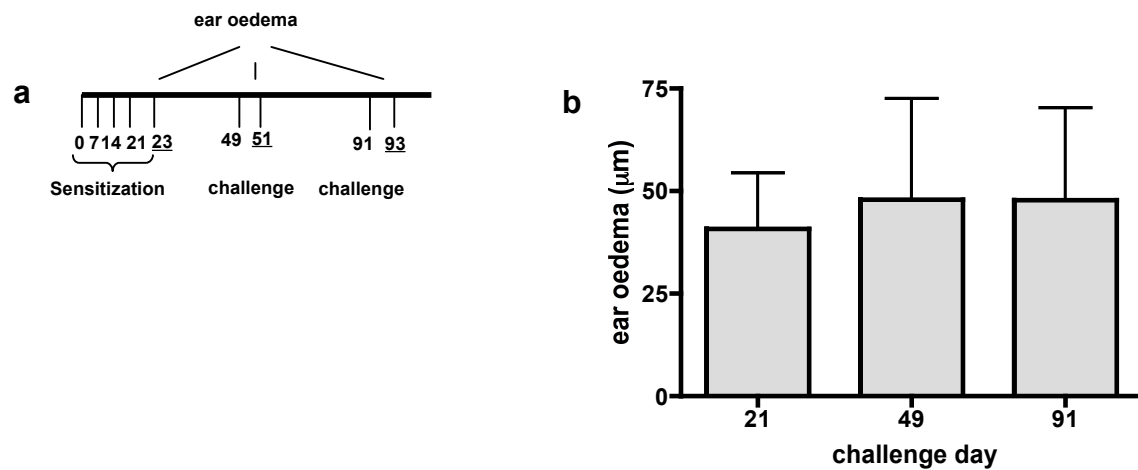
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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

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Supplemental figure 1. AD-like skin inflammation is induced upon DF challenge at day 49 or 91. A. Schematic protocol use for the study. B. DF-induced skin inflammation was analyzed at day 23, 51 and 93 (48 hours post challenge). Representative of three independent experiments .