

Immune mechanisms of sublingual immunotherapy: Are oral Langerhans cells the masters of tolerance?

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The route of administration of any vaccine determines, to some extent, its immunogenicity. This is because anatomic location determines the components of both the innate and adaptive immune systems with which antigens and adjuvants interact. In particular, antigen-presenting cells (APCs) that sense, sample, and present antigenic determinants have specialized functions depending on the tissues they survey. Historically, certain locations (eg, the testes and the anterior chamber of the eye) have been regarded as sites of “immune privilege” because it has been difficult to elicit inflammatory responses. More recently, we have come to understand that the privilege is merely a qualitatively different response providing the appropriate protection without inflammation. It has been hypothesized that the sublingual microenvironment might represent a mucosal site at which the default immune response to antigen encounter by local APCs, such as oral dendritic cells (DCs), is one of tolerance and that this might contribute to the efficacy of sublingual immunotherapy (SLIT).

A study by Allam et al¹ in this issue of the *Journal* addresses the potential role of DCs isolated from the vestibular region of the oral mucosa in tolerance associated with SLIT. In an earlier report these cells displayed typical characteristics of Langerhans cells (LCs) and were designated oral LCs (oLCs).² Unusually for DCs, high-affinity IgE receptors (FcεRI) were present in substantial numbers on oLCs from allergic and nonallergic subjects. Expression was higher in allergic subjects, probably because of surface retention by bound IgE.³ In the present study the authors assessed (1) the ability of oLCs to bind fluorescein isothiocyanate (FITC)-labeled antigen, (2) the consequences of that binding, (3) the maturation status of the cells, and (4) their ability to stimulate T-cell responses to alloantigen.

Understanding how specific allergen binding was determined is key to understanding this study. Purified native Phl p 5 was labeled with FITC and incubated with oral mucosal tissue for differing times and in differing doses to determine (1) the time dependence and (2) the concentration dependence of allergen binding. Exposure of tissue to allergen occurred at 4°C or 37°C and was followed by a 36-hour culture (37°C) during which oLCs migrated out of the tissue. Bound allergen was assessed on migrated CD1a⁺ cells by means of flow cytometry. Allergen binding occurred at both temperatures in a dose-dependent fashion but more so in the cells incubated with allergen initially at 37°C (see Fig 2 in Allam et al¹). Despite the absence of any obvious features identifying subsets of CD1a⁺ oLCs, only approximately 40% appeared to bind labeled allergen, and this was not different between donors with grass pollen allergy and nonallergic donors, despite higher FcεRI and increased specific serum IgE levels in the group with grass pollen allergy. Binding occurred rapidly, reaching the 40% plateau within 5 to 10 minutes.

As a binding specificity control, oral mucosal tissue was preincubated (37°C) with unlabeled Phl p 5 before addition of labeled allergen. Importantly, preincubation reduced staining intensities to levels observed on tissue exposed to allergen at 4°C. These data show that (1) additional staining signal obtained with a 37°C initial incubation was allergen specific, (2) specific allergen binding was probably saturating a surface receptor of some kind, and (3) binding to cells during the 4°C allergen incubation (observed also with keratinocytes) was nonspecific. On this basis, the authors calculated the specific binding of Phl p 5 by subtracting the percentage of FITC-positive CD1a⁺ cells at 4°C from the percentage of CD1a⁺ cells at 37°C.

In addition to increased staining intensity, the number of CD1a⁺ cells migrating out of mucosal tissue was significantly greater at 37°C than at 4°C. The number of migrated CD1a⁺ cells increased with time and was maximal after 48 hours. At this point, approximately 80% of all CD1a⁺ cells in the tissue had migrated into the culture medium, despite the fact that only 40% of these cells stained positive for allergen binding. Because a similar rate of migration over time was observed in control cultures (PBS, allergen at 4°C, and FITC-labeled dextran), it can be concluded that the time-dependent increase in migrated CD1a⁺ cell numbers was a nonspecific phenomenon, whereas the increased number of cells at each time point (37°C) was an allergen-specific finding. Once again, no difference was observed between donors with grass pollen allergy and nonallergic donors, suggesting a minimal role for specific IgE and FcεRI in this process.

The nature of the surface molecule or molecules bound by FITC-labeled Phl p 5 forms the basis of ongoing investigation. In light of recent findings describing specific interactions between certain allergens and molecules expressed by APCs, it is interesting to speculate that Phl p 5 might be another example of an allergen with intrinsic adjuvant activity by virtue of its ability to

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interact with components of the innate immune system.⁴ Recently, the house dust mite allergen Der p 2, which has homology with the MD-2 lipid-binding component of Toll-like receptor 4, was shown to facilitate Toll-like receptor 4 signaling.⁵ Similarly, the structure of Der p 7 has revealed characteristics of bacterial lipid-binding activity that might confer adjuvant activity.⁶ Carbohydrate moieties, such as β -glucan structures, of allergens have also been shown to have effects on DCs, including recruitment and the preferential induction of T_H2 responses.⁷ Carbohydrate receptors, such as lectins, have been shown to play a role in allergen uptake and activation of DCs, although they are unlikely to participate in Phl p 5 binding because this allergen is not glycosylated (personal communication, Andreas Nandy, Allergopharma, Germany).

On the basis that antigen uptake is typically associated with maturation of DCs, the authors analyzed maturation and migration markers on migrated oLCs. CD1a⁺ cells were divided into those that stained positively for FITC and those that did not. The phenotypic profile obtained is interesting from 2 perspectives. First, expression of the DC maturation marker CD83 was downregulated in LCs that bound allergen. Combined with unchanged levels of CD207 and CCR6, this observation implies that migrating oLCs retained an immature phenotype. Second, allergen-binding oLCs displayed markedly downregulated levels of CCR7. This is intriguing but initially counterintuitive because this chemokine receptor is traditionally associated with homing to local lymph nodes through interaction with the chemokines CCL21 (SLC) and CCL19 (ELC).⁸ The authors offer the intriguing speculation that this might be indicative of interactions between antigen-loaded oLCs and specific T cells in nonlymphoid regions of the oral mucosa, such as the interpapillary or "rete" ridges and lamina propria.

On the basis that SLIT is associated with desensitization, the authors also investigated intracellular expression of 2 cytokines commonly associated with immunologic tolerance, specifically TGF- β and IL-10. Staining of migrated CD1a⁺ oLCs revealed that tissue incubated with FITC-labeled allergen at 37°C (vs 4°C) displayed stronger staining for both cytokines. TGF- β has previously been shown to retard the maturation of human Langerhans-like cells, perhaps contributing to maintenance of the immature phenotype in the current study.⁹ Similarly, human DCs cultured in the presence of IL-10 had reduced ability to stimulate CD4 T cells and generated alloantigen-specific anergy.¹⁰ Thus the production of IL-10 by oLCs is consistent with IL-10 production from phenotypically immature DCs, which have tolerogenic capability. Additionally, the authors evaluated the ability of oLCs to induce IL-10-secreting T cells, TGF- β -secreting T cells, or both in an allogeneic stimulation setting. Previous studies demonstrated that repeated stimulation of T cells with immature allogeneic DCs can result in the induction of IL-10-producing T cells¹¹ and that induction of IL-10 is a feature of SLIT.¹² Consistent with these data, oLCs from mucosal tissue incubated with allergen at 37°C (vs 4°C) were able to induce higher levels of TGF- β and IL-10 staining in alloantigen-specific T cells. The authors conclude that allergen binding by oLCs within oral mucosal

tissue enforces the tolerogenic character of both the APCs and primed T cells.

Although the system described in this study constitutes an *in vitro* rather than an *in vivo* model, the observation that specific interaction appears to occur between oLCs and a major aeroallergen is intriguing. The resulting upregulation and apparent function of immunoregulatory cytokines in local APCs, together with transmission of a potentially tolerogenic phenotype to T cells, offers potentially important insights into local factors contributing to the immunologic mechanisms of SLIT. Several interesting questions remain to be addressed in future studies. Are tissue and cells from the vestibular region (which lies on the cheek side of the teeth) truly representative of the sublingual mucosa targeted in SLIT? Why, despite the fact that Fc ϵ RI levels were profoundly downregulated, does allergen-specific IgE not appear to contribute more to the phenomenon observed? Will T cells stimulated by allergen-loaded (rather than allogeneic) oLCs also express TGF- β and IL-10, and will those T cells act as regulatory cells? Finally, and perhaps most intriguingly, does the downregulation of CCR7 on migrating oLCs suggest that local antigen presentation occurs outside draining lymphoid tissue, thereby perhaps enhancing tolerogenic interactions? The answers to these and other questions arising from this work will undoubtedly clarify our understanding of the immunologic mechanisms of SLIT.

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