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# Modulation of dendritic cell innate and adaptive immune functions by oral and sublingual immunotherapy

Pamela A. Frischmeyer-Guerrerio[a,*], Corinne A. Keet[a], Anthony L. Guerrerio[b], Kristin L. Chichester[a], Anja P. Bieneman[c], Robert G. Hamilton[c], Robert A. Wood[a], John T. Schroeder[c]

[a] Division of Allergy and Immunology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Division of Gastroenterology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c] Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

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**Abstract** Sublingual (SLIT) and oral immunotherapy (OIT) are promising treatments for food allergy, but underlying mechanisms are poorly understood. Dendritic cells (DCs) induce and maintain Th2-type allergen-specific T cells, and also regulate innate immunity through their expression of Toll-like receptors (TLRs). We examined how SLIT and OIT influenced DC innate and adaptive immune responses in children with IgE-mediated cow's milk (CM) allergy. SLIT, but not OIT, decreased TLR-induced IL-6 secretion by myeloid DCs (mDCs). SLIT and OIT altered mDC IL-10 secretion, a potent inhibitor of FcεRI-dependent pro-inflammatory responses. OIT uniquely augmented IFN-α and decreased IL-6 secretion by plasmacytoid DCs (pDCs), which was associated with reduced TLR-induced IL-13 release in pDC-T cell co-cultures. Both SLIT and OIT decreased Th2 cytokine secretion to CM in pDC-T, but not mDC-T, co-cultures. Therefore, SLIT and OIT exert unique effects on DC-driven innate and adaptive immune responses, which may inhibit allergic inflammation and promote tolerance.


*Abbreviations:* DC, dendritic cell; TLR, Toll like receptor; IT, immunotherapy; SLIT, sublingual immunotherapy; OIT, oral immunotherapy; SCIT, subcutaneous immunotherapy; CM, cow's milk; pDC, plasmacytoid dendritic cell; mDC, myeloid dendritic cell; BDCA, Blood Dendritic Cell Antigen; Treg, T regulatory cell; APC, antigen presenting cell; DBPCFC, double blind placebo-controlled food challenge; OFC, oral food challenge; GEE, generalized estimating equation; poly (I:C), polyinosinic:polycytidylic acid.

* Corresponding author at: 600 North Wolfe Street, CMSC 1102, Baltimore, MD 21287-2631, USA. Fax: +1 410 955 0229.
*E-mail addresses:* pguerrerio@jhmi.edu (P.A. Frischmeyer-Guerrerio), ckeet1@jhmi.edu (C.A. Keet), aguerrerio@jhmi.edu (A.L. Guerrerio), kchiches@jhmi.edu (K.L. Chichester), abienema@jhmi.edu (A.P. Bieneman), rhamilto@jhmi.edu (R.G. Hamilton), rwood@jhmi.edu (R.A. Wood), schray@jhmi.edu (J.T. Schroeder).

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## 1. Introduction

Food allergy currently affects an estimated 15 million Americans [1,2]. Although there is no cure, novel forms of immunotherapy (IT) are emerging as viable effective treatments [3–6]. The mechanisms responsible for the clinical benefits of IT are incompletely understood. Prior studies have suggested that IT downregulates Th2 cytokine responses by $CD4^+$ T cells and induces antigen-specific T regulatory cells (Tregs) [7–9]. Dendritic cells (DCs) are professional antigen presenting cells (APCs) that take up antigens and prime T cell responses, and therefore likely play an instrumental role in directing these changes in T cell function. Two major classes of DCs have been identified in the peripheral blood of humans: 1) plasmacytoid DCs (pDCs), which are $CD123^+$ Blood Dendritic Cell Antigen-2 $(BDCA\text{-}2)^+$ $CD11c^-$ and express TLRs 7 and 9; these cells are a major source of IFN-α following microbial infection and 2) myeloid DCs (mDCs), which are $CD123^-$ $BDCA1^+$ $CD11c^+$ and express TLRs 2, 3, 4, and 8 [10–13]. Both subtypes regulate allergen-driven Th2 cytokine release by $CD4^+$ T cells, underscoring a critical role for these cells in the pathogenesis of allergic disease [14,15].

Recent studies have suggested that DC-derived cytokines released following TLR ligation can regulate T cell tolerance and Th2 differentiation. DC-derived IL-6 limits Th1 responses and promotes commitment to Th2 phenotypes, while IFN-α produced by pDCs potently inhibits IL-4 driven Th2 differentiation of $CD4^+$ T cells and destabilizes the Th2 phenotype by suppressing expression of GATA3 [16–18]. Pro-inflammatory cytokines secreted by DCs also have the capacity to break allergen-specific T cell tolerance, and lead to exaggerated Th2 (and Th17) responses [19].

We have previously found that DCs from children allergic to cow's milk produce significantly higher levels of pro-inflammatory cytokines, including IL-6 and TNF-α, following exposure to milk compared to DCs from nonallergic children [14]. Additionally, several studies have revealed global defects in innate immune pathways in individuals with allergic disease, including deficient IFN-α secretion following TLR7 and TLR9 stimulation [20–22]. Whether current treatments for allergic disorders, including immunotherapy, are able to modulate these abnormalities in DC responses is not well understood. Interestingly, subcutaneous immunotherapy (SCIT) was recently shown to restore TLR9-mediated IFN-α responses in DCs from ragweed allergic adults, suggesting IT may have the ability to modulate innate immune function and thereby promote tolerance in allergic individuals [23].

Sublingual (SLIT) and oral (OIT) immunotherapy are promising new treatments for food allergy. In SLIT, an allergen solution is first held under the tongue for a period of time and then swallowed, whereas in OIT the allergen is delivered in a food vehicle and swallowed immediately. The oral mucosa is relatively devoid of inflammatory cells but rich in tolerogenic APCs (specifically mDCs), suggesting SLIT may have the advantage of eliciting fewer adverse reactions while favoring tolerogenic responses [24]. On the other hand, higher doses of allergen can be delivered with OIT, which may be important for inducing tolerance. We recently reported that OIT was more efficacious for desensitization to cow's milk (CM) than SLIT alone but was accompanied by more systemic side effects [6]. In this study, we sought to examine how SLIT and OIT affect DC innate and adaptive immune functions, given the pivotal role these cells play in tolerance and Th2 differentiation.

## 2. Materials and methods

### 2.1. Study design and subject recruitment

The study was an open-label randomized trial of SLIT and OIT for the treatment of IgE-mediated CM allergy. Subjects were recruited from the Johns Hopkins University pediatric allergy clinic. Details of the study design, enrollment criteria, and clinical outcomes can be found in Ref. [6]. Baseline characteristics of subjects are summarized in Supplementary Table 1. Briefly, 24 subjects 6–17 years of age with documented IgE-mediated CM allergy underwent a double-blind, placebo-controlled food challenge (DBPCFC) to CM followed by initial SLIT escalation (minimum of four weeks) with an aqueous CM extract. Subjects were then randomized to continue SLIT escalation (n = 8) to a maximum dose of 7 mg of CM protein daily, or to begin OIT with a goal maintenance dose of either 1000 mg (n = 8) or 2000 mg (n = 8) of CM protein. These two OIT groups were combined for our analysis to increase power as their clinical courses were similar, and these subjects are herein referred to as "OIT". Key study time points were as follows: T1: baseline screening DBPCFC; T2: end of initial SLIT escalation and randomization to continue SLIT or switch to OIT; T3: end of dose escalation (median 10 weeks (range: 8–14) for SLIT and median 28 weeks (range: 20–41) for OIT); and T4: completion of at least 12 weeks of maintenance dosing. At T4, participants underwent a DBPCFC, and subjects in the OIT arm increased their daily maintenance dose to one half the tolerated dose (if greater than the target dose). All subjects then continued daily dosing, and underwent an open oral food challenge (OFC) to 8 g of CM protein at T5 (minimum 60 weeks on maintenance). If the subject passed this challenge without symptoms requiring medication, the subject was considered "desensitized" to CM and therapy was discontinued. A repeat OFC was done one week later at visit T6. If the subject passed this challenge, another OFC was done 5 weeks later at visit T7 (total of six weeks off therapy). All of the subjects in the study demonstrated an increase in their food challenge threshold, with a median fold increase of 7 for the SLIT group and 108 for the OIT group by visit T5. None of the SLIT subjects but 10/16 OIT subjects passed the desensitization challenge at T5. Only 5 of these 10 subjects passed the challenge at T7 after being off therapy for 6 weeks, and were therefore considered clinically tolerant. Two subjects, both in the OIT group, withdrew from the study during the course of therapy due to adverse events. This study was approved by the Johns Hopkins Institutional Review Board (study NA_00014511).

### 2.2. Cell preparation and cultures

Peripheral blood was collected in EDTA and subjected to double Percoll (Pharmacia Biotech, Inc., Piscataway, NJ) density (1.0751/1.081 g/mL) centrifugation. The upper fraction of cells in the double Percoll gradient consisted of basophil-depleted mononuclear cells that were used to isolate pDCs using $BDCA4^+$ magnetic bead selection (Miltenyi, Auburn, CA). Cells not retained on this column were then used to isolate mDCs with $BDCA1^+$ selection (Miltenyi) after depletion of $CD19^+$