



Contents lists available at ScienceDirect



Basic science for the clinician: T-cell biology in immunotherapy

John W. Steinke, PhD; and Monica G. Lawrence, MD

Asthma and Allergic Disease Center, Carter Immunology Center, University of Virginia Health Systems, Charlottesville, Virginia

ARTICLE INFO

Article history:

Received for publication November 1, 2013.

Received in revised form December 18, 2013.

Accepted for publication December 23, 2013.

ABSTRACT

Objective: This review discusses the current state of immunotherapy and how the CD4 T-cell response is pivotal in altering the allergic response.

Data Sources: PubMed literature review.

Study Selections: Articles pertaining to subcutaneous, sublingual, and oral immunotherapies, with specific emphasis on those describing the T-cell response.

Results: Although many drugs are available that help ameliorate allergic symptoms, the only intervention that has proved to provide long-term benefit and modulation of disease is immunotherapy. Many routes of immunotherapy are being pursued, including subcutaneous, sublingual, and oral immunotherapies; however, subcutaneous immunotherapy has the historical record of leading to immune changes that alter the immune response at subsequent allergen exposure. These changes are mediated by the induction of peripherally derived T-regulatory cells and appear to occur only after high-dose therapy for 3 to 5 years. Newer methods of sublingual and oral immunotherapies are currently being investigated, but their efficacy is not yet on par with subcutaneous immunotherapy.

Conclusion: The primary cells ultimately responsible for successful immunomodulation are CD4 T cells, specifically peripherally derived T-regulatory cells.

© 2014 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

The prevalence of allergy to venom, aeroallergens, or food continues to increase and results in considerable morbidity for the patient and increasing health care costs. Although many drugs are available that help ameliorate symptoms, the only intervention that has proved to provide long-term benefit and modulation of disease is immunotherapy (IT). The mainstay of IT treatment has been subcutaneous IT (SCIT), in which efficacy can be achieved after 3 to 5 years of high-dose treatment. Newer methods that target the mucosal immune system include sublingual IT (SLIT) and oral IT (OIT). With the recent approval of SLIT tablets by the Food and Drug Administration and other new approaches being pursued, it is important to understand the mechanistic action of each of these forms of IT. IT involves a decrease in mast cell and eosinophil numbers, a decrease in basophil activation, an increase in IgG4, and a shift in the cytokine milieu. However, the primary cells that are ultimately responsible for successful immunomodulation are CD4 T cells, specifically peripherally derived T-regulatory cells (Tregs). This review covers the current knowledge of various IT methods and how the T-cell response is altered to provide lasting benefit.

Methods

Primary literature on this topic was selected based on a PubMed search through October 2013 for articles in English using the key terms *subcutaneous immunotherapy (SCIT)*, *sublingual immunotherapy (SLIT)*, *oral immunotherapy (OIT)*, and *mechanism or T cell*. Studies on adults and children with IgE-mediated allergic rhinitis or food allergy were included, with specific emphasis on those describing the T-cell response. Preclinical trials using mouse models also were included when published clinical trials were not available.

Overview of Regulatory T-Lymphocyte Families

Numerous families of T cells have been identified; however it is the regulatory family that has been implicated in the successful response to IT and therefore is the main focus of this review. These include the thymic-derived and peripherally derived Tregs (tTregs and pTregs, respectively).^{1,2} The tTregs (also referred to in the literature as natural Tregs or nTregs) are characterized by their constitutive expression of the interleukin (IL)-2 receptor α chain (CD25) and the transcription factor forkhead box P3 (Foxp3). Although they can secrete IL-10, membrane transforming growth factor- β (TGF- β) appears to be primarily responsible for mediating their contact-dependent immune suppression. The tTregs are produced in the thymus in response to the expression of self-antigens and therefore are important in the prevention of autoimmunity. They are unlikely to be involved in tolerance to antigens

Reprints: John W. Steinke, PhD, Asthma and Allergic Disease Center, Carter Immunology Center, University of Virginia Health Systems, Charlottesville, VA 22908-1355; E-mail: js3ch@virginia.edu.

Disclosures: Dr Steinke received funding from UVA.

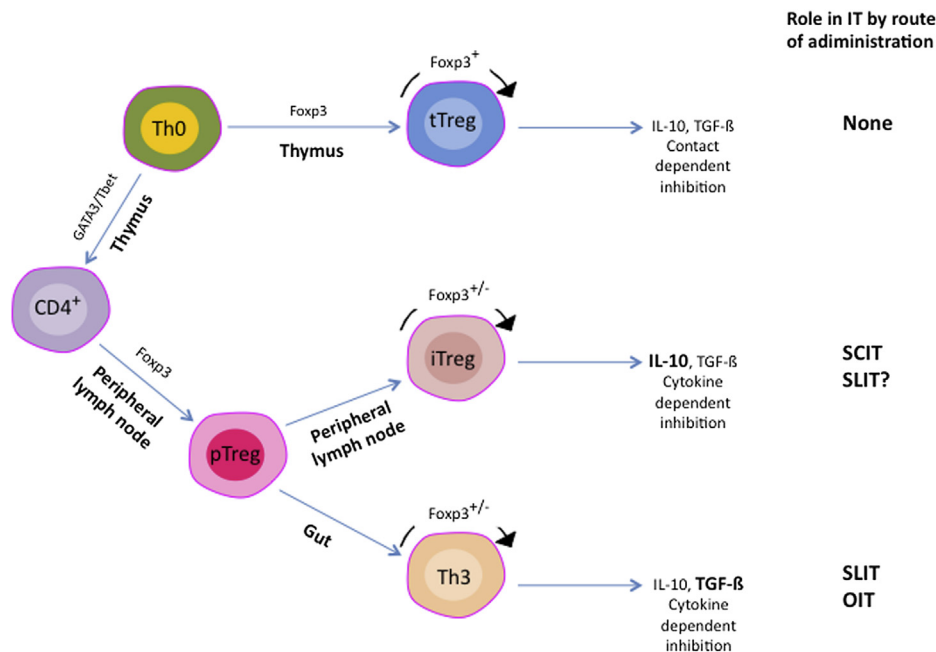


Figure 1. T-regulatory cell (Treg) populations and their involvement in immunotherapy (IT). Naive cells can develop into Tregs cells by 2 pathways. Those that develop in the thymus (tTregs) require and express forkhead box P3 (Foxp3) during development and when mature. The tTregs can secrete inhibitory cytokines but require cell-to-cell contact for inhibition. Because they are generated to inhibit responses to self-antigens, they are likely to have little role in IT. Peripheral Tregs (iTregs) develop outside of thymus and can express Foxp3, although expression is not absolute, and use secreted cytokines to mediate suppression (main inhibitory cytokine in *boldface*). The 2 main subpopulations of pTregs are shown and their role in IT is indicated. GATA3, GATA binding protein 3; IL-10, interleukin-10; OIT, oral immunotherapy; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; Tbet, T-box expressed in T cells; TGF- β , transforming growth factor- β ; Th0, T-helper type 0 cell.

not present in the thymus (eg, in tolerance to allergens in healthy subjects or in the immune benefits associated with allergen IT).

In contrast to the tTreg cells, an additional, less well-characterized class of inducible Tregs has been described that can develop in the periphery (pTregs; also referred to as inducible Tregs or iTregs).^{2,3} These pTreg cells are differentiated from pre-existing T-effector lymphocytes or from circulating naive T-helper type (T_H) 0 cells and are characterized by their prominent production of IL-10. The pTreg expression of Foxp3 and CD25 is controversial but does appear to occur. For example, it is unclear whether CD25 expression reflects the constitutive expression of this component of the IL-2 receptor, the signature characteristic of tTregs, or the derivation of pTregs from activated effector T cells that are transiently expressing CD25. The difficulty in using CD25 as a surface marker or Foxp3 as an intracellular marker of pTreg is that high levels of CD25 (IL-2 receptor- α) and Foxp3 are transiently expressed by CD4⁺ and CD8⁺ effector T cells after T-cell receptor activation and in response to IL-2 in a positive feedback loop.⁴ A subgroup of pTregs that are derived primarily from the gut and generate mucosal tolerance have been referred to as the T_H3 cells. Reflecting their prominent production of TGF- β , in addition to mediating tolerance, they are relevant to inducing secretory IgA production. It is the induction of IL-10-producing pTregs that plays a key role in decreasing allergen-specific T-cell responsiveness after IT^{5,6} (Fig 1).

Immunology of SCIT

Subcutaneous IT has been performed for more than 100 years, yet only in the past few years has the mechanism of efficacy been begun to be understood. A prominent role for IL-10-producing activated CD4⁺ cells was first described in studies involving bee venom SCIT.⁵ The use of whole bee venom extract or short peptides to phospholipase A₂, the major bee venom allergen, resulted in the induction of peripheral tolerance. Examination of the T-cell response demonstrated that T_H2 (IL-4, IL-5, and IL-13) and T_H1

(interferon- γ [IFN- γ]) cytokine production was decreased, whereas IL-10 levels were increased. These IL-10-producing T cells could suppress allergen-specific T-cell proliferation and activation and are currently recognized as pTregs.⁷ Although the appearance of the IL-10-producing pTregs is rapid, within 7 days of starting bee venom SCIT, full tolerance, if it is going to occur, requires 3 to 5 years of treatment.

Subsequent investigations with house dust mite (HDM) and birch pollen SCIT have extended the importance of IL-10 (and TGF- β) production by CD4⁺CD25⁺ T cells (pTregs) to inhalant allergy and confirmed that this occurs in parallel to the suppression of T_H2 and T_H1 proliferative responses and cytokine production.⁸ In that study, IL-10 responses in nonatopic subjects who had been exposed to allergen were similar to those in the SCIT-treated group, implying the restoration of tolerant T-cell responses in the atopic subject and that the normal immune response is geared toward nonresponsiveness or suppression rather than a shift toward a T_H1 response. Other studies also have shown IL-10 production by CD4⁺ cells, but without changes in grass pollen-induced proliferation or T_H2 cytokine production.⁶ Reasons for the differences in the studies could include the dose of the allergen used and differences related to the allergen itself and the ability to induce an immune response. However, what is consistent is that each of these studies has found cells capable of making high levels of IL-10 (with and without TGF- β) consistent with the pTreg type. A direct role for IL-10 in inducing the IgG4 antibody has been demonstrated, and, as such, the appearance of allergen-specific IgG4 in association with IT is consistent with it being a marker for pTreg induction.^{9,10} The protective effect of pTregs is evident from studies demonstrating that after 4 years of high-dose SCIT, subjects remained nonresponsive for at least 3 years after SCIT had been stopped, similar to those who had continued SCIT for the full 7-year period.¹¹

To examine the T-cell responses to SCIT in finer detail, MHC class II tetramers and epitope-guided mapping are being used. Once the HLA type to be studied is known, a synthetic MHC molecule of that

HLA type is designed and labeled with biotin. The biotin labeling allows crosslinking to streptavidin to which 4 of these synthetic MHC class II molecules will bind. Then, the MHC class II tetramers are added to pools of peptides derived from the allergen of interest and peptides that bind to the tetramers are identified using flow cytometry. This is repeated through several rounds of selection until a single peptide is isolated that recognizes that MHC class II molecule.¹² CD4⁺ T cells that bind to the peptide and MHC class II tetramer can subsequently be identified as allergen-specific T cells. The limitation of this approach is that only 1 epitope of the allergen is being examined, so the results obtained are likely an underestimation of the actual response.

Using these methods, the frequency of Fel d 1–specific T cells in patients with allergy has been shown to range from 1 in 7,000 to 1 in 30,000.¹³ Similar frequencies have been found after examining other allergen-specific T cells. Surprisingly, allergen-specific T cells also have been identified in subjects without allergy, although the numbers are smaller and the avidity of the T-cell receptor is decreased.¹⁴ When alder-specific T cells were examined, the phenotype of T cells from patients with allergy was characterized by the high expression of the cell surface markers CRT_{H2} (the prostaglandin D2 receptor that is a marker for T_{H2} cells) and CCR4 with low CXCR3 (chemokine receptors). In contrast, T cells from nonatopic subjects had high CXCR3, moderate CCR4, and low CRT_{H2} expressions.¹⁵ When the allergen-specific memory T-cell population was subjected to phenotyping, 2 populations could be separated based on the expression of CD27, a member of the tumor necrosis factor receptor superfamily that is expressed by naive and central memory T cells, with loss of expression on effector memory and terminally differentiated, exhausted T cells. The CD27[−] population had characteristics of classic T_{H2} cells in that they had high levels of CRT_{H2} and CCR4 on the cell surface and high intracellular levels of IL-4, IL-5, and IL-13. The CD27⁺ population had high levels of CXCR3, CCR7, and CD7 on the cell surface with high IFN- γ levels in the intracellular milieu, characteristics of T_{H1} cells.¹⁵ It was the ratio of CD27[−] to CD27⁺ cells that distinguished allergic from nonatopic subjects. These techniques were used to examine what happens to these T-cell populations after IT. Successful SCIT was associated with a loss of the CD27[−] population owing to apoptosis, which allowed the IL-10–secreting pTregs to develop. Previous studies have shown that STAT6 signaling in T_{H2} cells renders them resistant to Treg suppression,¹⁶ so the selective loss of the CD27[−] population (T_{H2}-like) allows restoration of Treg function.

Mechanisms of SLIT and Efficacy

Sublingual IT is administered by holding escalating doses of antigen drops or tablets under the tongue for a period of time. This allows for uptake of the antigen by oral antigen-presenting cells such as Langerhans cells.¹⁷ This local presentation of antigen is thought to result in local mucosal induction of IL-10– and TGF- β –producing T_{H3} cells.¹⁸

In one of the first mechanistic studies of SLIT in humans, peripheral blood mononuclear cells from patients treated with HDM SLIT for 3 years were shown to produce significantly more IL-10 after phytohemagglutinin stimulation than patients with untreated rhinitis.¹⁹ This provided indirect evidence for the immunoregulatory role of IL-10 in SLIT. Several years later, a small mechanistic study examining 9 patients undergoing birch SLIT suggested an early- and a late-phase response to therapy.²⁰ By 4 weeks of therapy, there was an increase in circulating pTregs accompanied by an increase in IL-10 and Foxp3 mRNA expression levels and nonspecific suppression of antigen-driven lymphocyte proliferation. After 52 weeks of therapy, the suppression of proliferation was specific to Bet v 1 (birch pollen) and the levels of circulating pTregs and IL-10 returned to baseline. However, there

was a persistent decrease of IL-4 mRNA and an increase in IFN- γ mRNA. This suggests that early in therapy IL-10–producing pTregs act nonspecifically to suppress lymphocyte proliferation in response to antigen, but over time this nonspecific response is replaced by an antigen-specific tolerance against the backdrop of a T_{H1}-skewed cytokine milieu.²¹ This may have important implications in determining the optimal duration of therapy.

Consistent with the transient induction of IL-10⁺ pTregs seen in that study, a randomized, double-blinded, placebo-controlled trial of 12 to 24 months of HDM SLIT showed a transient increase in the proportion of circulating Tregs, which were confirmed to have the ability to suppress HDM-specific effector T-cell proliferation and cytokine production. The study also showed significantly decreased HDM-induced CD4⁺ T-cell proliferation after 6, 12, and 24 months. There was a decrease in IL-5 levels accompanied by an increase in IL-10 and IFN- γ , but no change in IL-4, and no measurement of IL-9 or IL-13 cytokine levels.²²

Several other studies have confirmed the increase in pTregs after SLIT. In a study of patients treated with grass pollen SLIT for 12 to 18 months, the number of Foxp3⁺ cells in the sublingual epithelium was significantly increased in the treatment group compared with the placebo and nonatopic control groups.²³ However, the number of IL-10⁺ and TGF- β ⁺ mRNA cells did not change after SLIT, raising the possibility that these Foxp3⁺ cells were recently activated effector cells rather than true Tregs. Perhaps addressing these technical concerns, another later study of grass pollen SLIT demonstrated an increased production of IL-10 and TGF- β by oral Langerhans cells and an increased number of IL-10– and TGF- β –producing Tregs after therapy.¹⁸

Using the tetramer approach described earlier and a more robust double-blinded, placebo-controlled experimental model, Bonvalet et al²⁴ were unable to detect a significant decrease in grass allergen (Phl p 1 and Phl p 5)-specific CD4⁺ T cells during or after 4 months of SLIT or a change in surface phenotype markers. This was despite a significant 41.8% improvement in symptom score after grass pollen challenge in an allergen-exposure challenge. This raises the possibility that although clinical improvement may be seen as soon as 1 month, parallel immunologic changes involving the shift from a T_{H2} to a T_{H1} signature and upregulation of Tregs require longer treatment courses (up to 12 months).

Although it has been used in only a limited number of published trials on food allergy, the same increase in pTregs and cytokine shift away from T_{H2} has been shown in food SLIT. A study of patients receiving 12 months of peanut SLIT found significantly lower levels of serum IL-5 after SLIT than those in the placebo group. No significant difference between groups was found in IL-10 or IFN- γ levels after treatment. There was a trend toward an increased percentage of Tregs compared with placebo.²⁵

Mechanisms of OIT and Efficacy

Oral IT differs from SLIT in that the antigen is immediately swallowed and delivered to the gut-associated lymphoid tissues. Oral tolerance, or the development of tolerance within the gut-associated lymphoid tissues, has been recognized to occur in response to the repetitive oral ingestion of antigens since the early 1900s. Oral tolerance is thought to be mediated in large part by pTregs, in particular the T_{H3} subset, that produce primarily TGF- β (with and without IL-10) and promote the production of secretory IgA. This is supported by work that showed that tTregs are not required for the induction of oral tolerance,²⁶ and that deletion of pTregs in the DREG mouse model resulted in loss of oral tolerance.²⁷

Most published trials of OIT to date have focused on clinical end points, such as the ability to pass a blinded food challenge, skin prick wheal size, or serum level of allergen-specific IgE or IgG4.

Direct analysis of the cellular immune changes that occur with OIT in humans has been relatively limited to date and focused primarily on basophil activation, peripheral blood mononuclear cell cytokine production, and changes in the frequency of pTregs.²⁸

In 2 studies, peanut OIT was associated with a decrease in T_H2 cytokine (IL-4, IL-5, and/or IL-13) production from stimulated peripheral blood mononuclear cells.^{29,30} In contrast, other studies have shown increases in IL-5 production, and cytokine profile did not correlate with clinical outcome in most trials, suggesting considerable heterogeneity in the response.^{30,31}

Patients with milk allergy who can tolerate extensively heated milk have larger percentages of milk-specific pTregs than those who cannot tolerate heated milk or those with outgrown milk allergy,³² suggesting that the development of oral tolerance may involve expansion of the pTreg compartment just as it does in SLIT. Consistent with this finding, patients with peanut allergy who had undergone peanut OIT had an increased frequency of pTregs compared with controls.^{29,31} A study of 20 patients allergic to eggs and treated with egg OIT showed an increase in TGF- β ⁺ dendritic cells after OIT, which could induce the conversion of T effector cells to pTregs.³³ The combination of decreased numbers of food allergen-specific T-effector cells and increased allergen-specific pTregs is a possible mechanism for the development of tolerance in these patients.

Several recent studies have suggested intriguing possibilities for the future. The use of tetramers to identify peanut-reactive T cells has shown a significantly higher frequency of Ara h 1–specific T cells in patients with peanut allergy (9 cells per million) compared with healthy controls (1 cell per million).³⁴ An obvious application of this technique would be to monitor the frequency of these allergen-specific T cells during the course of OIT.

Two published preclinical trials used oral peanut plus a toll-like receptor-9 ligand agonist in peanut-sensitized mice and found increased T_H1 and decreased T_H2 cytokine production accompanied by decreased IgE levels and anaphylaxis.^{35,36} This T_H1 skewing and clinical benefit was even more pronounced than in those mice receiving oral peanut alone, suggesting that the use of adjuvants such as toll-like receptor agonists may be a powerful tool in increasing the efficacy of OIT.

Comparison between IT Regimens

There is evidence supporting the use of SCIT and SLIT for the treatment of rhinitis and asthma, but very few published trials have made a direct head-to-head comparison of the 2 IT routes. A recent meta-analysis performed by Dretzke et al³⁷ identified only 1 such well-controlled trial; this trial did not provide conclusive results in favor of SCIT or SLIT based on symptom score or medication use. This meta-analysis and another using indirect comparison of trials that used SCIT or SLIT showed a trend toward greater efficacy in the SCIT group.^{37,38} Caution must be made in interpreting this result because most included studies were heterogeneous in dose and duration of therapy, rather than direct head-to-head trials, which would provide more conclusive evidence favoring one route over another.

Because OIT and SLIT have shown clinical efficacy in the treatment of food allergy and have similar profiles in adverse events and ease of administration, one question that arises is which route of delivery is most clinically efficacious and whether this is supported by superior immunologic outcome data. A recently published head-to-head analysis attempted to address this by comparing 2 previously published double-blinded, placebo-controlled trials of 12 months of peanut OIT vs SLIT.³⁹ In addition to being 3 times more likely to pass a food challenge after treatment, patients in the OIT group had more significant decreases in the ratio of peanut-specific IgE to IgG4 and basophil activation. Interestingly, however, there

was no significant difference in the percentage of Tregs between the OIT and SLIT groups or between those who passed and those who failed the food challenge.

Future for Clinicians

Many routes of IT are being pursued. However, SCIT has the historical record of leading to immune changes that alter the immune response at subsequent allergen exposure. These changes are mediated by the induction of pTregs and appear to occur only after high-dose therapy for 3 to 5 years. Newer methods of SLIT and OIT are currently being investigated, but their efficacy compared with that of the more traditional method of SCIT is not known.^{38,40} Their mechanism of action is believed to be similar to that of SCIT, by the induction of pTregs, although this remains an active area of investigation. Obvious questions include the nature of the regulatory response in SLIT and OIT compared with SCIT and the optimal dose and duration of treatment required to achieve lasting immunomodulation. Once these issues are truly understood, allergists will have additional tools at their disposal to alter the morbidity associated with the increasing incidence of allergic disease.

References

- [1] Sakaguchi S. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell*. 2000;101:455–458.
- [2] Abbas AK, Benoist C, Bluestone JA, et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol*. 2013;14:307–308.
- [3] Akdis CA, Blaser K. IL-10–induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J*. 1999;13:603–609.
- [4] Kmiecik M, Gowda M, Graham L, et al. Human T cells express CD25 and Foxp3 upon activation and exhibit effector/memory phenotypes without any regulatory suppressor function. *J Trans Med*. 2009;7:89.
- [5] Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest*. 1998;102:98–106.
- [6] Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol*. 2003;111:1255–1261.
- [7] Muller U, Akdis CA, Fricker M, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol*. 1998;101:747–754.
- [8] Jutel M, Akdis M, Budak F, et al. IL-10 and TGF- β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol*. 2003;33:1205–1214.
- [9] Fellrath J-M, Kettner A, Dufour N, et al. Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J Allergy Clin Immunol*. 2003;111:854–861.
- [10] Satoguina JS, Weyand E, Larbi J, Hoerauf A. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol*. 2005;174:4718–4726.
- [11] Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med*. 1999;341:468–475.
- [12] Nepom GT. MHC class II tetramers. *J Immunol*. 2012;188:2477–2482.
- [13] Kwok WW, Roti M, Delong JH, et al. Direct ex vivo analysis of allergen-specific CD4+ T cells. *J Allergy Clin Immunol*. 2010;125:1407–1409.e1.
- [14] Kinnunen T, Nieminen A, Kwok WW, et al. Allergen-specific naive and memory CD4+ T cells exhibit functional and phenotypic differences between individuals with or without allergy. *Eur J Immunol*. 2010;40:2460–2469.
- [15] Wambre E, DeLong JH, James EA, LaFond RE, Robinson D, Kwok WW. Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during specific immunotherapy. *J Allergy Clin Immunol*. 2012;129:544–551.e1–7.
- [16] Pillemer BB, Qi Z, Melgert B, Oriss TB, Ray P, Ray A. STAT6 activation confers upon T helper cells resistance to suppression by regulatory T cells. *J Immunol*. 2009;183:155–163.
- [17] Smarr CB, Bryce PJ, Miller SD. Antigen-specific tolerance in immunotherapy of th2-associated allergic diseases. *Crit Rev Immunol*. 2013;33:389–414.
- [18] Allam JP, Wurtzen PA, Reinartz M, et al. Phl p 5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF- β 1 and IL-10–producing properties. *J Allergy Clin Immunol*. 2010;126:638–645.e1.
- [19] Ciprandi G, Fenoglio D, Cirillo I, et al. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol*. 2005;95:38–44.
- [20] Bohle B, Kinaciyan T, Gerstmayr M, Radakovic A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10–producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol*. 2007;120:707–713.

- [21] Frew AJ. How does sublingual immunotherapy work? *J Allergy Clin Immunol*. 2007;120:533–536.
- [22] O’Hehir RE, Gardner LM, de Leon MP, et al. House dust mite sublingual immunotherapy: the role for transforming growth factor-beta and functional regulatory T cells. *Am J Respir Crit Care Med*. 2009;180:936–947.
- [23] Scadding GW, Shamji MH, Jacobson MR, et al. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy*. 2010;40:598–606.
- [24] Bonvalet M, Moussu H, Wambre E, et al. Allergen-specific CD4+ T cell responses in peripheral blood do not predict the early onset of clinical efficacy during grass pollen sublingual immunotherapy. *Clin Exp Allergy*. 2012;42:1745–1755.
- [25] Kim EH, Bird JA, Kulis M, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol*. 2011;127:640–646.e1.
- [26] Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest*. 2005;115:1923–1933.
- [27] Hadis U, Wahl B, Schulz O, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity*. 2011;34:237–246.
- [28] Tang ML, Martino DJ. Oral immunotherapy and tolerance induction in childhood. *Pediatr Allergy Immunol*. 2013;24:512–520.
- [29] Varshney P, Jones SM, Scurlock AM, et al. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. *J Allergy Clin Immunol*. 2011;127:654–660.
- [30] Blumchen K, Ulbricht H, Staden U, et al. Oral peanut immunotherapy in children with peanut anaphylaxis. *J Allergy Clin Immunol*. 2010;126:83–91.e1.
- [31] Jones SM, Pons L, Roberts JL, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol*. 2009;124:292–300.e1–97.
- [32] Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. *J Allergy Clin Immunol*. 2009;123:43–52.e7.
- [33] Wu Z. Antigen specific immunotherapy generates CD27(+) CD35(+) tolerogenic dendritic cells. *Cell Immunol*. 2013;283:75–80.
- [34] DeLong JH, Simpson KH, Wambre E, James EA, Robinson D, Kwok WW. Ara h 1-reactive T cells in individuals with peanut allergy. *J Allergy Clin Immunol*. 2011;127:1211–1218.e3.
- [35] Zhu FG, Kandimalla ER, Yu D, Agrawal S. Oral administration of a synthetic agonist of Toll-like receptor 9 potentially modulates peanut-induced allergy in mice. *J Allergy Clin Immunol*. 2007;120:631–637.
- [36] Bashir ME, Louie S, Shi HN, Nagler-Anderson C. Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. *J Immunol*. 2004;172:6978–6987.
- [37] Dretzke J, Meadows A, Novielli N, Huissoon A, Fry-Smith A, Meads C. Subcutaneous and sublingual immunotherapy for seasonal allergic rhinitis: a systematic review and indirect comparison. *J Allergy Clin Immunol*. 2013;131:1361–1366.
- [38] Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. *J Allergy Clin Immunol*. 2012;130:1097–1107.e2.
- [39] Chin SJ, Vickery BP, Kulis MD, et al. Sublingual versus oral immunotherapy for peanut-allergic children: a retrospective comparison. *J Allergy Clin Immunol*. 2013;132:476–478.e2.
- [40] Khinchi MS, Poulsen LK, Carat F, Andre C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy*. 2004;59:45–53.