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Basic Science for the Practicing Clinician

Basic science for the clinician: Mechanisms of sublingual and subcutaneous immunotherapy

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ABSTRACT

Objective: To discuss the general immunologic changes that occur during immunotherapy, focusing on the differences between subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT).
Data Sources: PubMed literature review.
Study Selections: Articles pertaining to SCIT and SLIT, with specific emphasis on those that included immune mechanistic studies.
Results: Both SCIT and SLIT are characterized by the induction of regulatory B and T cells, decreased allergen-specific T-cell proliferation, a shift from a T_H2 to T_H1 cytokine milieu and from an IgE to an IgG4/IgA antibody response. These changes are accompanied by clinical improvement in symptoms.
Conclusion: Immunotherapy using allergen extracts administered via both subcutaneous and sublingual approaches have demonstrated efficacy in the treatment of allergic rhinoconjunctivitis and other allergic conditions. There are subtle differences between the approaches, and understanding these differences may help clinicians select a preferred route of therapy for particular patients or allergens, depending on the immune response that is being targeted.

Introduction

Subcutaneous immunotherapy (SCIT) has been in use since 1911 for the treatment of respiratory and venom allergies, and current practice involves administering escalating doses of antigen via 1 or more subcutaneous injections weekly until a maintenance dose is achieved. Maintenance SCIT is generally continued every 4 to 8 weeks for a period of 3 to 5 years, and multiple studies have found that it provides sustained benefit in reducing clinical symptoms to allergens and modulates the immune response to allergens.[1] Recently, newer routes of immunotherapy involving the mucosal immune system have emerged and gained popularity, including sublingual immunotherapy (SLIT) and oral immunotherapy (OIT).[2] SLIT is administered daily by holding antigen drops or tablets under the tongue, whereas OIT involves swallowing escalating daily doses of antigen. Characterizing the mechanism of action of immunotherapy has been the focus of intense investigation during the past several decades[1] and is important for practicing clinicians to understand as they consider the different routes of immunotherapy available to administer in clinical practice. This study discusses the general immunologic changes that occur during immunotherapy, focusing on the differences between SCIT and SLIT. We performed a PubMed literature review and selected articles pertaining to SCIT and SLIT, with specific emphasis on those that included immune mechanistic studies.

Changes in the Allergen-Specific T-Cell Response in SCIT and SLIT

Mechanisms of T-Cell modulation After Immunotherapy

The conventional theory regarding successful immunotherapy, be it SCIT or SLIT, has invoked the induction of regulatory T cells (Tregs), the nomenclature of which we will review briefly here.[3,4] Several types of Tregs have been described, including thymic-derived and peripherally derived Tregs.[5,6] Thymus-derived Tregs (tTregs; also referred to as natural Tregs) are characterized as $CD4^+CD25^+$ T cells that constitutively express the transcription factor Foxp3 and have a unique, hypomethylated CpG rich Foxp3 locus.[7–9] tTregs are produced in the thymus in response to expression of self-antigens and are therefore important in the prevention of autoimmunity. Although they constitute 5% to 10% of peripheral $CD4^+$ T cells, they are unlikely to be involved in tolerance

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to antigens not presented in the thymus, such as allergens. An additional Treg subset has been described that develops in the periphery (pTreg; also referred to as inducible Tregs).[6,10] These pTregs can differentiate from preexisting T-effector lymphocytes or circulating naive T cells and may or may not express Foxp3 and CD25 while possessing suppressive activity. These cells are difficult to identify and study based on using CD25 as a surface marker or Foxp3 as an intracellular marker because both of these markers can be transiently expressed by $CD4^+$ and $CD8^+$ effector T cells after T-cell receptor activation and in response to interleukin (IL) 2 via a positive feedback loop.[11] A subgroup of pTregs that is primarily gut derived and generates mucosal tolerance has been referred to as T_H3 cells. Reflecting their prominent production of transforming growth factor β (TGF-β), in addition to mediating tolerance, T_H3 cells are relevant to inducing secretory IgA production and oral tolerance. Suppression by Tregs occurs through contact inhibition; secretion of IL-10, TGF-β, and IL-35 that inhibit cellular activity; and surface interactions by negative costimulatory molecules, such as cytotoxic T-lymphocyte–associated antigen 4, or by competition for IL-2.[12,13]

An alternative mechanism for successful immunotherapy has been proposed that involves a shift in allergen-specific T cells from a T_H2 to a T_H1 phenotype.[14] The recent development of major histocompatibility class class II tetramers and epitope-guided mapping has allowed researchers to monitor the allergen-specific T-cell responses to immunotherapy. The frequency of Fel d 1–specific T cells in allergic individuals before immunotherapy ranges from 1 in 7,000 to 1 in 30,000, with similar frequencies found for other allergen-specific T cells.[15] Allergen-specific T cells have also been identified in nonallergic individuals, albeit with lower numbers and decreased avidity of the T-cell receptor.[16]

T-Cell Changes During SCIT

A prominent role for IL-10–producing pTregs was first described in studies that involved bee venom SCIT.[3] Induction of peripheral tolerance occurred with the use of either whole bee venom extract or short peptides to phospholipase A_2, the major bee venom allergen. The peripheral T-cell response demonstrated that both T_H2 (IL-4, IL-5, and IL-13) and T_H1 (interferon γ [IFN-γ] cytokine) production decreased, whereas IL-10 levels increased. These IL-10–producing T cells suppressed allergen-specific T-cell proliferation and activation and are now recognized as pTregs.[17]

Use of inhalant allergens in SCIT, including house dust mite and birch pollen, have confirmed the importance of IL-10 (and TGF-β) production by $CD4^+CD25^+$ T cells (pTregs) in the suppression of T_H2 and T_H1 proliferative responses and cytokine production.[18] Several studies have also found new or increased IL-10 production by $CD4^+$ cells in the absence of changes in allergen-induced proliferation but with decreases in T_H2 cytokine production, including IL-5 within 10 months of treatment.[4,19–21] Reasons for differences in T-cell responses could include dose of allergen used, different adjuvants added to the allergen, and differences related to the allergen itself and the ability to induce an immune response.

What is consistent is that each of these studies has found cells capable of making high levels of IL-10 (with or without TGF-β) consistent with the pTreg type. Although the appearance of the IL-10–producing pTregs in bee venom and inhalant allergen SCIT is rapid, within 7 days, full tolerance requires 3 to 5 years of sustained high-dose treatment. The tolerogenic effect of pTregs is evident from studies that found that after 4 years of high-dose SCIT, individuals remain nonresponsive for at least 3 years after SCIT has been stopped, similar to those who had continued SCIT for the full 7-year period.[22]

Use of the tetramer-guided approach described earlier to monitor the frequency and characteristics of alder pollen–specific $CD4^+$ T cells during immunotherapy led to the observation that 2 populations of allergen-specific memory T cells could be separated based on the expression of CD27. A terminally differentiated $CD27^-$ population has characteristics of classic T_H2 cells with high levels of CRT_H2 and CCR4 on the cell surface and high intracellular levels of IL-4, IL-5, and IL-13. In contrast, the $CD27^+$ population had high CXCR3, CCR7, and CD7 on the cell surface with high intracellular IFN-γ levels, characteristics of T_H1 cells, and suppressive IL-10.[23] It was the ratio of $CD27^-$ (T_H2) to $CD27^+$ (T_H1) cells that distinguished allergic from nonatopic individuals and suggested that this could be a biomarker for successful immunotherapy. Successful SCIT was associated with preferential loss of the pathogenic $CD27^-$ (T_H2) population, whereas the surviving IL-10 secreting $CD27^+$ T cells maintained a suppressed state and limited the development of new allergen-specific T_H2 cells.[24]

T-Cell Changes During SLIT

In an early mechanistic study of SLIT in humans, peripheral blood mononuclear cells from patients treated with house dust mite SLIT for 3 years produced more IL-10 after mitogen stimulation when compared with patients with untreated rhinitis.[25] Similar to SCIT, this provided indirect evidence of the immunoregulatory role of IL-10 in SLIT. A smaller mechanistic study that examined 9 patients undergoing birch SLIT suggested an early- and late-phase response to therapy.[26] By 4 weeks of therapy, there was an increase in circulating pTregs accompanied by an increase in IL-10 and Foxp3 messenger RNA (mRNA) expression levels and nonspecific suppression of antigen-driven lymphocyte proliferation. At the end of 52 weeks of therapy, suppression of proliferation was specific to Bet v 1 (birch pollen), and the levels of circulating pTregs and IL-10 returned to baseline. Persistent reduction of IL-4 mRNA and an increase in IFN-γ mRNA was maintained. This study 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 antigen-specific tolerance on the backdrop of a T_H1 skewed cytokine milieu.[27] Other studies have confirmed increased pTreg numbers, decreased allergen-specific T-cell proliferation, and a shift from a T_H2 to T_H1 cytokine milieu after SLIT, accompanied by clinical improvement in symptoms.[28,29]

Unique local immunomodulatory effects have also been noted with SLIT. In patients treated with grass pollen SLIT for 12 to 18 months, the number of $Foxp3^+$ cells in the sublingual epithelium significantly increased in the treatment group compared with the placebo group and nonatopic controls.[30] One caveat is that these cells may have been recently activated effector cells rather than true Tregs because the number of IL-10 and TGF-β mRNA-positive cells did not change after SLIT. In contrast, another study of grass pollen SLIT was able to demonstrate an increased number of IL-10 and TGF-β–producing Tregs after therapy.[31]

Bonvalet et al[32] revealed a significant (41.8%) improvement in symptom scores after grass pollen challenge in individuals given grass SLIT for 4 months. However, they were unable to detect a significant decrease in grass allergen (Phl p 1 and Phl p 5)–specific $CD4^+$ T cells numbers during treatment or 4 months after SLIT was stopped. There was a slight decrease in the number of $CD27^-$ or proallergic T_H2 cells.[32] This raises the possibility that although clinical improvement may be seen as soon as 1 month, parallel immunologic changes that involve the shift from a T_H2 signature and up-regulation of pTregs require longer treatment protocols (up to 12 months). Another explanation for the potential failure of immunotherapy is that the epigenetic changes noted in pTregs are unstable (ie, the FoxP3 locus is only transiently hypomethylated).[33,34]