

Review

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Mechanisms in AIT: Insights 2021

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Key words

allergen-specific immunotherapy (AIT) – regulatory T cells (Tregs) – regulatory B cells (Bregs) – regulatory innate lymphoid cells (ILCRs) – allergen-specific antibodies

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Abstract. Background: Allergen-specific immunotherapy (AIT) is currently the only treatment with potential long-term disease-modifying effects for patients suffering from allergic diseases such as allergic rhinitis, allergic asthma, venom allergy, or IgE-mediated food allergy. A better understanding of the molecular mechanisms underlying immune responses during successful AIT is of utmost importance and it may help to develop more effective and safer treatments.

Materials and methods: PubMed literature review was performed using keywords such as allergen-specific immunotherapy; regulatory T cells; regulatory B cells; regulatory innate lymphoid cells; and allergen-specific antibody from years 2018 to 2021. **Results:** The proposed mechanism of long-term tolerance induction in AIT, even upon treatment discontinuation, involves basophils, mast cells, innate lymphoid cells, dendritic cells, allergen-specific regulatory T and B cells, downregulation of effector type 2 responses, decrease in the production of IgE and increase in production of allergen-specific blocking antibodies, such as IgG2 and IgG4. **Conclusion:** We summarize the most recent advances related to mechanisms involved in the restoration of healthy immune responses to allergens during AIT. Our knowledge in this regard has significantly improved over the last years, which might well contribute to design novel and improved therapeutic approaches.

Introduction

Allergen-specific immunotherapy (AIT) is currently the only treatment that might influence the natural course of allergic diseases such as IgE-mediated food allergy, venom allergy, allergic rhinitis, or allergic asthma [1]. During the past decades, the prevalence of allergic diseases has dramatically increased worldwide, thus becoming one of the top global health issues. During this time, we have witnessed significant advances in the knowledge of the immunological mechanisms underlying allergic immune responses as well as those involved in allergen-specific tolerance induction. In this manuscript, we summarize the basic mechanisms underlying allergic diseases and highlight the immune mechanisms of action together with the up-to-date advancements in AIT.

Cellular and molecular mechanisms underlying successful AIT

Allergic inflammation is initiated when allergens are captured by antigen-presenting cells (APCs) in the skin, respiratory or gastro-

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intestinal mucosa. Epithelial cells produce alarmins such as IL-25, IL-33, and TSLP in response to allergens and other environmental insults. These allergens are subsequently processed into peptides and presented by APCs to naïve CD4⁺ T cells, which differentiate into T helper (Th) 2 cells and release type 2 cytokines such as IL-4, IL-5, IL-9, and IL-13. B cells are triggered to undergo IgE class-switching and differentiate into plasma cells secreting large amounts of allergen-specific IgE antibodies that bind to the high-affinity IgE receptor (FcεRI) on the surface of mast cells and basophils. Allergen re-exposure induces IgE-FcεRI complexes cross-linking, leading to the release of anaphylactogenic mediators such as histamine, leukotrienes, and cytokines. These mediators contribute to type-1 hypersensitivity reactions and occasionally lead to anaphylactic reactions. Late-phase reactions and multiple allergen exposures perpetuate allergic inflammation and contribute to tissue remodeling, fibrosis, and the most chronic manifestations of allergic diseases [1, 2].

To better understand the allergic immune cascade and tolerance induction mechanisms, the role of several immune cells including mast cells, basophils, innate lymphoid cells (ILCs), dendritic cells (DCs), regulatory T cells (Tregs), and regulatory B cells (Bregs) needs to be extensively monitored and investigated during the course of AIT and after treatment discontinuation.

Mast cells and basophils

Mast cells and basophils play a key role in the effector phases of allergy. The allergen-dependent cross-linking of the IgE bound to FcεRI on mast cells and basophils triggers the release of different mediators and type 2 cytokines that promote allergic inflammation. AIT induces very rapid desensitization of mast cells and basophils evidenced by low responsiveness to allergens despite the high allergen-specific IgE levels observed at the initiation of the treatment. Late effects of AIT are associated with a reduction of mast cell and basophil infiltration in the tissues and a reduced mediator release [3]. One possible mechanism is the increased production of allergen-specific IgG4 and the

elevated expression of the low-affinity IgG receptor (FcγRIIa and FcγRIIb) on mast cells and basophils. IgG4 competes with IgE in allergen binding, but also the binding of the FcγRIIb receptor by allergen-IgG complexes suppresses the IgE-mediated activation on mast cells. IgG-mediated inhibition also reduces Th2 cytokine release from mast cells and basophils. [4]

Another mechanism is the quick upregulation of histamine receptor 2, which has an inhibitory effect on FcεRI-mediated activation and degranulation of basophils. The decrease of basophil responsiveness has been documented during several studies of immunotherapy [5]. Interestingly, the analysis of basophil responses before, during, and after AIT can help to identify transient desensitization or maintained unresponsiveness.

Innate lymphoid cells

ILCs are recently described cells of the innate immune system. They are classified into two main prominent subsets that are cytotoxic and non-cytotoxic (helper) ILCs. The cytotoxic ILCs consist of natural killer (NK) cells that display functions of CD8⁺ cytotoxic T cells and lymphoid tissue inducer (LTI) cells that are involved in secondary lymphoid structure development. The helper ILCs have been subsequently separated into three different phenotypes: group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s). These three different subsets resemble the functions of Th1, Th2, and Th17 cells, respectively. Interestingly, new discoveries of IL-10-producing ILCs and retinoic acid (RA)-mediated pathway of IL-10-producing ILC2 induction has revealed the hidden immune regulatory properties of these cells [6, 7]. Golebski et al. [8] successfully demonstrated the insightful role of IL-10⁺ KLRG1⁺ ILC2s during AIT in patients with grass pollen allergy. Their results revealed that the competence of ILC2 to produce IL-10 was restored in patients who received grass-pollen sublingual immunotherapy. For this reason, IL-10⁺ ILC2s exhibit disease-modulating effects by AIT.

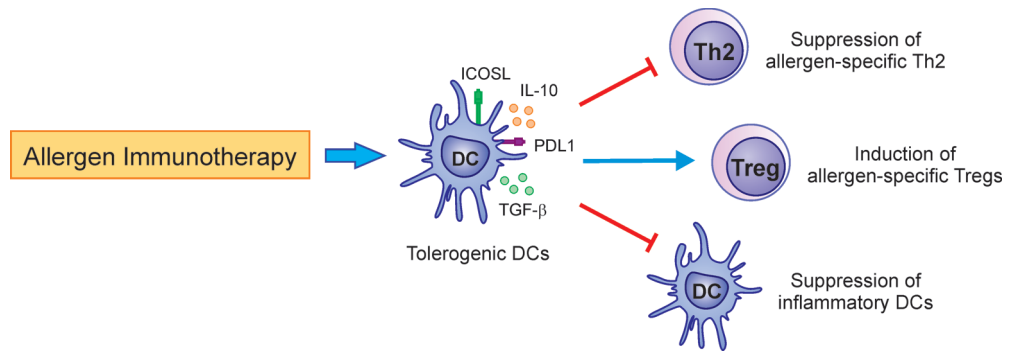


Figure 1. Mechanisms of immune tolerance induced by DCs during AIT. Tolerogenic DCs produce IL-10 and TGF- β , and express surface molecules that suppress Th2 and Th17 cells as well as inflammatory DCs, and induce allergen-specific Tregs.

Dendritic cells

DCs are APCs with the dual capacity to initiate and maintain allergic inflammation or to induce tolerance. DCs play an important role during the course of AIT. AIT induces an increase in plasmacytoid DCs (pDCs), which is a DC subset involved in the induction of Tregs and oral tolerance [9, 10]. A reduction in the frequency of CD11c⁺ conventional DCs (cDCs), which support Th2 responses in allergic patients, was also observed after AIT [9]. Mouse models have demonstrated that oral CD11b⁺ cDCs and macrophages transport sublingual allergens to lymph nodes and induce allergen-specific Tregs [3, 11]. Tolerogenic DCs represents a heterogeneous subset of DCs with an immature or mature phenotype that display increased capacity to produce IL-10. Allergoid-mannan conjugates generate tolerogenic IL-10-producing DCs and reprogram monocytes and macrophages into tolerogenic phenotypes [12, 13, 14]. Similarly, a nanovaccine produced by coupling PGLA-encapsulated ovalbumin with mannan induced in vitro IL-10-producing DCs that generated Tregs [15]. Cannabinoids also induce tolerogenic DCs able to generate Tregs by mechanisms depending on autophagy and metabolic reprogramming [16]. Another in vitro study showed the capacity of IL-27 produced by DCs to suppress grass pollen-stimulated peripheral blood mononuclear cell (PBMC) proliferation. Interestingly, a mouse model of allergic airway inflammation highlighted the critical role of IL-10 signaling in DCs to induce allergen-specific tolerance [17]. The scheme of tolerance-induced mechanisms by DCs during AIT is shown in Figure 1.

Regulatory T cells

Induction and maintenance of peripheral tolerance to allergens require the fine balance between Tregs and effector cells. Peripheral T-cell tolerance is characterized by an increase of AIT-induced Tregs and the shift of Th2 cell responses to Th1 [3]. Allergen-specific Tregs can be divided into thymic or natural Tregs (tTregs) and inducible Tregs (iTregs) including FOXP3-expressing iTregs, IL-10-secreting Tr1 cells, and TGF- β -producing Th3 cells [18, 19]. The suppression of different cell subsets by Tregs is required to completely establish cell-mediated tolerance. Tregs generated during and after AIT suppress effector cell function and induce blocking antibodies [1]. In AIT, four different mechanisms of Treg suppression have been described: secretion of inhibitory cytokines such as IL-10, TGF- β , and IL-35; metabolic disruption mechanisms; mechanisms involving surface molecules including programmed death 1 (PD1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG3), or inducible costimulatory molecule (ICOS); and cytotoxicity (Figure 2). Allergen-specific Tregs increase and Th2 cell reduction with the induction of natural or clinical tolerance in beekeepers during allergen exposure in the beekeeping season and AIT-treated allergic patients, respectively. Ex vivo analysis of human peripheral blood cells showed that house dust mite (HDM) AIT involves increase of functional allergen-specific Tregs and decrease of allergen-specific ILT3⁺ Treg cells, which display impaired suppressive function [20]. AIT generates long-term Treg production by inducing epigenetic changes in FOXP3 re-

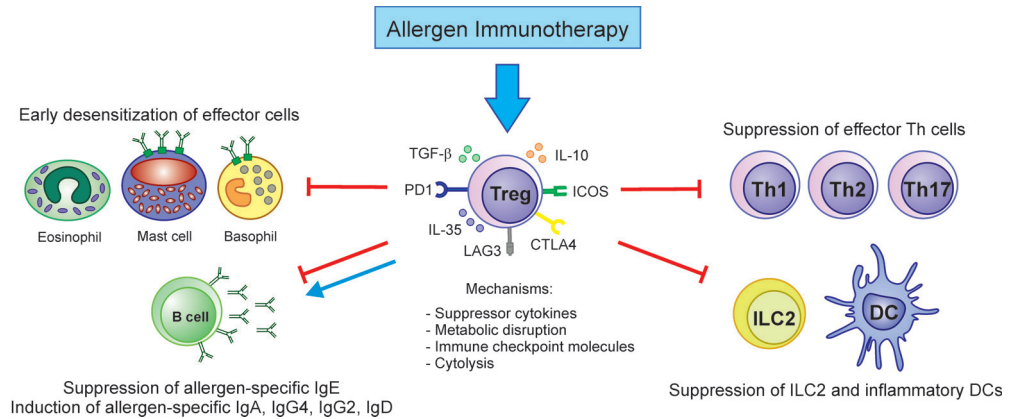


Figure 2. Mechanisms of immune tolerance induced by Tregs during AIT. Tregs produce immunosuppressive cytokines such as IL-10, TGF- β , and IL-35 that result in suppression of early desensitization of effector cells (eosinophils, mast cells, and basophils), effector Th cells (Th1, Th2, and Th17 cells), inflammatory DCs, ILC2, and allergen-specific IgE. Through IL-10, TGF- β , and IL-35 cytokine production, allergen-specific IgA, IgG2, IgG4, and IgD antibodies are released and exhibit IgE-blocking effects.

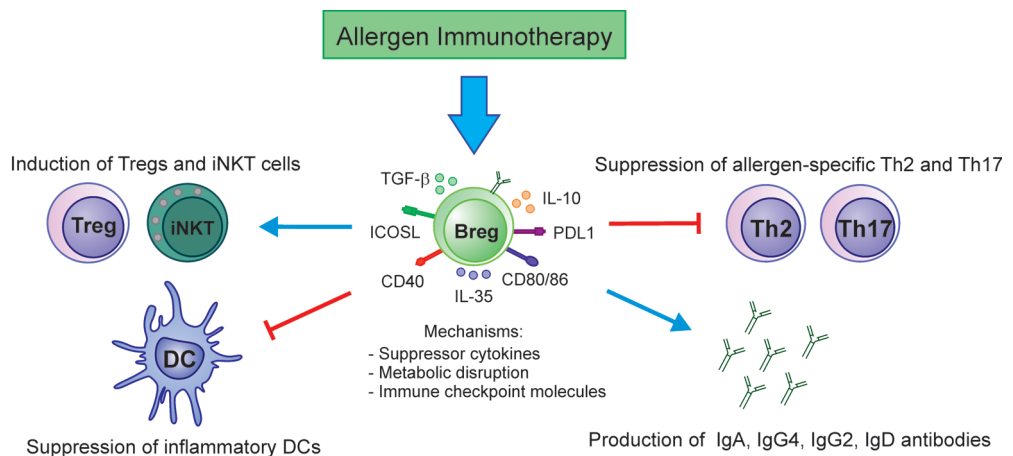


Figure 3. Mechanisms of immune tolerance induced by Bregs during AIT. Bregs suppress effector T cells (Th2 and Th17) and induce expansion of Treg cells via the release of IL-10, TGF- β , and IL-35 cytokines. Diverse surface molecules on Breg cells including BCR, PDL1, CD39, CD73, CD80/CD86, CD40, ICOS-L, and AhR are well-expressed and suppress the inflammatory responses. In autoimmune tolerance, Bregs activate iNKT cells with suppressive function. Moreover, Bregs are also the main producer of allergen-specific IgA, IgG2, IgG4, and IgD antibodies that compete with the crosslinking of allergen-specific IgE to effector cells.

gions in Tregs. IL-10-producing Tregs were induced after birch and grass pollen AIT. Interestingly, IL-35-inducible Tregs have been identified as a subset of iTregs with the capacity to reduce Th2 inflammation, T cell proliferation, and cytokines produced by ILC2s. Recent studies have demonstrated that successful subcutaneous immunotherapy (SCIT) increases IL-35 and IL-35-induced Tregs in blood [3]. All these studies have demonstrated the critical role of the tolerance induced by allergen-specific Tregs in successful AIT in humans.

Regulatory B cells

Although B cells play a major role in the immune system via the production of specific antibodies, recent evidence clearly revealed that B cells can also mediate immune responses through alternative mechanisms beyond antibody production [21]. Bregs play a crucial role in secreting the immunosuppressive cytokines including IL-10, IL-35, and TGF- β , and expressing immunosuppressive receptors such as cell membrane-bound molecules like B cell receptor (BCR), PDL1, CD39, CD73, CD80/CD86, CD40, inducible costimulatory ligand (ICOSL), and

aryl-hydrocarbon receptor (AhR) (Figure 3) [22]. Bregs can be stimulated by different factors, including inflammatory cytokines such as IL-6, IL-1 β , and IFN- α , microbial compounds such as TLR4 or TLR9 ligands, along with CD40 ligation [23]. During AIT, IL-10-secreting B regulatory 1 (Br1) cells specific to bee venom allergen phospholipase A₂ (PLA) suppress antigen-specific CD4⁺ T-cell proliferation and also produce IgG4 [24]. In the following study, IL-10⁺Br1 cells increased their frequency in allergic patients receiving AIT and naturally exposed to allergen [25]. In addition, immunotherapy-treated patients with HDM allergy exhibit a significantly increased population of Der p 1-specific B cells, plasmablasts, IL-10⁺, and IL-1RA⁺ Bregs [26]. Furthermore, Bregs present lipid antigen to sustain invariant natural Killer T cells (iNKT) cell homeostasis and activation in healthy individuals compared to systemic lupus erythematosus (SLE) patients [27]. Indeed, Bregs play an essential role in achieving immune tolerance during AIT.

Allergen-specific IgE, IgG4, IgA, and IgD responses

The secretion of immunoregulatory cytokine IL-10 from Tregs, Bregs, regulatory innate lymphoid cells (ILCregs) could suppress Th2 responses and shift towards the induction of allergen-specific IgA and IgG4 antibodies with inhibitory activity [28, 29, 30]. IgG4 antibodies attenuate allergic responses by inhibiting the activity of IgE to mast cell and basophil activation [31]. Similar to IgG4, an IgE-blocking effect has been observed in IgG2. Recent data reveals that both IgG2 and IgG4 are capable of suppressing IgE-mediated allergic responses in patients who received sublingual immunotherapy against grass pollen [32]. Although IgG2 and IgG4 are found to be mediating this protective effect in AIT [33], the tolerance induction from other IgG subclasses remains unclear. In order to clarify these roles of other IgG subclasses during AIT, further studies on this topic are required. Lastly, allergen-specific IgD was increased during the course of AIT in HDM-sensitized asthmatic patients [34]. In addition, allergen-specific IgD was increased in children consuming cow's milk compared

to children with cow's milk elimination, suggesting that food antigens may establish humoral IgD responses in humans [35].

Novel adjuvants

AIT adjuvant must be safe, stable, and induce Th1/Treg cell responses. Aluminium hydroxide is the most widely used adjuvant in humans. It stimulates innate immune cells and creates a reservoir of antigen at the site of injection (depot effect). In recent years, more and more studies focus on developing novel adjuvants that can increase proper immune responses in target cells (immunomodulators) or increase the capture of allergens by APCs (delivery systems). Calcium phosphate, a mineral salt, and microcrystalline tyrosine, a biodegradable amino acid, are immunomodulators also used in current AIT products due to their depot effect [36]. Among immunomodulators, TLR ligands have been also used in allergy vaccination. For example, poly I:C (TLR3 ligand), monophosphoryl lipid A (TLR4 ligand), resiquimod (TLR7/8 ligand), or unmethylated CpG motifs (TLR9 ligand) have been proposed as adjuvants for AIT due to their capacity of promoting tolerance in different allergy models. In addition, the incorporation of flagellin (TLR5 ligand) into allergens is a new interesting approach. Novel approaches targeting C-type lectin receptors have been developed. Allergoid-mannan conjugates were demonstrated to enhance allergen uptake capacity and to induce strong regulatory responses, thus acting simultaneously as immunomodulatory and delivery system adjuvant (Figure 4) [12, 13, 14, 37]. Remarkably, aluminium hydroxide impairs the tolerogenic properties imprinted by allergoid-mannan conjugates in human DCs [38]. Among delivery systems, viral-like particles (VLPs), micro-particles, and nano-particles have been investigated because the encapsulation of allergens in these systems prevents allergen degradation, achieves high concentrations of allergens, and impairs IgE recognition. Currently, other strategies to obtain hypoallergenic derivatives are widely investigated in clinical practice [36].

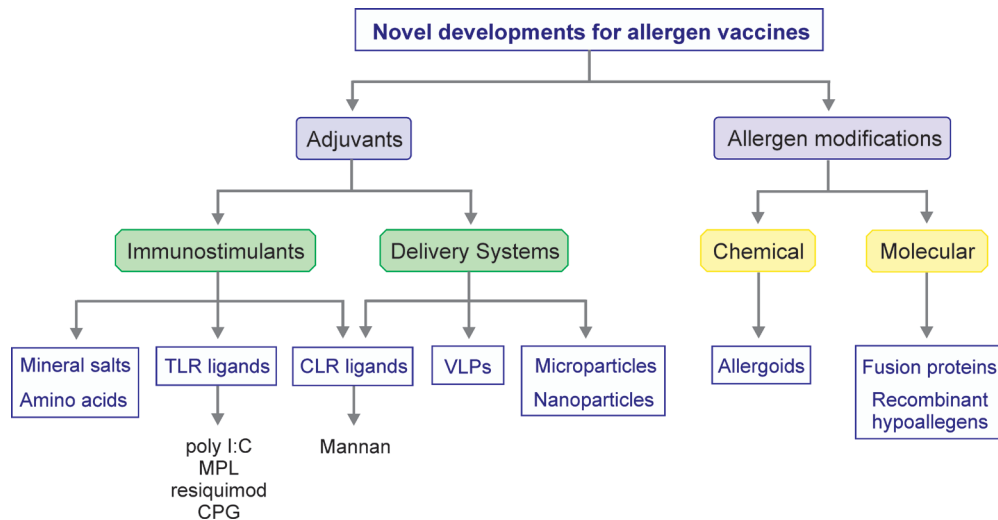


Figure 4. Novel adjuvants for AIT treatments. Adjuvants can be classified as immunostimulant substances including mineral salts, amino acids, TLR ligands or CLR ligands, or delivery systems such as VLPs or micro- or nanoparticles. Chemical and molecular modifications of allergens have also been developed for AIT.

Future perspectives of AIT vaccines

Future perspectives of AIT vaccines include the development of new adjuvants, hypoallergenic allergoids, and recombinant allergens, new application routes, and a better understanding of the mechanisms underlying successful AIT. Recently, the concepts of “innate trained immunity” and trained immunity-based vaccines (TibVs) were defined [39]. This describes the long-term functional reprogramming of innate immune cells which is induced by an insult and which promotes an altered response against a second insult. Mechanistically, trained immunity implies metabolic reprogramming and epigenetic changes. Trained immunity is clinically relevant when an exacerbated immune response drives disease progression, such as allergy. Therefore, targeting trained immunity could be a relevant therapeutic strategy for allergic diseases [40]. Recently, it has been demonstrated that allergoid-mannan conjugates can reprogram monocytes from both non-atopic and allergic donors into tolerogenic DCs via epigenetic and metabolic reprogramming [14]. Another recent study showed that SCIT for aeroallergens induces changes associated with tolerance in innate immune cells from allergic patients [9]. These data suggest a potential role of trained immunity in the mechanisms of tolerance induction during AIT.

Conclusion

Successful AIT establishes the induction of immune tolerance toward allergens, diminishes allergic symptoms, and improves clinical treatments in patients. After AIT, local infiltration of basophils and mast cells is reduced. In addition, induction of tolerogenic DC phenotype promotes the increase of Tregs which skew the immune response from Th2 to Th1 and induce the rise of Bregs. Consequently, Treg-derived IL-10 and Bregs prompt B-cell isotype switch toward IgG4 production. The greater understanding of underpinning mechanisms of AIT has improved medical treatment efficiency and better clinical outcomes in allergic patients and might well help to design better therapies in the near future.

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Conflict of interest

M.A. has received research grants from Swiss National Science Foundation, Bern; research grant from the Stanford University; Leading House for the Latin American Region, Seed Money Grant. She is in the Scientific Advisory Board member of Stanford University-Sean Parker Asthma Allergy Center, CA; Advisory Board member of LEO Foundation Skin Immunology Research Center, Copenhagen; and Scientific Co-Chair of World Allergy Congress (WAC) Istanbul, 2022. O.P. received research grants from MINECO, Ministerio de Ciencia e Innovación, CAM, Immunotek, and Novartis and fees for giving scientific lectures or participation in Advisory Boards from: Allergy Therapeutics, Amgen, AstraZeneca, Diater, Pfizer, Glaxo-SmithKline, Immunotek, Novartis, Sanofi-Genzyme, and Stallergenes. P.S. and A.A. declare no conflict of interest.

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