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Invited Review Article

Mechanisms of allergen-specific immunotherapy and allergen tolerance

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ABSTRACT

Allergen-specific immunotherapy (AIT) is the mainstay treatment for the cure of allergic disorders, with depicted efficacy and safety by several trials and meta-analysis. AIT impressively contributes to the management of allergic rhinitis, asthma and venom allergies. Food allergy is a new arena for AIT with promising results, especially via novel administration routes. Cell subsets with regulatory capacities are induced during AIT. IL-10 and transforming growth factor (TGF)-β are the main suppressor cytokines, in addition to surface molecules such as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1) within the *micro milieu*. Modified T- and B-cell responses and antibody isotypes, increased activity thresholds for eosinophils, basophils and mast cells and consequent limitation of inflammatory cascades altogether induce and maintain a state of sustained allergen-specific unresponsiveness. Established tolerance is reflected into the clinical perspectives as improvement of allergy symptoms together with reduced medication requirements and evolved disease severity. Long treatment durations, costs, reduced patient compliance and risk of severe, even life-threatening adverse reactions during treatment stand as major limiting factors for AIT. By development of purified non-allergenic, highly-immunogenic modified allergen extracts, and combinational usage of them with novel adjuvant molecules via new routes may shorten treatment durations and possibly reduce these drawbacks. AIT is the best model for custom-tailored therapy of allergic disorders. Better characterization of disease endotypes, definition of specific biomarkers for diagnosis and therapy follow-up, as well as precision medicine approaches may further contribute to success of AIT in management of allergic disorders.

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Introduction

The rapid rise in the frequency of allergic diseases during the last decades has triggered extensive research by the scientific community, which has mainly focused to understand the mechanisms of allergic immune responses and to establish new therapeutic strategies to cope with this allergy epidemics.[1–8]

Superior to allergen avoidance measures, two well-accepted approaches for management of allergic diseases are conventional pharmacotherapy and allergen-specific immunotherapy (AIT). In pharmacotherapy, several anti-mediators and anti-inflammatory agents such as anti-histamines, anti-leukotrienes, inhaled, topical and systemic corticosteroids, and recently approved novel biologicals as monoclonal antibodies can favorably control allergic manifestations.[9–12] However, upon cessation of medications, symptoms could generally relapse. To overcome this vicious cycle, AIT stands as the sole disease modifying therapeutic approach that promises tolerance induction and provides a long-term

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Abbreviations

AIT	Allergen-specific immunotherapy
$Al(OH)_3$	Aluminum hydroxide
AR	Allergic rhinitis
APC	Antigen presenting cell
Br1	Inducible IL-10 producing B cells
Breg	B regulatory cell
CTLA-4	Cytotoxic T-lymphocyte–associated antigen 4
DC	Dendritic cell
EoE	Eosinophilic esophagitis
EPIT	Epicutaneous immunotherapy
Foxp3	Forkhead box P3
HDM	House dust mite
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
ILCreg	Regulatory innate lymphoid cell
ILIT	Intralymphatic immunotherapy
ILT	Immunoglobulin-like transcript
MHC	Major histocompatibility complex
NK	Natural killer
OIT	Oral immunotherapy
PD-1	Programmed cell death protein-1
SCIT	Subcutaneous immunotherapy
SLIT	Sublingual immunotherapy
tDC	Tolerogenic dendritic cell
TGF	Transforming growth factor
Th	T helper
TLR	Toll like receptor
Tr1	Induced type T regulatory cell
Treg	T regulatory cell
VIT	Venom immunotherapy

unresponsiveness to allergens.[13–18] Since the initial publication by Noon *et al.* which reported improved symptoms of allergy following injection of grass pollen extract in 1911,[19] a great deal of work has increased our understanding of the immune mechanisms of allergic disorders and how AIT exerts its functions.

This manuscript summarizes the basic mechanisms underlying allergic diseases and mechanism of action of AIT, as well as reviewing the most recent advancements in the field.

Allergic inflammation

Allergic inflammation is an IgE-dependent hypersensitivity to allergens in atopic individuals. During initial encounter, an allergen captured by antigen presenting cells (APC) existent in the entry sites such as skin, respiratory tract or gastrointestinal mucosa is processed into peptides during migration of APC to the closest lymph node where the allergen peptides are presented to naïve $CD4^+$ T cells. In atopic individuals, if this peptide is recognized by $CD4^+$ T cells, with the dominance of IL-4 in the *micro milieu*, T helper (Th)-2 cells capable of producing Th2-type cytokines; IL-4, IL-5, IL-9 and IL-13 are induced. Consequently, B cells recognizing same peptides class-switch to IgE and start producing allergen-specific IgE antibodies. Mast cells, basophils and eosinophils; the effector cells of allergic inflammation possess high affinity surface receptors (FcεRI) to IgE antibodies. IgE-binding to the FcεRI is termed as '*sensitization*', meaning that a second exposure to this particular allergen could trigger activation of mast cells and basophils. This event will be concluded with instant degranulation of the cells, leading to rapid release of pre-formed mediators including histamine, tryptase, chymase and proteoglycans. Biogenic mediators like proteases, histamine, leukotrienes and also cytokines are produced subsequently. These events and mediators underlie allergic, type-1 hypersensitivity reactions that are presented as varying degrees from local isolated-to progressive life-threatening-reactions. Th2 cytokines are *sine qua non* mediators of allergic inflammation. While IL-4 and IL-13 activate B cells, IL-5 contributes to activation, recruitment and survival of eosinophils. IL-13 also contributes to epithelial maturation, mucus production, generation of extracellular matrix as well as contraction of smooth muscles (Fig. 1).[20–24] Upon continuous exposures, existing inflammation perpetuates signs and symptoms, which may lead to chronicity, tissue remodeling and fibrosis.[25,26]

Therapeutic approaches for allergic diseases, the need for allergen-specific immunotherapy

Besides allergen avoidance, the cure of allergic diseases basically relies on current pharmacotherapy regimens which target the mediators of allergy. There are favorable treatment outcomes during follow-up management, however, upon cessation, reactions may generally recur. Thus, a sustained non-responsiveness to allergens is a requisite. Efficacious AIT induces, establishes and maintains long-term clinical tolerance against allergens, resulting in amelioration of symptoms and decline in the need for pharmacotherapy, all of which improves allergy-sufferer's quality of life.[27–31] However, due to heterogeneous patient-responses to treatment regimens, well-described biomarkers to identify the most beneficial treatment approach for each individual patient are required.[32] In this point, personalized medicine approaches will provide advantage to find out these treatment responders.[33,34] Better understanding of the underlying mechanisms of allergen tolerance, the roles as well as interactions of cells will support the development of more rational, easily applicable, long-lasting, efficacious, safe and more patient-friendly therapeutic approaches.

Immune tolerance to allergens

Immune tolerance is the state of quiescence to self- or to harmless foreign antigens by means of multiple mechanisms. Dysregulated immune tolerance may lead to autoimmunity, cancer development, miscarriages, transplant rejection, and chronic infections, in addition to allergic diseases.[35] Unresponsiveness to otherwise harmless, ubiquitous allergens is provided by peripheral tolerance mechanisms.[36,37] From the allergy point of view, tolerance defines the induction and maintenance of the long-term unresponsiveness to allergens, which can be induced either by natural allergen exposure or by *in vivo* challenges. A suppressive, non-proliferative and non-inflammatory reaction should be established and sustained. AIT is an exemplification method of the latter by induction of tolerance to selected allergens. Altered allergen-specific T- and B-cell responses, decreased IgE-in addition to boosted IgG_4-production and decreased mast cell and basophil activation thresholds are the major outcomes of successful AIT (Fig. 2), consequently leading to sustained suppression of allergic manifestations.[38–40]

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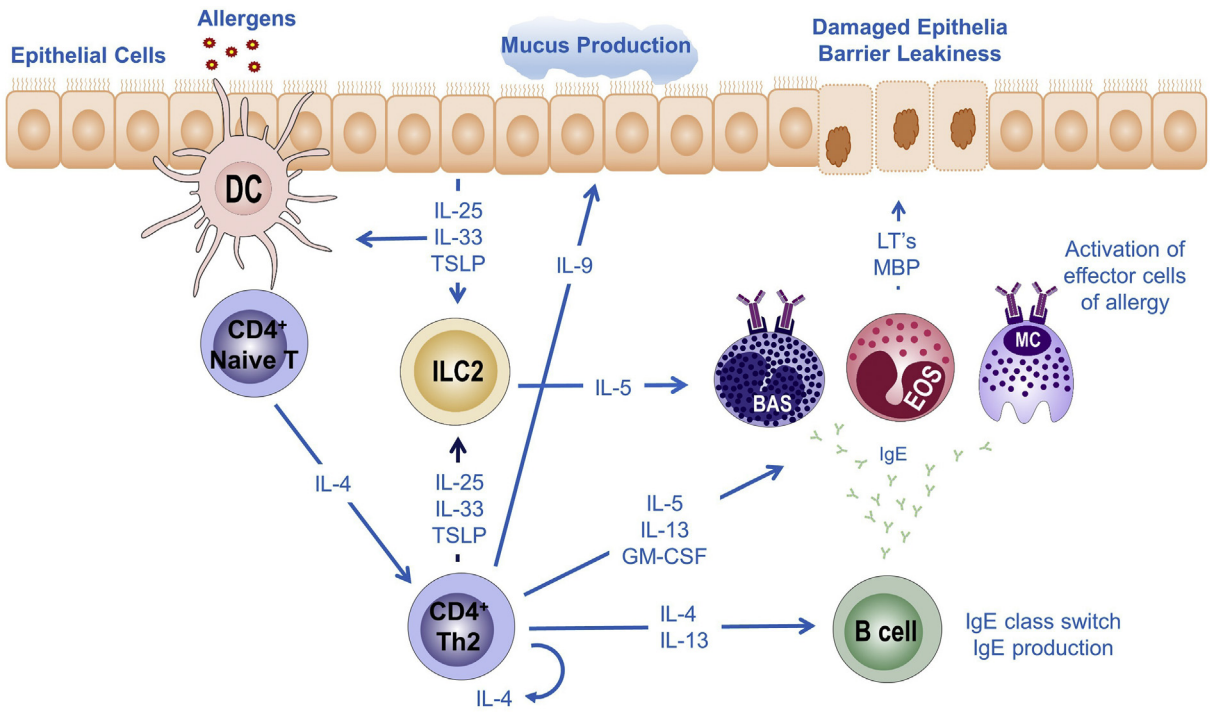

Fig. 1. Launch of an allergic response. Allergic immune responses are initiated with uptake and processing of allergens by dendritic cells, the professional antigen presenting cells. Processed allergen peptides are presented to naïve CD4$^+$ T cells that are differentiated to Th2 cells, with the presence of IL-4 in the micro milieu. Th2 cells produce the Th2 cytokines; IL-4, IL-5, IL-9 and IL-13. B cells consequently produce IgE which in turn binds to specific Fcε receptors on basophils and eosinophils; the effector cells of allergic inflammation. This event is called as sensitization. Upon recurrent encounter with the same allergen, immediate degranulation of these effector cells leads to discharge and production of histamine and leukotrienes, all of which lead to instant hypersensitivity reactions. Along with other functions in allergic inflammation, IL-4 acts in an autocrine manner for induction of CD4$^+$ Th2 cells. IL-5 contributes to activation, recruitment and survival of eosinophils. IL-9 induces increased mucus production while IL-13 and eosinophil products like major basic protein can induce epithelial damage and barrier leakiness. ILC2 contributes to allergic inflammation by production of Th2-type cytokines. The cytokines produced by epithelium; IL-25, IL-33 and TSLP can also be produced by Th2 cells and have capacity to activate ILC2. IL-25 induces DC activation. (BASO, Basophils; DC, Dendritic cells; EOS, Eosinophils; ILC, Innate lymphoid cells; LT, Leukotriene; MBP, Major basic protein; Th2, T helper type 2 cells; TSLP, Thymic stromal lymphopoietin.)

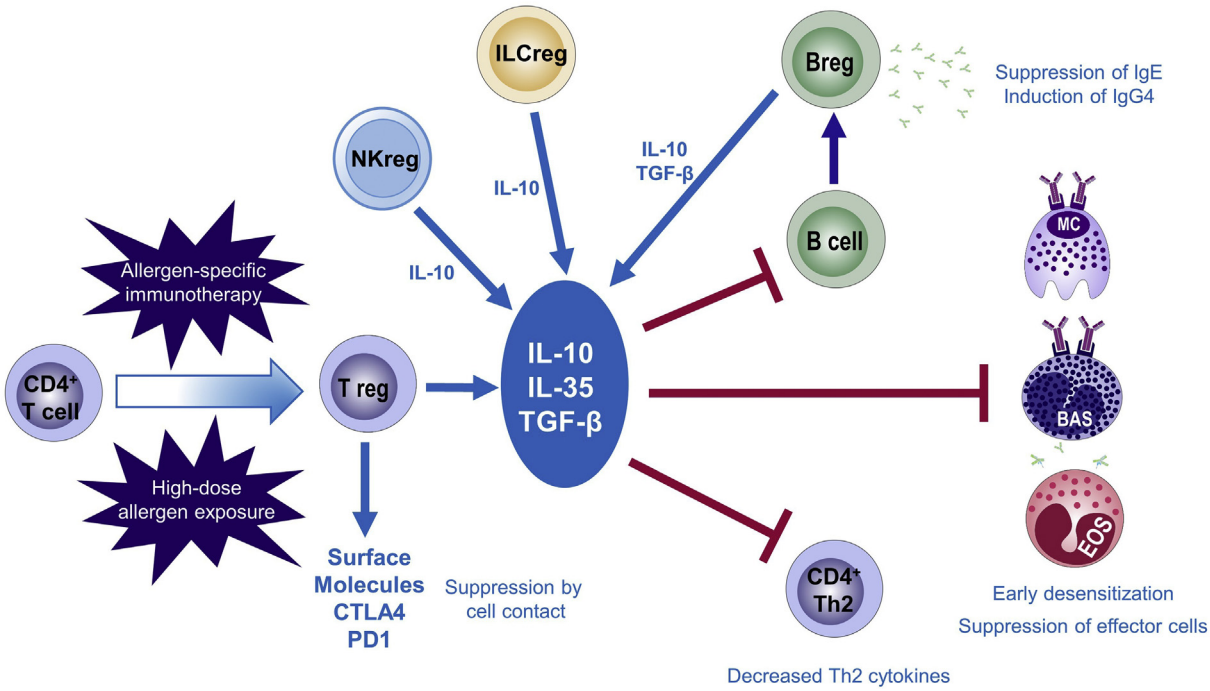

Fig. 2. Regulation of allergic immune responses following AIT. Allergen-specific CD4$^+$ Treg cells are induced either as a consequence of a successful AIT or following high dose allergen exposure. Treg cells have capacity to produce the regulatory cytokines; IL-10, TGF-β and IL-35 and also express surface molecules such as CTLA-4 and PD-1, all of which contribute to immune suppression of allergic responses. Treg cells suppress Th2 cells, eosinophils, basophils and can also induce allergen-specific Breg cells. The suppressive milieu restricts IgE production while inducing production of IgG_4 from B cells. Subsets of B cells, NK cells and ILCs with regulatory properties contribute to generation and conservation of allergen-specific tolerance. (BAS, Basophils; EOS, Eosinophils; ILCreg, Regulatory innate lymphoid cells; NKreg, Regulatory natural killer cells; Treg, Regulatory T cells.)

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Regulatory cells of the immune system

For a healthy immune response, it is crucial to establish a balance between the state of tolerance and intolerant reactions.[41,42] T regulatory cells (Treg) play the maestro function in conservation of this delicate balance. Naturally occurring, thymus-derived, Forkhead box P3 (Foxp3)$^+$ CD4$^+$CD25$^+$CD127$^-$ Tregs and peripherally-induced type 1 Tregs (Tr1) form the major subtypes of Tregs.[43,44] With the absence of co-stimulation from dendritic cells (DCs) T cell receptor triggering with the presence of transforming growth factor β (TGF-β) induces conversion of naive peripheral CD4$^+$CD25$^-$ T cells into suppressor Tr1 cells that are CD4$^+$CD25$^+$, CD45RB$^{-/low}$ and intracellular cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)$^+$.[45] Tregs with high levels of CD25, and FoxP3 have the highest suppressive capacity. Subsequent to high-dose allergen exposure of the beekeepers, FoxP3 expression increases in circulating Tregs, supporting the idea of the therapeutic use of bee venom.[46] Tregs are best known with their IL-10, IL-35 and TGF-β productions. IL-10, the major cytokine, suppresses IgE production through Treg cell–B cell interaction.[47,48] It simultaneously induces production of allergen-specific IgG_4, which is attributed as non-inflammatory within the frame of allergic inflammation. Together with IgG_2, IgG_4 induced following AIT competes with IgE for binding of allergens on mast cells and basophils.[49] Tregs also express suppressor molecules like CTLA-4 and PD-1, both of which contribute to immune tolerance.[50]

B cells contribute to immune responses by antibody production, together with secretion of various cytokines and expression of surface molecules. A recently revealed, heterogeneous subset termed as regulatory B cells (Breg) harbors immunosuppressive properties. IL-10 is the key cytokine of Bregs, while TGF-β and IL-35 exert crucial roles in B cell–mediated immunosuppression. The Breg subsets include CD27$^+$CD24^{high} B10 cells, CD24^{hi}CD38^{hi} immature transitional B cells, CD73$^-$CD25$^+$CD71$^+$ Br1 cells and a subset of plasma cells. Human Br1 with high IL-10 production capacity can suppress antigen-specific CD4$^+$ T cell proliferation, and also produce IgG_4 as a consequence of AIT.[51] The increased frequency of IL-10$^+$ Br1 cells in allergic patients receiving AIT[51] and in non-allergic beekeepers upon allergen exposure[52] support the *in vivo* existence of human Bregs. However, no difference was detected between patients with asthma and allergic rhinitis (AR) in terms of Breg subsets and IL-10 expression levels.[53]

Human Br1 cells selectively upregulate IgG_4 during their transition to plasma cells. This suggests an additional regulatory role due to the non-inflammatory and blocking function of IgG_4. In a human/mouse chimeric model of respiratory allergy, antibodies with IgE-blocking activity which contributed to reduction of airway inflammation were revealed to appear following AIT.[54] Taken together, Bregs appear to involve in mediation of allergen tolerance, but many open questions still remain to be answered.

Dendritic cells: decisive role players in crossroads of tolerance and immunity

Dendritic cells, a heterogeneous group of the APCs are most abundant at the mucosal sites and skin. The immature DCs attitude is strictly determined by the innate immune response-related elements present in the *micro milieu* during antigen uptake. Therefore, a DC can act either as tolerogenic or inflammatory, determined by the surrounding conditions, regardless of the antigen up-taken. Under inflammatory conditions, up-regulated expression levels of MHC-II, the co-stimulatory molecules; CD80 and CD86, and inflammatory cytokines; IL-1, IL-12 and IFN-γ will consequently lead to increased APC capacity to initiate most relevant adaptive T-cell response to a particular pathogen type and will drive induction of different Th subsets like Th1, Th2 and Th17.[55] The aberrant DC activation could lead to pathogenic conditions like autoimmunity, allergic diseases as well as graft rejection.[56,57] On the other hand, absence of inflammation during antigen uptake, the presence of suppressive cytokines like IL-10 and TGF-β, and the presence of TLR2-, TLR7-or TLR9-triggering molecules in the *micro milieu* will lead to conversion of the immature DCs to tolerogenic DCs (tDCs) with low expression of CD80, CD86 and MHC-II (Table 1). Production of TGF-β and IL-10 and expressions of suppressive molecules

Table 1
Contribution of innate immunity members in establishment and maintenance of allergen-specific tolerance.

Key players in innate immunity in AIT	Contribution in Allergen Tolerance	References
Dendritic Cells	• Cells which could determine between immune response and tolerance • Treg induction by down-regulated CD80, CD86 and MHC-II • Production of IL-10	55–63
Macrophages	• Production of TGF-β from M2 macrophages • Induce tolerance and tissue repair • M2b macrophages could produce IL-10	67–71
Innate lymphoid cells	• Substantial roles in between innate and adaptive arms of immunity • A regulatory subset of ILCs could produce IL-10 and contributes to maintenance of tolerance	79,99
Natural killer cells	• Cytotoxic cells responsible from anti-viral and anti-tumor responses • Forming a bridge between innate and adaptive arms of immunity by cytokine production • A regulatory subset of NK cells could produce IL-10 and contributes to maintenance of tolerance	101
Toll like receptors	• Receptors of innate immunity responsible from surveillance • Triggering of TLR2, TLR7 and TLR9 by PAMPs present in the *micro milieu* leads to conversion of DCs to a tolerogenic phenotype	58–60
Adjuvants	• Innate immune-response stimulants • Up-regulated co-stimulation and antigen presentation • Aluminum hydroxide and CpG are adjuvants utilized in AIT • TLR ligands, C-type lectin receptors, virus-like particles, dendrimers are all being investigated for exploitation in AIT	166,167,170,171
Trained immunity	• Up-regulated inflammatory responses of monocytes, macrophages and dendritic cells upon certain immune stimulants and environmental triggers such as BCG vaccination • Long-lasting • Protective in allergic responses	75,76

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like immunoglobulin-like transcript (ILT)-2, ILT3 and ILT4 by tDCs are vital for establishment and maintenance of tolerance. Vitamin D3, retinoic acid and indole-amine 2,3-dioxygenase support the induction of tDCs,[58–60] which could initiate induction of Tregs from naïve CD4$^+$ T cells and also can support the extension of the already present Treg populations.[61–63] tDCs in mouse can limit allergic inflammation.[64,65] Moreover, in a mouse model of allergic inflammation, when the DCs loose IL-10 signalization, tolerance induction becomes resistant and neutrophils infiltrate to lungs.[66] Better understanding of tDCs is essential for development of novel therapeutic strategies both in allergic diseases and also in autoimmunity and organ transplantation.[61–63]

Macrophages: candidate contributors of allergen-specific immunotherapy

Macrophages, the two-faceted cells of innate immunity possess phagocytosis, antigen presentation and cytokine-production. Thanks to having both pro- and anti-inflammatory competencies, macrophages impressively contribute to the immune homeostasis. Among the two activation patterns of macrophages, Th2-type cytokines induce the alternative route, leading to induction of M2 macrophage subsets with anti-inflammatory and healing properties by production of regulatory cytokines, including IL-10 and TGF-β. M2 macrophages were attributed to be a "double-edged sword" in allergic inflammation.[67] Both allergic and anti-allergic properties of M2 macrophages have been reported.[68–70] Subsets of M2 macrophages were revealed as; M2a, M2b, M2c and M2d, with different inductor stimulants as well as consequent products.[68] A regulatory subset of M2 macrophages that could take part in maintenance of allergen tolerance was demonstrated. Production of IgG_4 following AIT triggers *in vitro* conversion of M2a macrophages to M2b-like suppressive macrophages that were activated with the presence of IgG immune complexes and consequently produce IL-10.[71] The finding that acute stress-induced epinephrine could induce M2b differentiation from allergic M2a macrophages *in vitro* could explain relationship between stress and healing of allergic syndromes.[72] Hazy relationship between macrophages and tolerance deserves deeper investigation in order to better understand macrophage physiology and to authorize their possible employment in future therapeutic approaches.[73,74]

Stimulations such as BCG vaccination has capacity to increase inflammatory properties of macrophages, monocytes and DCs. This phenomenon is termed as 'trained immunity' and could induce a long-lasting up-regulated inflammatory response through epigenetic modifications. Along with these up-regulated responses, BCG may be attributed to affect the development of allergic disorders, as well as infections.[75,76]

Innate lymphoid cells: novel players in immune tolerance

Innate lymphoid cells (ILCs), recently recognized lymphocyte subclasses lack both lineage markers and B- and T-cell antigen receptors. ILCs are mucosal tissue resident cells with infrequent presence in peripheral blood. ILCs' crucial roles in immune defense, immune regulation and tissue remodeling are being illuminated.[77] Better characterization and understanding of ILCs highlight their contribution in almost all diseases, including allergic disorders. Shifts in the cytokine and chemokine profiles of the *micro milieu* initiated by tissue damage or inflammation can induce ILCs through their receptors for cytokines, microbial products and mediators.[78] ILCs exert substantial roles in between innate and adaptive immunity and involve in initiation and regulation of inflammation mainly through cytokine secretions.[79] A marked similarity in between developmental transcription factors of ILCs with that of T cells has been postulated.[77] ILCs designated subtypes were recognized as ILC1, ILC2 and ILC3, with comparable cytokine profiles to Th1, Th2 and Th17 subsets, respectively. Two more members of ILC family, due to their developmental and functional similarities, are known as NK cells and lymphoid tissue inducer cells, which overlaps with ILC1s and ILC3s, respectively.[80] In response to IL-12, IL-15, and IL-18, ILC1s can produce IFN-γ, which is essential in activation of macrophages.[81] ILCs can process antigens, thereby regulate the activity of antigen-specific T cells.[82] ILCs and T cells are also in a close interaction that regulates their activities mutually. IL-2 produced by T cells promotes activities of ILCs.[80]

ILC2s bear a resemblance to Th2 cells by sharing functional similarities such as production of IL-4, IL-5, IL-9 and IL-13, and also other effector molecules.[80,83] ILC2s involve in responses to extracellular parasites and allergens.[80] ILC2s can also respond to nonspecific cell-derived stimuli such as IL-25, IL-33, and thymic stromal lymphopoietin.[84] ILC2s are mainly positioned at the barrier surfaces as skin, airway and intestinal mucosa, and can promote inflammation in response to IL-33. ILC2 activation in mouse lungs depends on IL-33 via leukotriene B4 receptor 1.[85]

ILC2s contribute in allergic diseases by augmenting the Th2-type inflammation. The presence of ILC2s in the nasal lavage fluid of HDM-induced AR patients has been reported. Moreover, inferior nasal turbinate (INT) tissue-resident ILC2s produce abundant levels of IL-5 in response to IL-33, prostaglandin D2 or leukotriene D4, with the presence of IL-2.[86] Several trials have delineated critical roles of ILC2s in allergic diseases,[87–89] which were emphasized in virus-induced asthma exacerbations. IL-33, IL-33-responsive T cells and ILC2s were found to form key mechanistic links between viral infections and asthma exacerbations.[90] On the other hand, seasonal increases in peripheral ILC2s are reduced following AIT.[91]

Following resolution of inflammation, ILC2s can contribute to the re-establishment of epithelial barrier function and conservation of tissue homeostasis by production of amphiregulin, a member of epithelial growth factors.[92] Expression of amphiregulin has been observed in CCR10$^+$ ILC2s. Besides, the ligand of CCR10 (CCL27) is correlated with CCR10$^+$ ILC2 levels in allergic asthma patients, also plasma concentrations of CCL27 is correlated with asthma severity.[93]

ILC3s are characterized by their production of IL-17A, IL-17F, IL-22, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNF-α in response to IL-1β, IL-23, and aryl hydrocarbon receptor ligands. IL-22 and GM-CSF are main cytokines enrolled in epithelial barrier functions, mucus production and stimulation of intestinal macrophages.[80,94,95] On the other hand, lymphoid tissue inducer cells contribute in development of secondary lymph nodes and Peyer's patches,[80,96] and also involve in the maintenance of immune tolerance by induction of immature transitional regulatory B cells (itBreg).[97] Human ILC3s are primarily found in the intestinal lamina propria.[98] ILC3s exert crucial roles in the regulation of the homeostasis in the gastrointestinal tract.[79]

Although Foxp3-expressing ILCs have not been identified yet, a subset of ILCs with IL-10 production, maintained by autocrine TGFβ1 was designated as the regulatory ILC subset (ILCregs).[99] ILCregs are present in the intestinal tract and have a unique gene identity distinct from other ILC or Treg cell subsets. ILCregs exert their regulatory functions by limiting inflammation through secretion of IL-10 and TGF-β1. While IL-10 suppresses ILC1 and ILC3 activations, TGF-β1 sustains the expansion and maintenance of ILCregs, all of which enhances their inhibitory roles.[99] Retinoic acid has recently been reported to induce IL-10$^+$ ILCregs from ILC2s in human nasal tissues and also in the lung tissue samples of type-2 lung inflammation mouse model.[100] Along with ILCregs, a regulatory subset of NK cells with IL-10 production capacity has been revealed to suppress allergen-specific T-cell responses.[101]

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All above mentioned properties of ILC subsets may be attributed to highlight impacts of these cells in course of AIT and tolerance induction, which should further be investigated.

Current applications of allergen-specific immunotherapy

The primary objective of AIT is restoration of peripheral immune tolerance to allergens, inhibition of early- and late-phase allergic reactions, induction of allergen-specific regulatory cell subsets and their suppressor cytokines as IL-10 and TGF-β, suppressor molecules like PD-1 and CTLA-4, and limitation of IgE together with induction of IgG_4 production (Fig. 2).[43] In several human and mouse studies, upregulation of Tregs, decreased Th2 cells and their cytokines, histamine and tryptase levels and also eosinophil numbers have been highlighted as consequences of successful AIT.[28,102–105]

The success of AIT has been revealed with a great number of studies. AIT is being used for decades, mainly for AR, asthma, venom allergy and also for food allergy. A retrospective cohort analysis which investigated AR patients receiving birch pollen AIT has revealed that up to 6 years of follow-up, significantly more AIT patients were found to be AR and asthma medication-free. New-onset asthma risk was significantly reduced in the AIT-receiving group.[106] Additionally, a limited number of studies presented the real-life data on the progression of AR into asthma in patients receiving AIT.[107] AIT was proven to be beneficial for limited progression of allergic asthma severity.[108] Because food allergies become an emerging situation in pediatric age group, treatment intention has also been focused for these disorders. As avoidance of the allergenic food with strict elimination diets and symptomatic treatment measures cannot always be a part of solution, and life-threatening anaphylaxis and unwanted outcomes of limited diets are arising as major drawbacks, food AIT opens a new field in the management of food allergies. In persistent cases where there is a resistance to tolerance development to offending food allergen, food AIT arises as a life-saving method. There are several promising trials with oral, sublingual, subcutaneous and epicutaneous routes.[109,110]

Routes for allergen-specific immunotherapy

While subcutaneous and sublingual routes for AIT are being routinely utilized[111] studies are focused on establishment of novel routes for increasing efficacy and adherence as well as reducing the adverse reactions during AIT. **Subcutaneous immunotherapy** (SCIT) is the most widely used approach of AIT for more than a century. Subcutaneous injections of the particular allergen with increasing doses for a defined period best describes SCIT. The major drawbacks are discomfort from repeated injections, dependency for a health-care unit, long period required for tolerance establishment and also risk of severe hypersensitivity reactions. Substantial reductions in short-term symptom and medication scores in allergic asthma has been reported. Also, SCIT improves disease-specific quality of life in patients with allergic rhino-conjunctivitis.[13] House dust mite (HDM) SCIT for AR is effective in reduction of nasal syndrome and medication scores.[112] One of the well-accepted application of SCIT is insect venom allergy. Venom immunotherapy (VIT) is an effective method for prevention from further allergic reactions to insect stings.[113] Increased IgG_4 levels was reported to be correlated with both efficacy of VIT and protection from stings in patients with clonal mast cell disorders.[114] Although carrying a small but significant risk of systemic adverse reactions, VIT improves quality of life of patients with a previous history of systemic reaction, especially to bee stings.[115]

Sublingual immunotherapy (SLIT) is the administration of allergens in droplets or as tablets by sublingual route. SLIT is accepted as a viable alternative of SCIT for at least 30 years.[116] SLIT is a safer alternative in which home-application is available.[117–120] It is especially preferable in children due to avoidance from repeated injections. SLIT reduces the frequency and Th2-type cytokine production of allergen-reactive $CD4^+$ T cells in patients with HDM allergy.[121] Besides, SLIT for grass pollen induces tolerance by increasing specific serum IgG_2 and IgG_4 levels and allergen specific Tregs.[122] Several clinical trials have highlighted the efficacy of SLIT as an adjunct to pharmacotherapy in respiratory allergies.[123–125] According to a meta-analysis, SLIT provided significant symptom relief and reduced the medication requirements in pediatric patients with AR[126] and also decreased the symptoms of birch allergic patients with allergic rhino-conjunctivitis.[127] However, in a Cochrane analysis, due to usage of different invalidated symptom and medication scores and lack of data for important outcomes such as exacerbations and quality of life, a clinically precise conclusion cannot be reached for SLIT in asthma.[128] But, a recent meta-analysis has reported SLIT to be effective and safe both in children and adult patients with allergic asthma and AR.[129] Overall, a beneficial immunomodulatory effect of preventive SLIT with HDM has been reported to occur at a molecular level, by increased IgG_4 but unchanged IgE diversity.[130] In addition to Derp 2-specific IgG_4, specific-IgD levels were slightly increased during AIT in HDM asthmatic patients.[131] Further studies with larger cohorts are required to prove this candidate biomarker for monitoring success of AIT in HDM allergic patients. Also, dual application of SLIT for seasonal and perennial AR has recently been reported to be well-tolerated.[132]

Despite the fruitful outcomes by both SCIT and SLIT in respiratory allergies, there is limited evidence that AIT may be an effective option for other indications of allergy such as atopic dermatitis, which was highlighted in a recent Cochrane analysis.[133]

Oral immunotherapy is attributed as a potentially curative treatment for food allergy, especially in children mainly allergic to cow's milk, hen's egg and peanut.[134–139] During the active phase known as '*desensitization*', a non-reactive state has been established in which an increased amount of allergic food that the patient can tolerate without any reaction has been observed.[140] However, there are concerns about a sustained unresponsiveness and tolerance development after treatment. A recent meta-analysis of peanut OIT randomized trials found that OIT regimens considerably increase allergic and anaphylactic reactions over avoidance or placebo, despite effectively inducing desensitization, which has highlighted the requirement of safer methods of treatment.[141]

Intralymphatic immunotherapy (ILIT) is a novel method for direct administration of allergens into lymph nodes. The major benefit of ILIT is the reduced number of administrations of low-dose allergens. Only 3 intralymphatic injections are recommended 1 month apart during the entire treatment.[142] Though dependent on limited data, ILIT was found to be safe and the results of clinical trials are predominantly promising. In a recent review, among 6 clinical trials in which major allergens grass/birch pollens and cat dander were used, all but one has shown clinical improvement.[142] Besides, the latter one exhibited immunological change without any clinical improvement.[143] ILIT may increase compliance, which stands as a major drawback during AIT, and may possibly decrease treatment costs due to long treatment durations. On the other hand, requirement of experienced staff to administer allergens under ultrasound guidance looks like the marked disadvantage.

Epicutaneous immunotherapy (EPIT) is another novel AIT method in which allergens are applied to the skin via patches or after abrasion. Allergens captured by Langerhans cells (LCs) and DCs, in the epidermis and dermis, respectively, migrate to lymph nodes to prime $CD4^+$ T cells. Both LCs, DCs, in addition to

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keratinocytes and fibroblasts in cutaneous tissues can produce pro-inflammatory and anti-inflammatory cytokines that may skew T cell polarization into Th1 and Treg profiles and induce tolerance development. EPIT was revealed to lead to epigenetic changes through GATA3 only in Th2 cells, while it happens through FoxP3 in CD62L$^+$ Tregs in peanut-sensitized mouse.[144] Mast cells could also play roles in EPIT due to their presence in dermis. Risk of adverse reactions should always be considered, and thus hypoallergenic molecules should be preferred. A limited number of placebo-controlled trials have delineated the efficacy and safety of EPIT in pollen induced AR.[145,146] There are promising results in EPIT trials performed in cow's milk and peanut allergic children, as well.[109,110,147,148] Also, recently, in an open-label phase of an EPIT trial in patients with eosinophilic esophagitis (EoE), a reduction of eosinophil numbers in nearly half of subjects has been reported.[149]

Future expectations and conclusions

Being the sole long-term cure-providing option of allergic diseases, AIT still harbors a number of *Achilles heels*. Although being the sole disease-modifying treatment choice, long-term therapy schedules arise as a limiting factor. Both from the site of patient compliance and treatment related-costs, clinical- and bio-markers to increase the adherence to AIT are required. However, no definite cellular or humoral biomarkers has been able to predict the clinical outcome of AIT so far.[32,150] Among with already known biomarkers as IgE, IgG$_4$ and detection of IL-4 during the course of AIT, increased IL-10 mRNA expression levels following HDM SLIT was reported to be predictive of a better clinical response, and could be considered as a potential novel biomarker.[151] Estimation of CD4$^+$CD25$^+$FoxP3$^+$CD127$^-$ Treg frequencies was recently proposed as a candidate biomarker.[152] At the cellular level, basophil sensitivity was highlighted to be decreased during SCIT, which reflects long-term clinical outcome.[153] In monitorization of immunological kinetics after 2 years of discontinuation of a 3 year-AIT protocol, sustained Treg responses has been observed.[154] For VIT, monitorization of basophil responses by basophil activation test (BAT) was proposed as a potential indicator of tolerance.[155] Utilization of diverse OMIC approaches including genomics, proteomics and transcriptomics could be beneficial for demonstration of dysregulated molecular, cellular and serological factors in allergic diseases, and will contribute to immune monitoring of AIT by provision of novel biomarkers.[156]

Increasing evidence has shown that the regulation of follicular helper T cells (T_{FH}) plays a significant role in the success of AIT.[157] Recent reports indicate that $T_{FH}2$ cells are increased in the patients with AR. They are sharply decreased in response to AIT and correlated with symptom and medication score improvements.[157,158] Follicular regulatory T cells (T_{FR}) and Bregs modulate T_{FH} activities. T_{FR} cells represent a distinct population of CXCR5 expressing Foxp3$^+$ Tregs and suppress T_{FH} cell-mediated antibody production.[159,160] In patients with AR, T_{FR} were decreased and impaired in function. These T_{FR} defects have correlated with disease severity and IgE production. After AIT, circulating T_{FR} are elevated and their IgE-suppressing function is improved.[160] Therefore, T_{FH} and T_{FR} might be used as potential biomarkers to monitor the response to AIT.

There are several advantages and disadvantages of all administration routes (Table 2). OIT was found to have improved efficacy compared to SLIT and EPIT, while frequent office visits during up-dosing, risks of both adverse events and eosinophilic esophagitis (EoE) are disadvantages. SLIT has an improved safety profile compared to OIT, but patient compliance and risk of EoE again arise as disadvantages. Although increased dosing only once per week has led to increase in compliance, administration of allergens by injections is a limiting factor especially in pediatric age group.[161] On the other hand, although EPIT has best safety profile with ease of administration, there still is limited data to compare efficacy of EPIT with other routes.

The disease-modifying capacity puts AIT into the center, with proven specificity.[116,162–164] Efforts come together to increase the applicability of this miraculous approach (Fig. 3). The evolving concept in the field of AIT to increase its efficacy and safety is the administration of modified allergens with or without combination to novel molecules.[165] Adjuvants are being used in vaccine preparations to up-regulate inflammation for increased co-stimulation and antigen presentation (Table 1).[166] Adjuvants also have a depot effect at the injection site. In addition to enhancing immunogenicity, depot effect precipitates allergens, delays systemic absorption and simultaneously reduces the risk of severe anaphylactic reactions.[166] Aluminum hydroxide (Al(OH)$_3$) is the oldest and most commonly used adjuvant in human vaccines including AIT preparations.[166,167] Al(OH)$_3$ boosts adaptive immunity by inducing uric acid; an endogenous danger signal that activates inflammatory DCs.[168] Moreover, subcutaneous immunization of Al(OH)$_3$ with polymerized allergoids conjugated to mannan (PM) impairs the functional Tregs through PM-activated human DCs.[169] Else than Al(OH)$_3$, microcrystalline tyrosine, monophosphoryl lipid A and calcium phosphate are the other known adjuvants. TLR ligands, C-type lectin receptors, virus-

Table 2
Routes for allergen-specific immunotherapy.

Route of AIT	Advantages	Drawbacks	References
Subcutaneous (SCIT)	• Proven efficacy and safety • Possible applications in venom immunotherapy • Increased efficacy in head to head comparisons	• Repeated injections • Healthcare unit dependency for injections • Long duration • Risk of severe hypersensitivity reactions	13,111–115
Sublingual (SLIT)	• Proven efficacy and safety • No injections • Preferable in children • Less dependency for healthcare unit • Potential for home application	• Could have less efficacy in comparison with SCIT	116–133
Oral (OIT)	• A potential curative treatment for food allergies	• Safety and efficacy concerns	134–141
Intralymphatic (ILIT)	• Reduced number of injections • Reduced treatment duration	• Requirement of experienced staff for injections under ultrasound guidance • Risk for side effects due to leakage of allergens from the injected lymph nodes	142,143
Epicutaneous (EPIT)	• Applicability in food and aero allergens • Good safety profile • Studies revealed promising results	• Risk of adverse reactions	109,110 144–149

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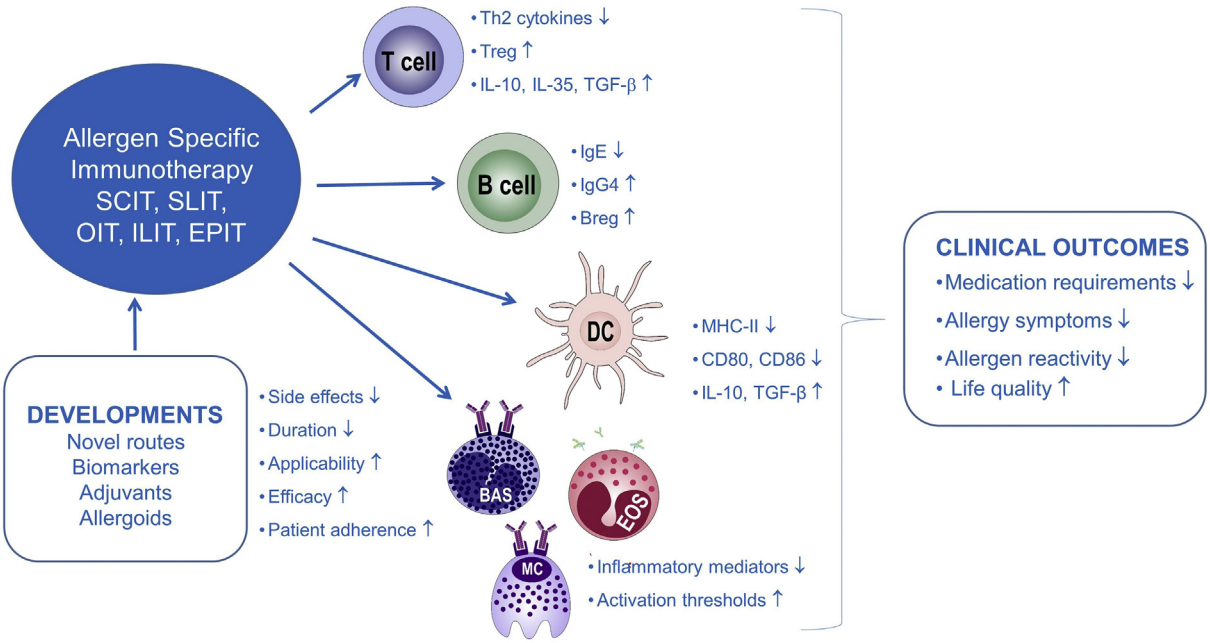

Fig. 3. Allergen-specific immunotherapy, proceedings and cellular responses. Allergen-specific immunotherapy is the only option for long-term cure of allergic diseases. While SCIT and SLIT has been used for a long time, OIT, ILIT and EPIT routes are recently developed strategies of AIT. Recent developments in this particular area of allergology and immunology aim to provide increased efficacy, applicability and patient adherence to therapy joined with diminished side effects and duration. Novel administration routes, definition of biomarkers for better monitorization of therapy success, novel adjuvants for increased AIT efficacy and development of allergen preparations with increased antigenicity and decreased allergenicity, such as allergoids all serve for these important goals. The net result of AIT is termed as allergen-specific tolerance, which is an active immune response state resulting from functional changes in immune cells. The clinical outcomes of a successful AIT could be listed as decreased medication requirements, allergen reactivity and allergy symptoms joined with increased life quality. (BAS, Basophils; EOS, Eosinophils; DC, dendritic cells; EPIT, epi-cutaneous immunotherapy; ILIT, intra-lymphoid immunotherapy; OIT, oral immunotherapy; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.)

like particles, dendrimers are being investigated for exploitation in AIT.[170] Clinical superiority of CpG-adjuvanted allergen preparations to be utilized in peanut AIT was reported in a mouse model of nasal AIT, whose activity is based on transition of Th2 cells to IFN-γ-producing Th1 cells, together with IL-10 producing non-Th2 cells.[171]

Allergoids are chemically modified allergen extracts created with treatment of native allergens with formaldehyde or glutaraldehyde. This modification conserves immunogenic linear T-cell epitopes while disrupting the conformational IgE epitopes.[172–174] Allergoids' antigenicity is increased, but due to limited capacity to bind to IgE, expectation of hypersensitivity reactions are greatly diminished. Their mechanism of action is still not fully illuminated. A recent study investigating AIT with glutaraldehyde-modified birch pollen allergoid revealed modified humoral and T-cell responses, leading to formation of augmented IL-10-producing Tr1 cells.[174] Efficacy of allergoids are being questioned nowadays.[175] According to the results of a retrospective cohort analysis with 2350 patients, HDM allergoid AIT in AR and asthma was reported to be beneficial and was revealed to limit probability of asthma development.[176]

Peptide immunotherapy has been a promising developing area in AIT. Recent studies have shown that short-term peptide immunotherapy has fewer side effects and is a safe and clinically effective alternative. *Lolium perenne* peptide (LLP) administered just before the grass pollen season significantly reduced seasonal symptoms and conjunctival provocation test (CPT) reactivity and increased protective specific antibodies. These responses were seen as early as four weeks after treatment initiation and indicated the development of tolerance. Similarly, treatment with purified protein was revealed to increase clinical efficacy of SCIT, in mouse. TGFβ1 mimetic peptide regulated Th1 and Th2 responses via the modulation of cytokines and antibodies, induced Treg cell differentiation, and inhibited basophil degranulation.[177–183]

Novel routes of administration that aim to increase patient compliance, shorten treatment durations and reduce health costs draw attention into focus both from the research and clinical points of views. As our understanding of the underlying mechanisms of AIT is increasing day-by-day through figuring out detailed interrelations in between effector cells and their products within the *micro milieu*, our conceptual approach to allergic disorders is also gaining improvement progressively, which may favor for other clinical conditions such as in autoimmunity, chronic infections, organ transplantation and cancer, as well.

Conflict of interest

The authors have no conflict of interest to declare.

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