



Modulation of immune responses by immunotherapy in allergic diseases

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Allergen immunotherapy (AIT) has been used for 100 years and until now different immunoregulatory pathways have been shown to take place in its mechanisms of action. It is characterized by administration of the causative allergen and is shown to be clinically efficient even after discontinuation of therapy particularly in allergic respiratory diseases, bee venom allergy, and food allergy. Generation of antigen/allergen-specific peripheral tolerance is the key mechanism during immunotherapy. It is mediated by development of T and B regulatory cells, IgG4 isotype allergen-specific antibodies and the involvement of multiple suppressor factors, which lead to decreased tissue inflammation, early and late phase responses. Describing novel regulatory mechanisms in the process of immune tolerance induction will help to identify treatment modalities not only for allergic disorders, but also for autoimmune diseases, organ transplantation, chronic infections, and cancer.

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Introduction

Allergen-specific immune response develops from a series of complex mechanisms, such as structural features and dose of the allergen, route and timing of its exposure, existence of innate immune response stimulants within the allergen and genetic susceptibility of the host. All of these factors also contribute to the development and maintenance of immune tolerance [1]. In the process of immune tolerance induction the balance between allergen-specific effector T helper 2 (Th2) cell and T

regulatory (Treg) cell is achieved through several immunologic mechanisms such as alterations and decrement in response of effector T and B cells to allergen, generation of specific antiinflammatory antibody isotypes, alleviation in the activity of cell types other than lymphocytes like basophils, eosinophils, and mast cells which play an important role in the emergence of both immediate and late phase reactions in response to allergens (Figure 1). In addition to the suppressive and regulatory action of Treg cells, recently described IL-10-producing B regulatory cells [2,3] were also shown to contribute to development of tolerance in venom allergic individuals undergoing immunotherapy [4*].

Allergen immunotherapy (AIT) is the only treatment option given by subcutaneous (SCIT) and sublingual (SLIT) routes modulating the response of the patient against allergen for long term periods through prompting peripheral immune tolerance mechanisms [5]. In a conventional SCIT, this can be achieved by repeatedly administering the allergen in gradually increasing doses at certain time intervals for aeroallergen-mediated rhinitis and asthma [5]. In addition, oral immunotherapy (OIT) is used for food allergy [6] and intralymphnode, cutaneous routes are under investigations [7,8]. AIT not only induces tolerance but also hampers the process of development of asthma in allergic rhinitis patients and emergence of new allergies in monosensitized individuals [9]. In this review, the immunological mechanisms that underpin AIT are reviewed with the highlight of recent advances. Additionally, the possible use of biologicals in association with AIT is briefly emphasized.

The mechanism of generation and function of inducible T regulatory cells

AIT induces a major change in allergen-specific T cell subsets. The proportion of IL-4-secreting Th2 cells decreases, meanwhile IL-10-secreting inducible Treg cells specific for the same allergenic epitope increase in number and function similar to the immune status observed in nonallergic healthy individuals [3]. This appears to be one of the milestones in the development of peripheral tolerance to allergens. A significant correlation is found between improvement of symptoms and the increase in Treg cell numbers during immunotherapy [10]. Inducible type of Treg (iTreg) cells, which are derived from naive CD4⁺ T lymphocytes in the periphery predominantly take part in the induction of tolerance [11,12]. Deficiency of extrathymically generated iTreg cells results in the augmentation of Th2 type allergic

Glossary

AIT: allergen immunotherapy
CTLA-4: cytotoxic T lymphocyte antigen-4
FOXP-3: Forkhead box protein 3
GITR: glucocorticoid induced tumor necrosis factor receptor
OIT: oral immunotherapy
PD-1: programmed death-1
pDC: plasmacytoid dendritic
R: receptor
SCIT: subcutaneous immunotherapy
SLIT: sublingual immunotherapy
T helper 2: Th2
Treg: T regulatory
iTreg: inducible type of Treg
nTreg: natural CD4⁺CD25⁺FOXP3⁺ Treg

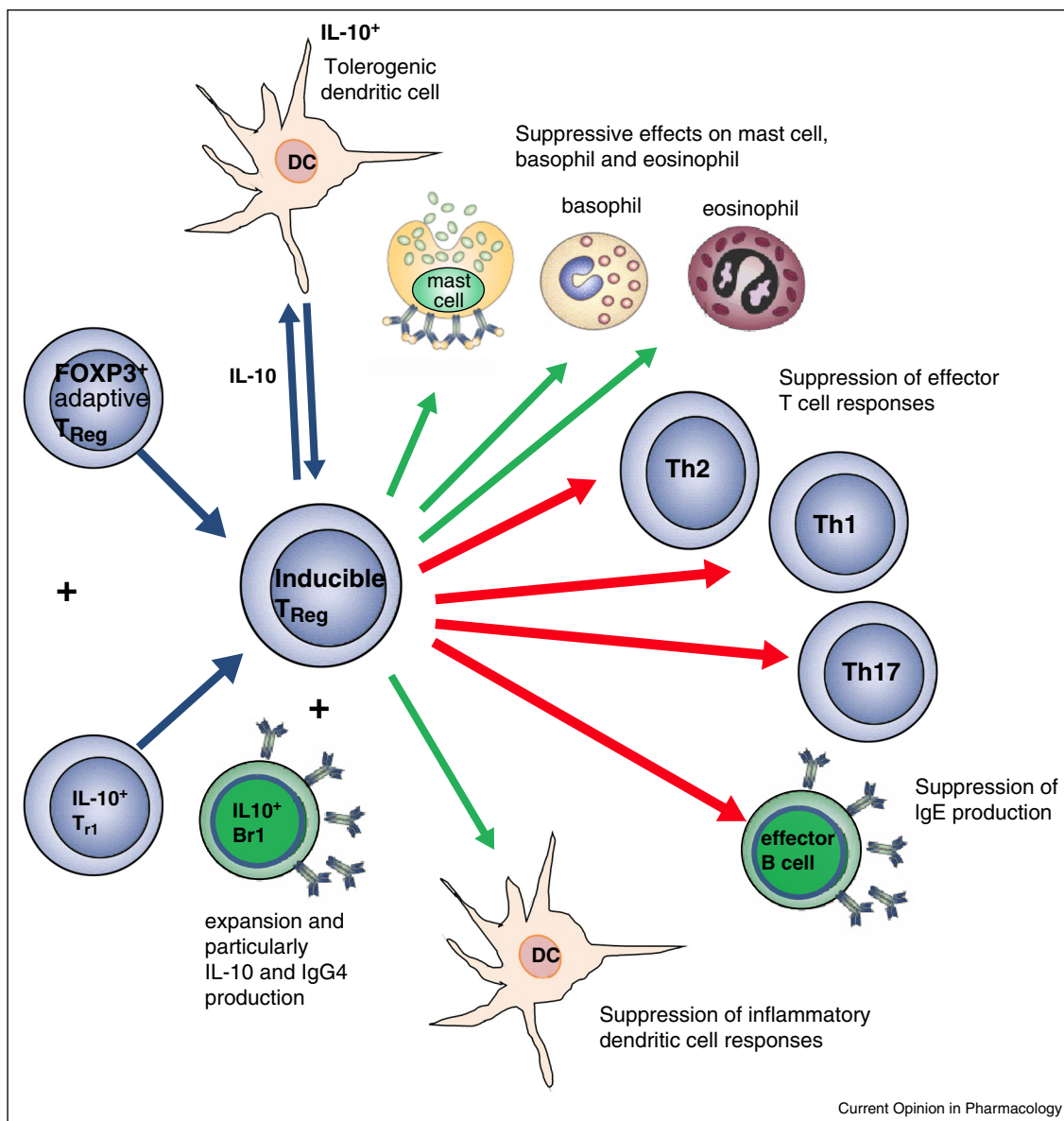
inflammation at gastrointestinal tract and lungs, but does not result in generation of an autoimmune pathology [13]. It seems that there are overlapping roles of iTreg cells and natural CD4⁺CD25⁺FOXP3⁺ Treg (nTreg) cells, which are generated in the periphery or originate from thymus [14]. iTreg cells are composed of FOXP3⁺ (Forkhead box protein 3) adaptive Treg cells and FOXP3 negative but IL-10-producing Tr1 cells [15]. FOXP3⁺ adaptive Treg cells were suggested to exert their function via cell contact mechanisms initially, but it is clarified that they also express high levels of suppressive cytokines IL-10 and TGF-beta similar to Tr1 cells, which utilize same suppressive cytokines to induce tolerance. Studies investigating the role of different types of Treg cells during AIT have shown contradictory results, which may arise from different timing schedule for the assessment of immunological changes or assessing the alterations at different organs. For example, during a one year birch pollen induced SCIT trial, the number of IL-10-secreting Tr1 cells were significantly increased by three months and stayed increased by 12 months in peripheral blood, whereas the number of CD4⁺CD25⁺CD127^{low}FOXP3⁺ adaptive Treg cells did not change [16]. In contrast, two years of grass pollen immunotherapy resulted in increased number of both FOXP3⁺ T cells and FOXP3⁺IL10⁺CD25⁺ T cells in nasal mucosa of patients with allergic rhinitis [17]. Overall, these data suggest overlapping functions of iTreg cells, CD25 Treg cells and Tr1 cells for the induction of tolerance during AIT.

The existence of FOXP3 expression and its level is also important for the suppressor activity of iTreg cells [14], which acquire FOXP3 expression in the periphery. They are generated in gut-associated lymphoid tissue, spleen, lymph nodes and inflamed tissues, where they have different TCR repertoires according to the anatomical locations and distinct repertoires from that of other antigen-experienced T cells within the same location [18]. High affinity TCR signaling [19] together with TGF-beta and IL-2 [15] and costimulation of increased cytotoxic T lymphocyte antigen-4 (CTLA-4 or CD152 on T cell surface) [20] and low CD28 signaling are optimum conditions for FOXP3 induction and iTreg cell generation

[14]. In addition to CTLA-4 signaling, the function of programmed death-1 (PD-1) on T cell surface is also important for the generation of suppressor activity of iTregs, which has been recently shown for a mouse model of cockroach allergen-induced asthma [21]. Additionally, the transcription factors RUNX1 and RUNX3 both have an effect on TGF-beta-mediated FOXP3 expression of iTreg cells in humans. They bind to FOXP3 promoter and activate Treg cells. The level of intracellular FOXP3 expression decreases to the lowest level, when RUNX1 and RUNX3 were both silenced, however there was no effect on the expression of the specific transcription factors of other T cell subsets, such as Th1 or Th2 [3]. As demonstrated recently, stimulation of ICOS results in increase in the number and function of peripheral iTreg cells [22]. Another surface marker existing on Treg cells is neuropilin-1, the expression of which correlates with expression of FOXP3 and suppressive function of Treg cells. However, this surface marker is selectively expressed on nTreg cells and not on iTreg cells in a mouse model of autoimmune pathology [23]. Besides these molecules, two other receptors namely OX-40 and glucocorticoid-induced tumor necrosis factor receptor (GITR), members of the TNF receptor super family are also found on Treg cells, but they are not specific to these cells. Studies regarding the activation and function of GITR on Treg cells reveal contradicting results, depending on the environmental status of the host and activation status of both effector and regulatory T cells [24]. The stimulation of OX-40 on naive CD4⁺ T cells prevented the expression of FOXP3 and generation of iTreg cells in response to TGF-beta, whereas blocking of OX40-OX40 ligand interaction resulted in generation of iTreg cells in a mouse model of allergen-induced airway disease [25]. There are numerous studies showing the function of these molecules and related molecular pathways performed in humans, suggesting that therapies targeting them may be involved in future therapeutic approaches for the modulation of immune tolerance in allergic diseases and asthma.

Once iTreg cell is generated, it further elicits IL-10-producing dendritic cells favoring further suppressive mechanisms [26]. Dendritic cells have been known to express a primary role in triggering naive T cells to activate and differentiate into different effector subtypes for many years. Later their dual capacity was noticed. According to their maturity stages and microenvironment, they are able to induce either inflammatory responses or immune tolerance [27]. Interleukin-10-secreting tolerogenic dendritic cells are semimature dendritic cells with a reduced cell surface expression of CD80, CD86, HLA-DR, CD40, and CD54 [28]. However, the expression of MHC-II on their surface is required for induction of Treg cells [29]. Besides semimature dendritic cells, ICOS-L-harboring mature plasmacytoid dendritic cells (pDC) have been also shown to differentiate naive CD4⁺ T cells into Treg cells [30]. It has been recently shown that pDC

Figure 1



Generation and function of Treg cells during AIT. FOXP3⁺ adaptive Treg cells together with Tr1 cells make up inducible Treg cell population during peripheral tolerance induction and IL-10⁺ tolerogenic dendritic cells contribute to their generation. Red and green arrows indicate the inhibitory and regulatory pathways of inducible Treg cells on several other cell types, respectively. Suppression of effector T cell types is the key point and several other mechanisms take place to induce tolerance.

colocalized with a high percentage of Treg cells in human palatine and lingual tonsils [31^{*}]. The authors have pointed that there is a significant correlation between Treg cell number and pDC number in human tonsils. Additionally, they have proven the ability of pDCs of human tonsil cells to generate CD4⁺CD25⁺CD127⁻FOXP3⁺ functional Treg cells which further supports the concept of tolerogenic function of dendritic cells [31^{*}]. Apparently, findings in humans and mice do not fully concur, and may show differences depending on the affected target organ and experimental conditions. For example CD103⁺ DCs, but

not pDCs induced FOXP3 expression in naive CD4⁺ T lymphocytes for pulmonary tolerance induction. In addition, CD103⁺ DCs, but not pDCs upregulated retinaldehyde dehydrogenase 2, which results in production of retinoic acid in a mouse model [32]. Further studies *in vivo* are needed to fully elucidate the role for different types of dendritic cells and helper molecules for the generation of iTreg cells in humans.

Another important question was recently focused on mechanisms of how allergen tolerance can be broken,

suggesting a mechanism of allergy development in a healthy individual. It was demonstrated that stimulation of allergen-specific T cells with certain toll-like receptors (TLR) and proinflammatory cytokines can induce *in vitro* CD4⁺ T cell proliferation in peripheral lymphocytes of individuals, who are tolerant. In this context, stimulation with IL-1 β , IL-6, TLR-4, TLR-8 of myeloid dendritic cell breaks allergen-specific CD4⁺ T cell tolerance [33].

Very recently the phenotypical expression of B regulatory cells in humans for an allergic disease was described. Apart from IL-10-secreting DCs, there exist IL-10-secreting allergen-specific Breg cells, which are shown to exist in bee venom tolerant beekeepers and bee venom allergic individuals, who had undergone venom immunotherapy [4*]. They are characterized as CD73⁺CD25⁺CD71⁺ B cells, which have a suppressive function on antigen-specific CD4⁺ T cells and have the capacity to specifically produce IgG4.

Besides Treg and B reg cells, human IL-10-secreting natural killer regulatory cell is shown to suppress allergen stimulated T cell proliferation and [34] may be a candidate to investigate in future studies of tolerance induction as well as other regulatory cell subsets.

What do iTreg cells do during the induction of tolerance? Examples from *in vivo* and *in vitro* studies of aeroallergen immunotherapy

Both cellular and humoral regulatory mechanisms take place in AIT (Table 1) [1]. The suppression of effector T cell subsets (Th2, Th1, and Th17 cells), inflammatory dendritic cells, mast cells, eosinophils and basophils, IgE production by B cells as well as induction of IL-10-producing dendritic cells and IgG4-producing B cells utilize multiple mechanisms. These mechanisms can be listed as cell contact dependent mechanisms via CTLA-4 and PD-1 receptors, because of cytokine signaling of IL-10 or TGF-beta or increased tryptophan metabolism under the supervision of iTreg cells [1,3]. In beekeepers it was shown that IL-10 secreting Treg cells inhibited the proliferation of PLA-specific effector

T cells seven days after the beginning of bee venom season. Additionally, this suppressive effect was inhibited by blocking CTLA-4, PD-1, and IL-10 receptors either as single molecules or simultaneously [35]. As an additional mechanism, tryptophan catabolising enzyme indoleamine 2,3-dioxygenase in dendritic cells is induced by Treg cells to generate immunoregulatory catabolites and to convert inflammatory dendritic cells into regulatory dendritic cells [36]. Despite the fact that the time points for the initiation of these immunologic events during AIT are based on injection schedules and individuals, a decrease in mast cell and basophil degranulation takes place initially. This is hypothetically dependent on the usage of three dimensional structure intact natural allergens, and may not happen in peptide immunotherapy. This is called 'early desensitization effect'. Afterwards allergen-specific iTreg cells start to be generated in an increasing manner with the capacity of suppression of effector T cells. Decrease in tissue mast cells and eosinophils and reduction in release of their mediators together with attenuation of late phase responses starts to occur after one to several months of AIT as a late effect [37]. During a modified cluster SCIT with *Phleum pratense*, increase in IL-10 production at 4th week, increase in allergen-specific IgG4 and IgA levels, reduction in early phase responses at 12th week were detected, and show significant changes compared to respective baseline values [38]. All these changes continued during the whole one year period. However, there was no reduction in specific IgE levels during the same time interval [38]. Generally, there is an early increase followed by a gradual, but late decrease over months or years in allergen specific IgE levels during AIT, which doesn't correlate with the reduction in degree of symptoms.

As a suggestive mechanism of early desensitization, there is a decrease in the number of basophils [39*], together with a loss of stored mediators and inhibition of their release from mast cells and basophils in the beginning of AIT. This may occur in a few hours or days, particularly if rush and ultrarush type of AIT is used [40]. This basophil suppression can be achieved through rapid upregulation

Table 1

Mechanisms of immune tolerance by Treg cells

Inhibitory and suppressive mechanisms	Regulatory and inductive mechanisms
Suppression of allergen specific Th2 cells and other effector cell types and their cytokines	Induction of allergen-specific inducible Treg cells
Decrease in degranulation and mediator release of mast cells, basophils, eosinophils	Induction of IL-10 secreting Br1 cells
Reduction in tissue numbers of mast cells, eosinophils and effector T cells and neutrophils	Further induction of tolerogenic dendritic cells
Decrease in proinflammatory cytokine production of mast cells and basophils	Increased secretion of IL-10 and TGF- β
Suppression of allergen specific IgE production	Increased FOXP3 expression
Suppression of IgE facilitated antigen presentation by B cells and dendritic cells	Increased allergen specific IgG4 and IgA production by B cells
Suppression of late phase allergic responses	Rapid upregulation of H2 receptors on basophils

of H2 receptors (R) in the first six hours and stimulation of these receptors on basophils *in vivo* [39^{*}]. By activation of H2R, allergen-induced FcεRI-mediated degranulation of basophil and release of mediators, histamine and sulfidoleukotriens are hampered. In addition, as a similar allergy suppression mechanism, H2R is predominantly expressed on allergen-specific Th2 cells compared to H1R. In allergen-stimulated cultures of human Th2 cells, the secretion of Th2 type cytokines IL-4 and IL-13 were found to be reduced upon stimulation with histamin and via H2R [41].

Another way for the prevention of basophil and mast cell degranulation during AIT is the synthesis and functional persistence of allergen-specific IgG4 [42], which blocks allergen-IgE interaction, inhibits IgE-facilitated allergen presentation to T cells and allergen-induced boost of memory IgE production [16,38,43,44^{**}]. IgG4 antibody is not able to activate complement cascade and cannot cross-link the antigen to form immune complexes, therefore has an immune suppressive function. IL-10 inhibits the production of total IgE and specific IgE, but enhances IgG4 and it is actively and directly involved in the suppression of allergen-specific T effector cells [45]. After one year of a cluster SCIT with *Der p*, patients showed significantly higher proportions of IL-10-producing Tr1 cells, IL-10 and IgG4 levels compared to baseline levels and nonallergic controls', and enhanced suppression of *Der p* 1 induced stimulation by Tr1 cells [46]. Although the concentration of blocking antibodies decrease in number after two years of discontinuation of SCIT in grass pollen allergic patients, their function remain to be maintained [47]. Apparently the mechanism of action of Treg cells is nearly the same in SCIT and SLIT, however, relatively bigger studies are needed to investigate it in more detail (Table 2).

Apart from SCIT and SLIT there is a relatively new way for application of AIT namely intralymphatic immunotherapy that has been shown to be efficient clinically for cat allergic individuals [8]. It has a novel feature that when cat allergen is applied intralymphatically with the vaccine containing MAT molecule, the required allergen dose for tolerance induction is 1000 times lower compared to the conventional SIT. This is suggested to be due to increased cellular internalization of the allergen and activation of inflammasome with the aid of MAT molecule [48^{**}]. Intralymphatic route for tolerance induction seems to work but further long term human studies with these novel molecules are needed clarifying the exact immunomodulatory mechanism in order to insert intralymphatic immunotherapy in daily clinical practice.

Immunotherapy combined with novel biologicals

Omalizumab is a humanized monoclonal antibody binding to IgE through its Fc portion. Omalizumab not only decreases the serum concentrations of unbound IgE molecule, but also decreases the expression of high affinity FcεRI on basophils, dendritic cells, and monocytes. It prevents the cross-linking of the allergen on mast cell and basophil surface. Thereby, it aids the improvement of symptoms in poorly controlled IgE-mediated respiratory diseases treated with conventional treatment. Omalizumab allows the patients to discontinue their inhaled corticosteroid during the course of its usage [7]. Several studies focused on efficacy and safety of omalizumab on AIT assessed at different time points during and after the end of the immunotherapy protocol [49,50,51]. In a cluster AIT protocol for allergic asthma, the use of omalizumab for 16 consecutive weeks before AIT and for three more weeks during the induction phase resulted in fewer systemic allergic reactions related with AIT and higher rates of patients reaching to the targeted

Table 2

Comparison of immunological changes during and after successful SCIT and SLIT in human studies

Immunological changes	SCIT	SLIT
Generation of allergen specific Treg cells	+	+
Increase in local FOXP3 ⁺ Treg cells	+ (nasal)	+ (oral)
Generation of allergen specific Breg cells	+	Not known
Increase in IgG4 level	+	+
Increase in IgA level	+	+
Decrease in IgE facilitated antigen binding	+	+
Increase in IL-10	+	+
Increase in TGF-β	+	+
Decrease in serum Th2 type cytokine response	+	+
Increase in serum Th1 type cytokine response	+ or no change	+ or no change
Reduction in number of tissue mast cell, eosinophil and basophils	+ (nasal)	+ (nasal)
Rapid desensitization	+	Not known
Early increase in allergen specific IgE	+	+
Decrease in spe IgE	+	+ or no change
Decrease in skin prick test reactivity	+	+ or no change
Seasonal blunting of IgE increase	+	+
Local action of particular dendritic cells to induce Treg cell generation	Not known	+ (oral mucosa)

maintenance dose at the end of initial therapy [51]. In another study, concurrent Omalizumab usage during 18 weeks of induction and maintenance phases of allergoid immunotherapy for grass pollen revealed same symptom severity, rescue drug usage rates and quality of life, but improved FEV1 levels compared to placebo group two years after the beginning of immunotherapy [50]. Omalizumab is also used before and during peanut and cow's milk AIT in clinical trials and increased the desensitization rates rapidly among high risk patients [52,53]. In contrast to studies showing its beneficial effects, a placebo controlled trial to assess the omalizumab in peanut immunotherapy was stopped due to severe anaphylactic reactions [54].

Food allergen immunotherapy

SLIT and OIT are the preferred routes of allergen administration in food AIT. Increase in Treg cells, IgG4 levels, reduction in skin prick test reactivity, basophil activation, basophil histamine release, food specific IgE levels were detected at the end of peanut and cow's milk OIT [55,56,57]. OIT for peanut allergy resulted in demethylation of FOXP3 CpG sites in antigen-induced Treg cells [58]. However, there are still some concerns regarding AIT for food allergy so that SLIT and OIT are not recommended for widespread clinical use due to safety concerns, and the lack of sustained immune tolerance [5].

An essential question in food AIT is the maintenance of clinical tolerance after stopping AIT. In other words, the majority of individuals receiving egg, cow's milk and peanut are desensitized to related food during AIT, but maintenance of unresponsiveness could not be achieved for longer periods. A local desensitization effect seems to take place, but long-term immune regulation leading to a long-term cure has not been confirmed so far. Therefore, OIT and SLIT for food AIT are still experimental and more research is needed for safe and efficient induction of tolerance to allergenic foods [6].

Conclusion

Generation of a functional T-reg cell response is the key point for the induction of peripheral tolerance to allergens. In addition to Treg cells, several other mechanisms related with different regulatory and non-lymphocyte lineage cell types, receptors, cytokines and regulatory pathways are shown to contribute to the development of peripheral tolerance in allergic diseases. There are several open points in the molecular mechanisms of induction of allergen tolerance and the elucidation of novel molecular pathways such as microRNAs in immune regulation can be foreseen [59]. As an additional important mechanism, defective mucosal surface epithelial barrier function may be one of the main players for relapses and inefficient responses to the therapy [60]. Although SLIT and SCIT can be used for respiratory allergens and

SCIT for bee venom allergens in daily clinical practice, so far their routine usage was not possible due to some safety concerns and natural history of food allergy. Nevertheless, the discovery of new biomarkers showing the immune tolerance capacity with new pathways and molecules for the mechanism of AIT and defining the efficacy of concurrent use of new biologicals during or before the initiation of protocol is expected to facilitate, appropriate responder patient selection and determining safer and more efficient routes of AIT.

Conflict of interest

M. Akdis has received grants from the Swiss National Science Foundation, EF7 McDALL, and EF7 Predicta. C. A. Akdis has consultant arrangements with Actellion, Aventis, Stallergenes, Allergopharma, and Circacia; is employed by the Swiss Institute of Allergy and Asthma Research, University of Zurich; and has received grants from Novartis, PREDICTA, the Swiss National Science Foundation, McDALL, and the Christine Kühne Center for Allergy Research and Education Research. Dr. Cavkaytar has no any potential conflict of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Akdis M, Akdis AC: **Immune tolerance**. In *Middleton's Allergy: Principle and Practice*. Edited by Adkinson Jr BSB, Busse BW, Holgate WW, Lemanske Jr ST, O'Hehir RE RF. Elsevier Saunders Press; 2014:45-64.
 2. Mauri C, Bosma A: **Immune regulatory function of B cells**. *Annu Rev Immunol* 2012, **30**:221-241.
 3. Akdis M, Akdis CA: **Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens**. *J Allergy Clin Immunol* 2014, **133**:621-631.
 4. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, Ruckert B, Akdis CA, Akdis M: **IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses**. *J Allergy Clin Immunol* 2013, **131**:1204-1212.
- IL-10+ IgG4 producing allergen specific B regulatory (Br1) cells are phenotypically and functionally defined in human peripheral blood mononuclear cells for the first time in nonallergic beekeepers and in venom allergic individuals before and after immunotherapy.
5. Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M, Nelson H, Akdis CA: **Update on allergy immunotherapy: American Academy of Allergy Asthma & Immunology/ European Academy of Allergy and Clinical Immunology/ PRACTALL consensus report**. *J Allergy Clin Immunol* 2013, **131**:1288-1296 e3.
 6. Syed A, Kohli A, Nadeau KC: **Food allergy diagnosis and therapy: where are we now?** *Immunotherapy* 2013, **5**:931-944.

7. Akdis CA: **Therapies for allergic inflammation: refining strategies to induce tolerance.** *Nat Med* 2012, **18**:736-749.
 8. Senti G, Cramer R, Kuster D, Johansen P, Martinez-Gomez JM, Graf N, Steiner M, Hothorn LA, Grönlund H, Tivig C *et al.*: **Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections.** *J Allergy Clin Immunol* 2012, **129**:1290-1296.
 9. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, Koivikko A, Norberg LA, Valovirta E, Wahn U *et al.*: **Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study.** *Allergy* 2007, **62**:943-948.
 10. Lou W, Wang C, Wang Y, Han D, Zhang L: **Responses of CD4(+) CD25(+) Foxp3(+) and IL-10-secreting type 1 T regulatory cells to cluster-specific immunotherapy for allergic rhinitis in children.** *Pediatr Allergy Immunol* 2012, **23**:140-149.
 11. Aslam A, Chan H, Warrell DA, Misbah S, Ogg GS: **Tracking antigen-specific T-cells during clinical tolerance induction in humans.** *PLoS ONE* 2010, **5**:e11028.
 12. Suarez-Fueyo A, Ramos T, Galan A, Jimeno L, Wurtzen PA, Marin A, de Frutos C, Blanco C, Carrera AC, Barber D *et al.*: **Grass tablet sublingual immunotherapy downregulates the TH2 cytokine response followed by regulatory T-cell generation.** *J Allergy Clin Immunol* 2014, **133**:130-138 e2.
 13. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY: **Extrathymically generated regulatory T cells control mucosal TH2 inflammation.** *Nature* 2012, **482**:395-399.
 14. Josefowicz SZ, Lu LF, Rudensky AY: **Regulatory T cells: mechanisms of differentiation and function.** *Annu Rev Immunol* 2012, **30**:531-564.
 15. Curotto de Lafaille MA, Lafaille JJ: **Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor?** *Immunity* 2009, **30**:626-635.
 16. Mobs C, Slotosch C, Löffler H, Jakob T, Hertl M, Pfützner W: **Birch pollen immunotherapy leads to differential induction of regulatory T cells and delayed helper T cell immune deviation.** *J Immunol* 2010, **184**:2194-2203.
 17. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT: **Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa.** *J Allergy Clin Immunol* 2008, **121**:1467-1472 e1.
 18. Lathrop SK, Santacruz NA, Pham D, Luo J, Hsieh CS: **Antigen-specific peripheral shaping of the natural regulatory T cell population.** *J Exp Med* 2008, **205**:3105-3117.
 19. Gottschalk RA, Corse E, Allison JP: **TCR ligand density and affinity determine peripheral induction of Foxp3 in vivo.** *J Exp Med* 2010, **207**:1701-1711.
 20. Zheng SG, Wang JH, Stohl W, Kim KS, Gray JD, Horwitz DA: **TGF-beta requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive CD4+CD25+ regulatory cells.** *J Immunol* 2006, **176**:3321-3329.
 21. McGee HS, Yagita H, Shao Z, Agrawal DK: **Programmed death-1 antibody blocks therapeutic effects of T-regulatory cells in cockroach antigen-induced allergic asthma.** *Am J Respir Cell Mol Biol* 2010, **43**:432-442.
 22. Busse M, Krech M, Meyer-Bahlburg A, Hennig C, Hansen G: **ICOS mediates the generation and function of CD4+CD25+Foxp3+ regulatory T cells conveying respiratory tolerance.** *J Immunol* 2012, **189**:1975-1982.
 23. Yadav M, Louvet C, Davini D, Gardner JM, Martinez-Llordella M, Bailey-Bucktrout S, Anthony BA, Sverdrup FM, Head R, Kuster DJ *et al.*: **Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo.** *J Exp Med* 2012, **209**:1713-1722 S1-S19.
 24. Ephrem A, Epstein AL, Stephens GL, Thornton AM, Glass D, Shevach EM: **Modulation of Treg cells/T effector function by GTR signaling is context-dependent.** *Eur J Immunol* 2013, **43**:2421-2429.
 25. Duan W, So T, Croft M: **Antagonism of airway tolerance by endotoxin/lipopolysaccharide through promoting OX40L and suppressing antigen-specific Foxp3+ T regulatory cells.** *J Immunol* 2008, **181**:8650-8659.
 26. Wisniewski J, Agrawal R, Woodfolk JA: **Mechanisms of tolerance induction in allergic disease: integrating current and emerging concepts.** *Clin Exp Allergy* 2013, **43**:164-176.
 27. Saei A, Hadjati J: **Tolerogenic dendritic cells: key regulators of peripheral tolerance in health and disease.** *Int Arch Allergy Immunol* 2013, **161**:293-303.
 28. Li X, Yang A, Huang H, Zhang X, Town J, Davis B, Cockcroft DW, Gordon JR: **Induction of type 2 T helper cell allergen tolerance by IL-10-differentiated regulatory dendritic cells.** *Am J Respir Cell Mol Biol* 2010, **42**:190-199.
 29. Huang H, Dawicki W, Lu M, Nayyar A, Zhang X, Gordon JR: **Regulatory dendritic cell expression of MHCII and IL-10 are jointly requisite for induction of tolerance in a murine model of OVA-asthma.** *Allergy* 2013, **68**:1126-1135.
 30. Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA, Qin XF, Liu YJ, Gilliet M: **Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand.** *J Exp Med* 2007, **204**:105-115.
 31. Palomares O, Ruckert B, Jartti T, Kucuksezer UC, Puhakka T, Gomez E, Fahrner HB, Speiser A, Jung A, Kwok WW *et al.*: **Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance.** *J Allergy Clin Immunol* 2012, **129**:510-520 e1-9.
- The function and phenotypical features of Treg cells residing in human palatine and lingual tonsils are characterized for the first time and their frequency is compared with that of peripheral blood.
32. Khare A, Krishnamoorthy N, Oriss TB, Fei M, Ray P, Ray A: **Cutting edge: inhaled antigen upregulates retinaldehyde dehydrogenase in lung CD103+ but not plasmacytoid dendritic cells to induce Foxp3 de novo in CD4+ T cells and promote airway tolerance.** *J Immunol* 2013, **191**:25-29.
 33. Kucuksezer UC, Palomares O, Ruckert B, Jartti T, Puhakka T, Nandy A, Gemicioglu B, Fahrner HB, Jung A, Deniz G *et al.*: **Triggering of specific Toll-like receptors and proinflammatory cytokines breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood.** *J Allergy Clin Immunol* 2013, **131**:875-885.
 34. Deniz G, van de Veen W, Akdis M: **Natural killer cells in patients with allergic diseases.** *J Allergy Clin Immunol* 2013, **132**:527-535.
 35. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M: **In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure.** *J Exp Med* 2008, **205**:2887-2898.
 36. Fallarino F, Grohmann U: **Using an ancient tool for igniting and propagating immune tolerance: IDO as an inducer and amplifier of regulatory T cell functions.** *Curr Med Chem* 2011, **18**:2215-2221.
 37. Akdis CA, Akdis M: **Mechanisms of allergen-specific immunotherapy.** *J Allergy Clin Immunol* 2011, **127**:18-27 quiz 28-19.
 38. Francis JN, James LK, Paraskevopoulos G, Wong C, Calderon MA, Durham SR, Till SJ: **Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity.** *J Allergy Clin Immunol* 2008, **121**:1120-1125 e2.
 39. Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, Zumkehr J, Jutel M, Akdis C: **Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2.** *J Allergy Clin Immunol* 2012, **130**:1153-1158 e2.
- The suppressor function of H2 receptor activation is revealed for the first time in pathophysiology of initial early desensitization of rush immunotherapy protocol.
40. Maintz L, Bussmann C, Bieber T, Novak N: **Contribution of histamine metabolism to tachyphylaxis during the buildup phase of rush immunotherapy.** *J Allergy Clin Immunol* 2009, **123**:701-703.

41. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, Zak-Nejmark T, Koga R, Kobayashi T, Blaser K *et al.*: **Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors.** *Nature* 2001, **413**:420-425.
 42. James LK, Bowen H, Calvert RA, Dodev TS, Shamji MH, Beavil AJ, McDonnell JM, Durham SR, Gould HJ: **Allergen specificity of IgG(4)-expressing B cells in patients with grass pollen allergy undergoing immunotherapy.** *J Allergy Clin Immunol* 2012, **130**:663-670 e3.
 43. Mobs C, Ipsen H, Mayer L, Slotosch C, Petersen A, Wurtzen PA, Hertl M, Pfutzner W: **Birch pollen immunotherapy results in long-term loss of Bet v 1-specific TH2 responses, transient TR1 activation, and synthesis of IgE-blocking antibodies.** *J Allergy Clin Immunol* 2012, **130**:1108-1116 e6.
 44. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, Staple SQ, Aalberse RC, Till SJ, Durham SR: **Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity.** *J Immunol* 2004, **172**:3252-3259.
- The pathophysiological mechanism that underlines the longstanding effect of aeroallergen immunotherapy is shown. Although the number of IgG4 antibodies decrease in number their inhibitory function against IgE-allergen binding goes on after two years of discontinuation.
45. Akdis M, Burgler S, Cramer R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O *et al.*: **Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases.** *J Allergy Clin Immunol* 2011, **127**:701-721 e1-70.
 46. Lou W, Wang C, Wang Y, Han D, Zhang L: **Enhancement of the frequency and function of IL-10-secreting type I T regulatory cells after 1 year of cluster allergen-specific immunotherapy.** *Int Arch Allergy Immunol* 2012, **159**:391-398.
 47. James LK, Shamji MH, Walker SM, Wilson DR, Wachholz PA, Francis JN, Jacobson MR, Kimber I, Till SJ, Durham SR: **Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies.** *J Allergy Clin Immunol* 2011, **127**:509-516 e1-5.
 48. Zaleska A, Eiwegger T, Soyer O, van de Veen W, Rhyner C, Soyka MB, Bekpen C, Demiröz D, Treis A, Söllner S *et al.*: **Immune regulation by intralymphatic immunotherapy with modular allergen-translocation MAT vaccine.** *Allergy* 2014 <http://dx.doi.org/10.1111/all.12461>.
- The mechanisms for the efficient immune regulation by MAT vaccine used for tolerance induction in cat allergic individuals are established for the first time with this study.
49. Kopp MV, Hamelmann E, Zielen S, Kamin W, Bergmann KC, Sieder C, Stenglein S, Seyfried S, Wahn U, group Ds: **Combination of omalizumab and specific immunotherapy is superior to immunotherapy in patients with seasonal allergic rhinoconjunctivitis and co-morbid seasonal allergic asthma.** *Clin Exp Allergy* 2009, **39**:271-279.
 50. Kopp MV, Hamelmann E, Bendiks M, Zielen S, Kamin W, Bergmann KC, Klein C, Wahn U, group Ds: **Transient impact of omalizumab in pollen allergic patients undergoing specific immunotherapy.** *Pediatr Allergy Immunol* 2013, **24**:427-433.
 51. Massanari M, Nelson H, Casale T, Busse W, Kianifard F, Geba GP, Zeldin RK: **Effect of pretreatment with omalizumab on the tolerability of specific immunotherapy in allergic asthma.** *J Allergy Clin Immunol* 2010, **125**:383-389.
 52. Schneider LC, Rachid R, LeBovidge J, Blood E, Mittal M, Umetsu DT: **A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients.** *J Allergy Clin Immunol* 2013, **132**:1368-1374.
 53. Nadeau KC, Schneider LC, Hoyte L, Borrás I, Umetsu DT: **Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy.** *J Allergy Clin Immunol* 2011, **127**:1622-1624.
 54. Sampson HA, Leung DY, Burks AW, Lack G, Bahna SL, Jones SM, Wong DA: **A phase II, randomized, doubleblind, parallelgroup, placebocontrolled oral food challenge trial of Xolair (omalizumab) in peanut allergy.** *J Allergy Clin Immunol* 2011, **127**:1309-1310 e1.
 55. Keet CA, Frischmeyer-Guerrero PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, Steele P, Driggers S, Burks AW, Wood RA: **The safety and efficacy of sublingual and oral immunotherapy for milk allergy.** *J Allergy Clin Immunol* 2012, **129**:448-455 e1-5.
 56. Varshney P, Jones SM, Scurlock AM, Perry TT, Kemper A, Steele P, Hiegel A, Kamilaris J, Carlisle S, Yue X *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.
 57. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, Shreffler WG, Steele P, Henry KA, Adair M *et al.*: **Clinical efficacy and immune regulation with peanut oral immunotherapy.** *J Allergy Clin Immunol* 2009, **124**:292-300 e1-7.
 58. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, Berglund JP, Tsai M, Maecker H, O'Riordan G *et al.*: **Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3).** *J Allergy Clin Immunol* 2014, **133**:500-510.
- The first study to show allergen induced Treg cells with enhanced suppressive and chemotactic functions and epigenetic changes within the FOXP3 locus of these Treg cells in food AIT.
59. Rebana A, Akdis CA: **MicroRNAs: essential players in the regulation of inflammation.** *J Allergy Clin Immunol* 2013, **132**:15-26.
 60. Soyka MB, Wawrzyniak P, Eiwegger T, Holzmann D, Treis A, Wanke K, Kast JI, Akdis CA: **Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN-gamma and IL-4.** *J Allergy Clin Immunol* 2012, **130**:1087-1096 e10.