Mechanisms of immune tolerance to allergens in children

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ABSTRACT:

As the prevalence of allergic diseases has significantly increased over the last years, understanding the causes and mechanisms of these disorders gains great interest and as a result, intense investigations are achieved in this field. The current knowledge pinpoints immune tolerance mechanisms as indispensable for healthy immune response to allergens in daily life. It is evident that the generation and maintenance of allergen-specific T-cell tolerance is of vital importance for a healthy immune response to allergens, which is gained spontaneously by dose-dependent exposures to allergens in nature or by allergen-specific immunotherapy. Allergen-specific immunotherapy induces regulatory T cells with capacity to secrete IL-10 and TGF-β, limits activation of effector cells of allergic inflammation (such as mast cells and basophils), and switches antibody isotype from IgE to a non-inflammatory type IgG4. However, although allergen specific immunotherapy stands as the only method of tolerance induction in allergic individuals, several factors like long duration of treatment, compliance problems and life threatening side effects has limited the widespread applicability of this immuno-modulatory treatment. Current research concerns the introduction of allergens in more efficient and safer ways to overcome these limitations. Defining the endotypes and phenotypes of allergic diseases may provide selection chance of the ideal patients; additionally novel biomarkers will ensure new custom-tailored therapy modalities.
**Introduction**

Immune tolerance is the essential way of continuity of homeostasis. In the network of immune regulation a continuous process of stimuli-response interaction takes place in a harmony with functional tolerance mechanisms. Contact of the host with plenty of antigens in daily life, especially through mucosal surfaces, bears a great burden on the highly reactive immune system, which shall result with healthy unresponsiveness to these stimuli as seen in *tolerance*. It is clear that this unresponsiveness is mutual for the well-being of the host, as well. What happens in the state of responsiveness, as in *intolerance*? External antigens or allergens can trigger hypersensitivity reactions, which harm the host and may present clinically as allergic rhinitis, asthma, atopic dermatitis, food allergy or anaphylaxis. This is also valid for internal antigens. Intolerance to self will result with the development of autoimmune disorders. On the other hand, excessive tolerance is another state of health problem, which may lead to invasion by microorganisms, parasites, or to cancer development. Clearly, regulation of tolerance is essential for the continuity of life. The best example for this paramount regulation is the state of pregnancy, in which tolerance to paternal and fetal antigens has to go on without any immune reactions until labor.

Tolerance development might be therapeutically manipulated to restore normal immunity in conditions such as allergic and autoimmune disorders. Allergen-specific immunotherapy is one of the best models for visualizing the tolerance induction to external antigens [1]. Understanding the key steps in allergen-specific immunotherapy may also shed light on the development of novel therapeutic approaches in the field of autoimmune disorders and cancer area [2].

**Allergic immune response**

The allergic immune response is directed against various environmental allergens and manifests clinically as allergic rhinoconjunctivitis, allergic asthma, atopic dermatitis, food allergy and/or anaphylaxis. In atopic individuals under the influence of genes and microenvironment, it is proposed that a tendency to develop T helper
(Th)-2 type immune response is prominent [3]. Subsets of immune and inflammatory cells interact via cytokines and the key cytokines responsible for this allergic response include interleukin (IL)-4, IL-13 and IL-5 [4]. Specific recognition of antigenic determinants (epitopes) of allergens by T and B lymphocytes elicits the immune response [5]. This recognition is controlled by highly specialized antigen presenting cells located in strategic positions like mucosal surfaces (gastrointestinal mucosa, airway epithelium) and dermis. Processing and presentation of allergenic epitopes to T-helper (Th) lymphocytes in the presence of relevant co-stimulatory cytokines, chemokines, signals, vitamins, histamine-adenosine-like small molecules and other cells in the micro milieu shape the immune response [6, 7]. Especially in the presence of IL-4, naïve T cells activated by antigen-presenting cells differentiate into Th2 cells. In the presence of IL-4 and IL-13, class-switching in B cells promotes the synthesis of IgE antibodies. Allergen-specific IgE antibodies bind to high-affinity FcεRI receptors that are expressed on mast cells and basophils, in which re-exposure to the sensitizing allergen activates these cells to release and produce biogenic mediators (release of histamine and proteases, the synthesis and release of newly generated lipid-derived mediators, such as leukotrienes and cytokines), responsible for the symptoms and signs of type I hypersensitivity allergic reactions. Thereafter, in the late-phase response, 6- to 12-hours after allergen exposure, a cell-driven process with infiltration of eosinophils, neutrophils, basophils, T lymphocytes, and macrophages occur and release of additional inflammatory mediators and cytokines, perpetuating the proinflammatory response take place [8, 9]. In this phase, especially the role of IL-5, the cytokine responsible for the activation, survival and tissue recruitment of eosinophils, is marked. This late-phase response is thought to be responsible for the persistent, chronic signs and symptoms of allergic diseases and continued allergen exposure often establishes a state of chronicity [10], (Figure 1).

**Allergic immune response in clinical perspectives**

Allergic immune response and atopic status of children bear a great area of interest in today’s science as childhood period gives an opportunity to understand the developmental steps of allergic disorders and natural history of allergic disorders. In
the most widely-known **atopic march** concept, in atopic individuals, introduction to new antigens (possible allergens) during weaning from breast feeding may present clinically as atopic dermatitis or gastrointestinal intolerability. With the evolving immune response, a state of tolerance develops to most common food allergens within time. Thereafter atopic child starts to develop immune hypersensitivity to aeroallergens, especially in the very early nursery years. These children generally suffer from allergic rhino-sinusitis and/or asthma symptoms in those years, requiring long periods of treatment regimens, with concomitant decreased quality of life [11]. Although allergen sensitization is known as a common cause of these disorders, substandard avoidance measures may not resolve the problem. Even inadequacy of conventional pharmacotherapy regimens proposed by highly cited guidelines for the disease control is another issue. In addition to multiple factors enrolled in triggering, different phenotypes and endotypes of allergic disorders need to be clarified for a better understanding of allergic immune response. Even developments in this field in future may enlighten novel customized therapeutic approaches in the field of pharmacotherapy of allergy. Several meta-analyses have also been already delineated the impact of allergen specific immunotherapy in tolerance development. The intersection of allergic disorders in some time periods of growing child, shaping of immune response depends on multi-factorial stimuli. These include our genes, microenvironment of the organism and concomitant triggering events, age of the organism as for the natural history of atopic disorders.

**Regulatory T cells**

Humans can act to allergens in various ways. For a healthy immune response, unresponsiveness to allergens is of vital importance. Immune tolerance to allergens can be defined as the formation of long-term clinical tolerance against allergens, which is sustained by changes in memory-type, allergen-specific T and B cell responses and up-regulation of mast cell and basophil activation thresholds with the eventual control of symptoms of allergy [8, 12-14]. Regulatory T (Treg) cells are a subset of T cells with immune regulatory properties. The naturally occurring, thymus-selected $CD^+CD^{25+}FOXP^+$ Treg cells and the inducible type-1, IL-10-secreting Treg cells (Tr1
cells) are the major subsets of Treg cells. Tr1 cells are responsible from maintenance of peripheral tolerance. Forkhead box P3 (FoxP3) is the lineage-specific transcription factor for CD4⁺CD25⁺ Treg cells, which has a functional master regulator role in development of Treg cells [15]. Various suppressor and regulatory mechanisms have roles in maintenance of immune homeostasis by Treg cells, which helps to inhibit the development of allergen-specific Th2 and Th1 cell responses, and also directly or indirectly suppress effector cells of allergic inflammation [16] (Figure 2). Impaired allergen-specific suppressive function of CD4⁺CD25⁺ Treg cells from allergic patients has been shown in comparison with non-allergic controls. Circulating CD4⁺CD25⁺ Treg cells in non-allergic healthy individuals have been shown to suppress proliferation of allergen-specific effector cells on exposure to allergens, whereas Treg cells from sensitized individuals are not able to do so to the same extent [17].

**Induction of allergen-specific peripheral tolerance**

It is evident that peripheral T-cell tolerance induction through the generation of allergen-specific regulatory Tr1 cells is of vital importance in healthy immune responses to allergens [18-20]. Normally, central tolerance is the major mechanism for the determination of overall T-cell numbers, however some T-cells may escape from thymic deletion and for this reason, peripheral tolerance is developed in peripheral lymphoid organs, and is regulated by facts like T-cell anergy, apoptosis, Treg cells, suppressive cytokines and antigen presenting cells (APCs) [21]. Studies have shown that a peripheral T cell repertoire, which recognizes same T cell epitopes of allergens as allergic patients also exists in healthy individuals and the precise balance between the frequency of allergen-specific Th2 cells and allergen-specific Tr1 cells to common environmental allergens is shown to define an allergic or a healthy outcome [10]. These findings underline the important contribution of Tr1 cells to the active regulation, which is important in inducing and maintaining specific unresponsiveness to allergens. This unresponsiveness is mainly maintained by the production of IL-10 and TGF-β, which are produced by antigen-specific Treg cells. Both naturally occurring FOXP3⁺ and inducible Tr1 cells are claimed to take part in establishment of peripheral T cell tolerance to allergens [22]. Establishment of
clinical tolerance in human subjects has been shown to be associated with the loss of IL-4-producing T cells and increase of IL-10-producing antigen-specific Treg cells [23]. In addition to IL-10, Treg cells produce TGF-β, another suppressive cytokine. Moreover, there is a switch in the cytokine production frequencies of antigen-specific T-cells. Reduction in production of IL-4 and increase in IL-10-production are marked [23]. IL-10 and related cytokines IL-19, IL-20, IL-22, IL-24 and IL-26 participate in T cell-mediated diseases by distinct regulation of T cell cytokine profiles [24]. In addition to IL-10, expressions of co-stimulatory molecules CD80, CD86 and programmed cell death ligand 1 (PD-L1) are reduced, which may have down-modulatory effects on T-cell immune responses [25]. Treg cells also express CTLA-4 strongly, which inhibits activation of T cells in contrast to CD28 [26]. TGF-β is an important suppressive cytokine, which is essential for the maintenance of immunologic self-tolerance [27]. Naïve CD4⁺ T cell–Treg conversion requires induction of FOXP3 by TGF-β, which is required for expansion and suppressive capacity of Treg cells at the same time [28, 29].

**Impact of allergen presentation**

Dendritic cells (DCs) are important members of immunity and play a pivotal role in the orchestration of immune responses by linking innate and adaptive immunity [30]. Circulating DCs in human subjects can be divided into two main groups: myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC), both of which are equipped with distinct repertoire of Toll-like receptors (TLRs) [31, 32]. pDCs express TLR7 and TLR9, whereas mDCs express TLR8 [33]. Loss of allergen-specific peripheral T-cell tolerance in response to TLR8-ligand(L) stimulation but not to TLR7L or TLR9L stimulation underlines the contribution of pDCs to tolerance induction [34], which is in line with the findings supporting the important roles of pDCs in the induction and maintenance of peripheral tolerance to food and inhalant allergens in human tonsils [35]. Else than DCs, TLRs also regulate B-cell responses. Stimulation of B cells with ligands for TLR3, TLR7 and TLR9 induce memory B-cell generation as well as IgG1, IgA and IgG4 production. Mammalian telomeric oligodeoxynucleotide (ODN) suppresses activation of B cells, production of antibodies and generation of memory. All these findings support roles of TLR-triggering in the regulation of allergic responses [36].
**The other players of immunity**

Subsets of antigen-presenting cells as DCs and B-cells with IL-10 production capability were identified, which were shown to contribute to the suppressive effects of IL-10 [37, 38]. Natural killer (NK) cells are important members of innate immunity. Circulating NK cells retain effector subsets with distinct cytokine profiles as NK1 and NK2, similar to Th1 and Th2-cells. These cells are claimed to display different inflammatory properties [39]. Recent studies have revealed a NK cell subset with IL-10 production, which has suppressive effects on allergen-specific T-cell responses [40]. A B cell subset capable of producing IL-10, expressing IgG4 and suppressing allergen-specific CD4+ T cell proliferation has been very recently demonstrated, which is named as B-regulatory 1 (BR1) cells [41]. Suppression by IL-10 occurs in various ways. T cells are suppressed by blocking of CD2, CD28 and inducible co-stimulator (ICOS) co-stimulatory signals by IL-10, through the use of Src homology 2 domain-containing tyrosine phosphatase (SHP-1), which rapidly binds to CD28 and ICOS and de-phosphorylates them [42, 43]. IL-10 has inhibitory effects also on activated monocytes and macrophages, by suppression of co-stimulatory molecules on these cells and by down-regulation of MHC class II molecules and antigen-presenting cell capacity [44, 45]. It has been shown that expression of the suppressor of cytokine-signaling 3 gene (SOCS3) was induced by IL-10, pointing the possible contribution of IL-10 by the inhibition of IFN-γ-induced tyrosine phosphorylation of signal transducer and activator 1 (STAT1) [46].

**Antibody responses during tolerance development**

Induction of peripheral tolerance also induces changes in antibody isotypes. Serum allergen-specific IgE levels decrease gradually, while allergen-specific IgG4 type blocking antibodies increase during allergen-SIT [9, 47, 48]. In contrast to T cells, B cells do not become fully suppressed to antigenic stimuli, but they switch to produce not IgE but IgG4 instead [47]. IL-10 contributes to the regulation of specific antibody isotypes towards a non-inflammatory phenotype [49, 50]. Important blocking effects of IgG4 is maintained by its ability to compete with binding of antigens to the IgE on the Fcc receptors, which are expressed on surface of mast cells and basophils, and consequently limits effector cell activation and degranulation [51]. In addition,
blocking antibodies can also prevent the activation of CD4$^+$ cells by inhibiting CD23-mediated, IgE facilitated presentation of antigens [52].

**Immune regulatory role of histamine**

Histamine has functional roles on immune regulation through four distinct histamine receptors (HR). Among these, HR2, which is relatively highly expressed on Th2 cells, induces Treg cells and positively interferes with the peripheral antigen tolerance. Up-regulation of HR2 increases IL-10 levels, suppresses T cell stimulation and enhances the suppressive action of TGF-β on T cells. On the other hand, HR4 modulates migration of eosinophils and recruitment of mast cells, involves in DC activation and T-cell differentiation as well [7, 53].

**Conventional routes for immunotherapy; subcutaneous and sublingual ways**

The way how immune tolerance is induced has been investigated deeply in recent years. The therapeutical induction of immune tolerance either as in allergen-specific immunotherapy (SIT) or spontaneous induction as in bee keepers or as in cat owners has great importance and contributions to this field. Complex mechanisms are claimed to be responsible for the establishment of peripheral tolerance [50, 54, 55]. SIT is known to be the only available curative treatment of the allergic diseases. Peripheral tolerance induction by the generation of allergen-specific Treg cells, which have suppressive proliferative and cytokine response capabilities, is the consequence of SIT [19, 49]. Subcutaneous (SCIT) and sublingual (SLIT) routes are major routes for immunotherapy, both of which are being applied successfully in allergic rhinitis and asthma patients, both in children and adults, in addition to bee venom injection immunotherapy [56, 57]. Increased production of IFN-γ following SLIT has been correlated with the success of immunotherapy [58].

Peripheral tolerance can also be established as a consequence of natural, high dose allergen exposure. In healthy non-allergic bee keepers and cat owners [55, 59], Treg cells specific for the relevant major allergens become the major T cell subset of these individuals, which utilize multiple suppressive mechanisms like IL-10 and TGF-β as secreted cytokines, and CTLA-4 and PD-1 as surface molecules. High-dose
exposure models as in bee-keepers brighten our understanding of the nature of Treg responses in immune tolerance induction [22]

**Novel therapeutic approaches of allergen specific immunotherapy**

The side effects and long duration of treatment in allergen specific immunotherapy are undeniable negative impacts on the preference of this strategic treatment. Improvements in the ease of administration and safety may open possible new fields of indications as atopic dermatitis, food allergies and for large local reactions after bee stings. Modified allergens and routes are promising developments in the field of allergen specific immunotherapy. Knowledge of the influence of IgE-facilitated antigen presentation on allergen-specific Th2 responses have lead to investigate ways to generate non-IgE-binding allergens [60]. By genetic engineering approaches, two bee venom major allergens, phospholipase A2 and hyaluronidase, have been fused with the aim of deleting B-cell epitopes to prevent IgE cross-linking and to preserve T-cell epitopes due to conformational changes. Novel fusion protein Api M (1/2) causes abolished IgE reactivity, reduced basophil degranulation and emerged type-1 skin reactivity [61]. In addition to allergen modification, routes of introduction are also under intense investigation. A novel method to introduce the allergen in a more efficient way is recently described. Modular antigen translocating (MAT) molecules present the allergen to the major histocompatibility class-II pathway intracellularly, which enhances antigen presentation. The rapid translocation of the MAT-fused allergens in the cytoplasm bears a great advantage. Intracytoplasmic accumulation of the MAT–fused allergens can induce stronger proliferation responses to the corresponding allergens even nearly 10-100 times lower concentrations. Cytokine responses in PBMC cultures reveal marked increases in IL-10 and IFN-γ secretion and decreases in IL-4 and IL-5 responses compared with those induced by the corresponding recombinant allergen [62].

As a promising route, intralymphatic allergen administration under ultrasound guidance can induce tolerance simply in three injections, which provides a great advantage on classical multiple injections of subcutaneous immunotherapy. Recently, in a randomized double-blind study safety and efficacy of intralymphatic immunotherapy (ILIT) with recombinant major cat dander allergen Fel d 1-MAT
molecule had been shown. The increase in nasal reactivity to the allergen in the ILIT group is pronounced than the placebo group. Also, it has been shown that in the ILIT group T regulatory cell response had been induced in addition to IL-10 response, as well as increased cat dander specific IgG4 levels [63]. In another double-blind, placebo-controlled study, clinical improvement in nasal allergic symptoms has been reported in patients with allergic rhinitis, who had been treated with 3 intralymphatic injections of birch or grass pollen [64]. Similarly, epicutaneous immunotherapy (EPIT) is another novel approach in which highly immunogenic skin is the target. Although the first successful intervention had been held in 1921 in a horse dander allergic asthmatic patient [65], nowadays this route again gains attention. Recently in a placebo-controlled, double-blind study the safety and efficacy of EPIT in grass pollen allergic rhinitis patients has been shown in a dose-dependent manner after 6 patches only [66]. In another prospective double-blind trial in grass pollen allergic children, transcutaneous administration of the allergens has been shown to be effective in reducing symptoms and also the use of antihistamines [67]. Progresses in EPIT are promising as this method can be a non-invasive alternative for immunotherapy in patients with injection-phobia, especially in children.

**Loss of peripheral tolerance with the view of inflammation:**
Allergen-specific tolerance is maintained long-term, once established. However, observation of allergic exacerbations followed by infections, especially viral ones, triggered performance of studies investigating breakage of peripheral tolerance. Recent findings revealed that activation of innate immunity by specific activators condition specific adaptive immune responses to allergens. IL-1β and IL-6 cytokines with pro-inflammatory properties as well as triggering of TLR4 and TLR8, which recognize microbial particles as danger signals have been shown to lead to proliferation of allergen-specific CD4+ T cells in peripheral bloods of normally unresponsive individuals by various possible ways like forming an inflammatory milieu, potentiating mDCs or by rendering T cells unresponsive to Treg suppression [34]. These findings may shed light on how healthy subjects develop allergic diseases upon encountering microbes or inflammatory conditions.
**Future perspectives**

It is evident that allergen specific immunotherapy is still keeping its leading position in the long-term management of allergic disorders. Fall-short of strict allergen avoidance measures and failure of sustainability of clinical responsiveness to pharmacotherapies increase the demand for more efficient and safer allergen immunotherapy regimens. With the increasing understanding of the roles of cytokines, chemokines, pathways, networks and cellular interactions, novel modulated routes are tested and allergens are being developed in the pipeline for more successful immunotherapy courses. However, the same immunotherapy regimens can show variable clinical response in allergic individuals. This may be due to different endotypes and phenotypes of allergic diseases. Assignment of biomarkers to identify these endotypes and phenotypes may provide optimum patient selection for the relevant immunotherapy regimen. Definition of these phenotypes and endotypes will bring out the opportunity to select the best patient for more efficient treatment [68]. When the key questions; ‘In which patient?, Which route?, Which allergen? For how long duration should immunotherapy be given?’ are also being answered, then the field of applicability of allergen specific immunotherapy will be broadened. Thus the importance of establishing data to describe these differences in between endotypes and phenotypes is crucial. Mechanistic studies aiming better understanding of diseases and risk factors, to guide towards an improved diagnosis and therapy should be performed [69]. Characterization and increased understanding of the underlying disease mechanisms in allergic diseases will warrant possible use of individualized combined applications of immune response modifiers with allergen-SIT.

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References:


Figure legends

**Figure 1. Initiation of allergy:** DCs present peptides of allergens to naïve CD4+ T cells and with the existence of IL-4 in the milieu, consequent induction of Th2 cells occurs. Th2 cells produce cytokines: IL-3, IL-4, IL-5, IL-9, IL-13, which are named as Th2-type cytokines. B cells switch to produce IgE and binds to specific Fce receptors on mast cells and basophils. This is known as sensitization and upon encountering the same allergen for the next time, degranulation of mast cells and basopils takes place, leading to immediate hypersensitivity. Th2-type cytokines are important survival signals for mast cells basophils and eosinophils.

**Figure 2. Mechanisms of tolerance to allergens:** Allergen specific immunotherapy as well as high dose encountering with allergens induce Treg cells, which results with peripheral tolerance development. The effector cells of allergic inflammation are regulated by regulatory and suppressive functions of Treg cells in various ways. Treg cells suppress Th2 cells and their cytokine productions (IL-3, IL-4, IL-5, IL-9 and IL-13), which are indispensible for the differentiation, survival and activity of mast cells, basophils, eosinophils and mucus producing cells as well as tissue homing of Th2 cells. IL-10 and TGF-β suppress IgE production and meanwhile induce IgG4, which is a non-inflammatory Ig isotype.
Induction of Th2 cells

Naïve T

Th2

IL-4

Induction of IgE

B cell

Production of Th2-type cytokines

IL-3
IL-4
IL-5
IL-9
IL-13

Th2-type effector functions

basophil

eosinophil

Activation of effector cells of allergy

IgE

Figure 1
Allergen entry via:
- Natural high dose
- Immunotherapy routes

Induction of allergen-specific Treg cells

Th2

IL-10, TGF-β

IL-4, IL-13

Th2 cell homing

IL-3, IL-4, IL-5

Mast cell
Basophil
Eosinophil

Suppression of mucus production

Early desensitization of Mast cells & basophils

TReg

IL-10, TGF-β

IL-4, IL-13

IgE production

Mucus production

Endothelial cells

Suppression of Th2 cell homing to tissues

Early induction of IgG4
Late decrease in IgE