Cellular and Humoral Mechanisms of Immune Tolerance in Immediate-Type Allergy Induced by Specific Immunotherapy

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Abstract
The management of immediate-type allergy (ITA) is based on allergen avoidance, symptomatic pharmacological therapy and specific immunotherapy (SIT). Among these, SIT presents the only curative treatment. The efficacy of SIT in the treatment of IgE-mediated ITA has been proven in numerous clinical studies and is well established. This review discusses the relevance of immunoregulative humoral and cellular mechanisms leading to immune tolerance in ITA. Special focus is placed on the role of antibodies potentially interfering with the IgE-mediated immune reaction and regulatory T (T reg) cells including their immunosuppressive cytokines, which play a critical role in shifting the T helper 2 cell-driven allergic immune response towards allergen tolerance. Distinct subsets of constitutive and inducible T reg cells have been identified inhibiting the activation of allergen-specific effector T cells via cell contact- or cytokine-dependent suppression. Current research suggests that both inducible interleukin-10-producing CD4+ T reg cells and naturally occurring CD4+CD25+ T reg cells actively control allergic responses and that the disturbance of their function or number may contribute to the development or progression of allergy. Thus, the fine balance between allergen-specific T helper 2 and T reg cells constitutes a critical factor for the successful treatment of ITA by SIT.

For a long time, specific immunotherapy (SIT), the only approved curative treatment capable of modifying the natural history of individuals suffering from immediate-type allergy (ITA), was based merely on experience. Pioneered by Noon and Freeman almost a century ago, who treated patients suffering from allergic rhinoconjunctivitis with a low-dose incremental schedule of pollen injections [1], the basic principles of SIT have stayed the same. In 1998, the WHO accepted the therapeutic benefit of SIT [2], and recommendations were published regarding the mode of application and the modalities of SIT treatment. Until now, the effects of SIT have been documented in numerous controlled clinical studies with different allergens, such as birch and grass pollen [3, 4]. Only recently, the cellular and molecular mechanisms of SIT became more and more the objective of research studies.
**Immediate-Type Allergy**

ITA, classified as type 1 allergy by Coombs and Gell [5], is characterized by the production of allergen-specific IgE antibodies. The initial step in the development of ITA is the priming of allergen-specific CD4+ T helper 2 (Th2) cells [6]. Production of Th2 cell cytokines, such as interleukin (IL)-4, IL-5, IL-9 and IL-13, is essential in this process since T cell activation in the presence of IL-4 increases the differentiation of naive T cells into Th2 cells [7–9]. In addition, the secretion of IL-4 and IL-13 by Th2 cells in the presence of allergen recognized by naive B cells leads to the synthesis and secretion of allergen-specific IgE by plasma cells [10]. The central role of IgE antibodies in ITA such as allergic rhinoconjunctivitis, asthma or anaphylaxis is well known. Cross-linking of IgE receptors on the surface of effector cells such as basophils and mast cells by complexes of IgE antibodies and captured allergen results in an immediate-type immune reaction characterized by the release of histamine and the synthesis of prostaglandins, leukotrienes, proinflammatory cytokines and other mediators of the allergic response.

**SIT: Humoral Mechanisms**

During the course of SIT, a progressive decrease in allergen-specific IgE serum levels (following an initial transient increase) to preimmunotherapy levels or lower [11, 12] as well as moderate or no alterations in allergen-specific IgE antibody titers can be observed [13, 14].

**Allergen-Specific IgG Antibodies**

Apart from changes in IgE levels, an increase in allergen-specific IgG antibodies has been reported [13, 15]. These antibodies can block IgE-mediated anaphylaxis in vivo and seem to inhibit not only the allergen-induced release of inflammatory mediators from basophils and mast cells, but also IgE-mediated allergen-presentation to T cells [16].

Among the SIT-induced IgG antibodies, allergen-specific IgG4 has been proposed to play an important ‘protective’ role, since it competes with allergen-specific IgE antibodies for binding to administered allergen and can prevent the activation of CD4+ T cells by inhibition of IgE-mediated antigen presentation [17–19]. Furthermore, the IgG4 subset, which is secreted in significant quantities after prolonged SIT and remains stable during long intervals of continued immunotherapy [20, 21], promotes a significant reduction in mast cells and eosinophils accompanied by a diminished release of inflammatory mediators [22–24]. It was thus suggested, that increasing IgG4 antibody levels correlate with the clinical efficacy of SIT [25, 26] and may be considered as a marker of SIT-induced immune tolerance and decreased allergen sensitivity [2, 15, 27].

However, in a number of studies, subsets of patients with good clinical relief had no significant IgG antibody production, and some patients with increased IgG antibody titers showed no clinical response [28, 29], raising the possibility that the synthesis of IgG antibodies may rather reflect high allergen exposure than play a causal role in successful immunotherapy. Since there is a great interindividual variability in IgG4 levels among SIT-treated allergic patients not strictly correlating with the clinical outcome of SIT [30], allergen-IgG/IgG4 antibody complexes are apparently controversially discussed in modulating the allergic immune response and preventing IgE-mediated reactions.

**Anti-IgE Antibodies**

With regard to allergen-specific antibody responses before and during SIT treatment, anti-IgE antibody therapy seems to be a complementary approach to treat allergic diseases. In light of recent demonstrations that binding of IgE to FceRI receptors on mast cells not only elicits an allergic effector phase but also contributes to an amplified allergic reaction by enhancing mast cell survival and FceRI expression [31, 32], the interaction between IgE and specific IgE receptors provides an attractive target for the inhibition of ITA. It has been shown that anti-IgE antibodies are able to interfere with IgE binding to FceRI receptors by capturing free serum IgE, thus resulting in reduced leukotriene release after stimulating mast cells with allergen [33, 34]. Taken together, anti-IgE monoclonal antibody therapy represents an effective approach to treat ITA, which can be implemented as a monotherapy as well as in combination with SIT [35, 36].

**SIT: Cellular Mechanisms**

SIT affects different components of the immune system (fig. 1). Cellular modifications consist of a reduction in allergen-induced T cell proliferation, indicating the induction of peripheral tolerance in allergen-specific T cells, and a decrease in the initially disease-eliciting, antigen-specific Th2-dominated immune response towards
a Th1 reaction with increased interferon-γ production [37, 38]. The key cell type responsible for orchestrating this switch of T cell activity is a T cell population called T\textsubscript{reg} cells.

\textbf{T\textsubscript{reg} Cells}

In healthy individuals, T\textsubscript{reg} cells are usually present at a higher frequency than their effector counterparts [39]. They are able to inhibit the development of allergic Th2 responses [40], capable of directly suppressing the proliferation and cytokine expression of Th1 and Th2 cells, and exhibit inhibitory activity on antigen-presenting cells such as dendritic cells and macrophages [41]. A wealth of studies indicate that the ratio of Th2 cells to T\textsubscript{reg} cells seems to be decisive for the success of SIT [15, 39, 42]. As a result, the disturbed balance of T\textsubscript{reg} and effector cells in allergic individuals is re-established. Several distinct subsets of T\textsubscript{reg} cells have been identified based on phenotypic and functional markers (table 1). Recent evidence of the role of different T\textsubscript{reg} cell subsets in allergy will be discussed below.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Cellular and humoral network in ITA and tolerance induction by SIT. Upon SIT, different subsets of T\textsubscript{reg} cells are activated by antigen-presenting dendritic cells (DC). Action of both naturally occurring T\textsubscript{reg} cells (T\textsubscript{reg}) and induced T\textsubscript{reg} cells (Tr1, Th3) influences the pattern of antibody synthesis by B cells and cytokine expression by T helper cells (Th1 and Th2) through cell-cell contact and cytokine-dependent mechanisms. This results in inhibition of the effector cells of ITA, mast cells and eosinophils and leads to immune tolerance. Ag = Allergen; IFN = interferon.}
\end{figure}
**Type 1 T<sub>reg</sub> Cells**

On the cellular level, presumably, the most contributing factor to successful allergen immunotherapy is the peripheral induction of type 1 T<sub>reg</sub> (Tr1) cells. This is an inducible subset of T<sub>reg</sub> cells, which was first isolated after cloning human T cells activated with alloantigens in the presence of IL-10 [43]. The diversity of Tr1 cells compared with Th1 and Th2 cells is characterized by the production of high amounts of IL-10, with or without transforming growth factor (TGF-β), interferon-γ and IL-5, and low or no IL-2 and IL-4 [43, 44].

Current concepts evidenced the critical role of Tr1 cells in the maintenance of tolerance against allergens and in the modulation of allergic diseases due to the fact that skewing of allergen-specific T cells to a Tr1 cell-like phenotype seems to be a pivotal event in successful SIT. This contention goes along with findings that allergen-specific Th2 cells are also found in healthy individuals [45], but compared with allergic patients, are balanced by the dominant subset of Tr1 cells supporting a regulatory network which prevents initiation of clinical symptoms [39, 46, 47].

Functional studies of Tr1 cells specific to distinct antigens revealed that the modulation of Th1 and Th2 responses by Tr1 cells mostly depends on the secretion of the immunosuppressive cytokine, IL-10 [48–51]. IL-10 inhibits the proliferative response of peripheral T cells against distinct antigens [52] and plays a crucial role in the induction of T cell anergy by blocking CD28 tyrosine phosphorylation and CD28 binding to downstream signaling molecules [53]. In vitro, it facilitates the expression of the regulatory transcription factor Foxp3 under IL-4/IL-12-neutralizing conditions [54]. Other important effects of IL-10 on ITA include the modulation of eosinophil function [55] and the reduction in proinflammatory mediators released by mast cells [56]. Nevertheless, Tr1 cells may also directly influence the activity of allergen-specific T cells as demonstrated by birch pollen al-lergen (Bet v 1)-specific IL-10-producing T<sub>reg</sub> cells suppressing the proliferation of effector Th cell clones by cell-to-cell contact, independent of cytokine secretion [57].

**CD4+CD25+ T<sub>reg</sub> Cells**

The major subset of T<sub>reg</sub> cells is a subpopulation of CD4+ T cells that constitutively express the activation marker CD25 (α-chain of the IL-2 receptor), referred to as ‘naturally’ occurring T<sub>reg</sub> cells [58–61]. In addition to CD25, these cells are characterized by expression of the glucocorticoid-induced tumor necrosis factor receptor and the most definitive T<sub>reg</sub> cell marker, the forkhead winged-helix transcriptional factor Box p3 (Foxp3) [59, 62–64]. A significant property of CD4+CD25+ T<sub>reg</sub> cells is their ability to convey suppressive activity to other CD4+ T cells, a phenomenon termed ‘infectious tolerance’ [65–68]. This suppressive activity has been shown to be IL-10 [66] and TGF-β dependent [65]. Additionally, CD4+CD25+ T<sub>reg</sub> cells partially promote differentiation of IL-10-secreting, contact-independent Tr1-like cells in a contact-dependent manner [66].

In atopic individuals, the suppressive function of CD4+CD25+ T<sub>reg</sub> cells is diminished under certain conditions when compared with cells from nonatopic healthy controls [69, 70], and CD4+CD25+ cells from birch pollen-allergic patients exhibit an impaired suppression of birch pollen-stimulated CD4+CD25− cells [71]. In light of a putative increase in CD4+CD25+ T<sub>reg</sub> cells during SIT [42, 72], the relationship between inducible Tr1 cells

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Origin</th>
<th>Subset-specific markers</th>
<th>Foxp3 expression</th>
<th>Mode of action</th>
<th>Cytokine secretion</th>
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<tbody>
<tr>
<td>Th1</td>
<td>periphery</td>
<td>CD4+&lt;sup&gt;1&lt;/sup&gt;, T-bet</td>
<td>–</td>
<td>promotes chronic inflammation and persistence of the effector phase, antagonizes Th2 cells</td>
<td>IFN-γ, IL-12</td>
</tr>
<tr>
<td>Th2</td>
<td>periphery</td>
<td>CD4+&lt;sup&gt;1&lt;/sup&gt;, GATA-3</td>
<td>–</td>
<td>promotes allergy, antagonizes Th1 cells, induces IgE and IgG4</td>
<td>IL-4, IL-5, IL-13</td>
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<tr>
<td>T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>thymus</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;CD25&lt;sup&gt;+&lt;/sup&gt;, GITR</td>
<td>+</td>
<td>contact dependent, maintenance of tolerance against allergens, inhibits effector T cells</td>
<td>TGF-β, IL-10&lt;sup&gt;low&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tr1</td>
<td>periphery</td>
<td>CD4+&lt;sup&gt;1&lt;/sup&gt;</td>
<td>–/+</td>
<td>contact independent, IL-10 and TGF-β dependent, induces a switch from IgE to IgG4, suppresses effector T cells</td>
<td>IL-10, TGF-β, IL-5, IFN-γ, IL-2&lt;sup&gt;low&lt;/sup&gt;, IL-4&lt;sup&gt;low&lt;/sup&gt;</td>
</tr>
<tr>
<td>Th3</td>
<td>periphery</td>
<td>CD4+&lt;sup&gt;1&lt;/sup&gt;</td>
<td>?</td>
<td>induces oral tolerance</td>
<td>TGF-β, IL-10&lt;sup&gt;low&lt;/sup&gt;, IL-4&lt;sup&gt;low&lt;/sup&gt;</td>
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IFN-γ = Interferon-γ; GITR = glucocorticoid-induced tumor necrosis factor receptor.

<sup>1</sup> CD25+ upon activation and in vitro culture.

<sup>2</sup> Constitutive CD25+ expression (in contrast to the other T cell subsets).
and naturally occurring T_{reg} cells in induction of tolerance has to be further elucidated. Recent evidence suggests that after exposure to antigen in the periphery, CD4+CD25+ thymus-derived T_{reg} cells can terminally differentiate into Tr1 cells producing IL-10 and/or TGF-β. On the other hand, Tr1 cells may just be another distinct subset of T_{reg} cells, with similar regulatory functions to CD4+CD25+ T_{reg} cells [73, 74].

**T Helper 3 Cells**

Another subset of ‘adaptive’ T_{reg} cells, which is characterized by secretion of high amounts of TGF-β but low levels of IL-4 and IL-10, has been designated as T helper 3 (Th3) cells [75, 76]. This T_{reg} population was identified in experimental models of oral tolerance and has been investigated in several human autoimmune diseases including multiple sclerosis, arthritis and diabetes as well as in contact sensitivity and allergy [77]. The exact classification of Th3 cells is still debated. Immunologic features suggest that they may be of the same T cell subset as Tr1 cells, the other subset of adaptive T_{reg} cells [78]. However, Th3 cells could also represent activated naturally occurring T_{reg} cells [79].

Faria and Weiner [77] see the amount of TGF-β as a primary link between the distinct T_{reg} populations and proposed that Th3 cells – which secrete high levels of TGF-β – may represent master regulators for the induction of T_{reg} cells in the periphery. Besides inhibiting the Th1- and Th2-promoting transcription factors T-bet and GATA-3 [80–82], TGF-β has been shown to stimulate Foxp3 expression [83–85] and the expansion of CD4+CD25+ T_{reg} cells in vivo [86–88]. In line with the latter findings, a recent study demonstrated that Th3 cells might mediate peripheral immune tolerance not only by direct immunosuppressive effects but also by the induction of CD4+CD25+ T_{reg} cells [89].

Finally, a specific role of Th3 cells in inducing oral tolerance has been assumed. TGF-β can function as a switching and promotion factor for B cells towards the induction of IgA secretion [90, 91], and both increased TGF-β serum levels and secretion of TGF-β-driven allergen-specific IgA have been found in allergic patients treated by oral SIT [15, 92, 93]. Nevertheless, since other reports failed to show that T_{reg} cells induced by oral SIT fulfill criteria typical of Th3 cells [42, 94, 95], the precise function of Th3 cells in the regulatory network of immunotolerance remains to be determined.

**Summary**

ITA is characterized by an increased production of allergen-specific IgE antibodies and a predominant Th2 cell-driven cytokine pattern caused by an impaired balance of different T lymphocyte subsets. Therapeutic strategies for the treatment of ITA have to affect both the humoral and cellular disturbances. While utilization of anti-IgE antibodies presents a new, promising strategy to successfully inhibit the unwanted effects of enhanced IgE synthesis in ITA, it does not correct the underlying cellular pathomechanisms. SIT promotes the activation and expansion of different subsets of regulatory T cells, which actively regulate and rebalance the distribution of T helper cells and their cytokine pattern. While the humoral and cellular mechanisms underlying this effect are only partially understood, carefully designed clinical studies of SIT-treated patients will help to further elucidate the immunological processes involved in achieving immune tolerance.

**References**


