

# **Allergen Immunotherapy and Tolerance**

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### **Peripheral Tolerance Mechanisms**

The concept of inducing immune tolerance has become a prime target for prevention and treatment strategies for many diseases such as allergy, asthma, autoimmunity, organ transplantation and infertility in which dysregulation of the immune system plays an essential role. Immune tolerance to allergens is characterized by establishment of a long-term clinical tolerance<sup>1,2</sup>. In addition to the immune responses induced by various modes of allergen-SIT, the development of a healthy immune response during high dose of allergen exposure in beekeepers and cat owners has been intensively studied to understand mechanisms of allergen tolerance in humans<sup>3,4</sup>. Although there remains several points to be elucidated, mechanisms of venom immunotherapy is similar to aeroallergen immunotherapy and includes changes in the profile of allergen-specific memory T and B cell responses, the production of specific antibody isotypes to skew the immune response towards a non inflammatory direction, as well as decreased activation, tissue migration and degranulation of mast cells, basophils and eosinophils.

During an immune response, CD4+ T cells normally receive signals activated through the engagement of the TCR, which recognizes peptides of specific antigens presented on the surface of antigen-presenting cells (APC) by MHC-II molecules. In addition, costimulatory receptors (e.g. CD28, ICOS, CD2), which recognize ligands (e.g. B7 proteins, CD80, CD86, ICOSL, LFA-1) expressed on the surface of APCs are triggered and contribute to the activation of the T cell by decreasing the threshold of TCR triggers required for activation<sup>5,6</sup>. In response to this antigen-specific stimulation, activated T cells produce IL-2 and proliferate. However, when T cells receive stimulus only through the TCR without any engagement of co-stimulatory receptors, they enter in a status of unresponsiveness characterized by their inability to produce IL-2 or proliferate upon re-stimulation. This status has been termed T cell anergy.

After the discovery of Th1 and Th2 cell subsets in 1986, it was thought that these two subsets regulate each other and this is sufficient to explain all adaptive T cell related immunological events. Th1 cells were thought to play a role in infections and autoimmunity and Th2 cells in allergic disease. Both subsets were thought to have reciprocal roles in the counter regulation of the other. After the introduction of Treg cells it was demonstrated during the last 18 years that although there is reciprocal regulation between individual Th cell subsets, Treg cells play a major role in immune tolerance in allergy, including hymenoptera venom tolerance in venom-SIT and

beekeepers, autoimmunity, organ transplantation, cancer, pregnancy and chronic infections<sup>7</sup>.

### **Antigen presentation, dendritic cells and immune tolerance**

Innate immune response is based on recognition of microbial molecular patterns by pattern recognition receptors (PRR) such as toll like receptors (TLR) on epithelial cells, DC and many other cells. There is 10 functional TLRs in humans. TLR 1, 2, 4, 5, 6 and 10 expressed on the cell surface and migrate to phagosomes after activation and TLR 3, 7, 8 and 9 expressed in the endosomes and the endoplasmic reticulum<sup>8</sup>. TLR distinguish specific microbial components of bacteria, protozoa, viruses and fungi and stimulate APC to produce different responses. Unmethylated cytosine-phosphate-guanine (CpG) oligonucleotides in microbial DNA sequences activate TLR 9 to induce Th1 and Treg cells. Inhalation of synthetic oligonucleotide containing CpG motif by allergic patients with asthma leads to increased expression of interferon  $\gamma$ , but it does not reduce allergen-induced sputum eosinophils and Th2-related gene expression in sputum cells<sup>9</sup>. Immunostimulatory oligonucleotides suppress Th2 responses by disturbance of APC to present antigen to Th2 cells and inhibition of IL-4 release from mast cells and basophils and attenuate remodelling in animal models of asthma<sup>10,11</sup>.

Different subsets of dendritic cells are important in the induction of both protective immunity and tolerance development. In humans, myeloid DC, plasmacytoid DC and Langerhans cells exist as DC subsets<sup>12</sup>. After exposure to allergen, DC cells mature and migrate from periphery to the T cell areas of lymphoid tissues, where they produce T cell differentiation related cytokines and prime naive T cells<sup>13</sup>. DC present antigenic peptide to T cells through major histocompatibility complex (MHC) coupled to T cell receptor along with costimulatory signals as CD80/CD86 with CD28 or CTLA-4 and CD40 with CD40L, OX40 with OX40L and ICOSL with ICOS interactions<sup>14</sup>. Allergen-induced pro-Th2 cytokines including thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 are released from epithelial cells and dendritic cells for the development of Th2 responses and function of DC requires instructions from neighboring cells<sup>15</sup>.

In healthy immune response, monocytes differentiate to tolerogenic DC in the presence of granulocyte-macrophage colony-stimulating factor and CCL18 and prime Treg cells to induce tolerance. In allergic subjects, DC bind less CCL18 than healthy individuals which is responsible for absence of tolerogenic effects of CCL18 in allergic patients<sup>16</sup>. Most of the functional studies on dendritic cells have been performed in mice. IL-10 differentiated DC (DC10) treat mouse lung inflammation by decreasing airway hyperreactivity, by increasing IL-4, IL-5 and IL-13, decreasing

allergen-specific IgE and IgG1 responses and activation of CD4<sup>+</sup> CD25<sup>+</sup> Treg cells in a mouse model of house dust mite allergic-asthma<sup>17,18</sup>.

### **Regulatory T cells**

The existence of suppressor cells, which limit ongoing immune responses and prevent autoimmune disease was postulated over 30 years ago<sup>19</sup>. The recent phenotypic and functional characterisation of these cells, has led to a resurgence of interest in their therapeutic application in a number of immune-mediated diseases. Two broad subsets of CD3<sup>+</sup>CD4<sup>+</sup> suppressive or regulatory T (Treg) cells have been described. These are constitutive or naturally occurring versus adaptive or inducible Treg cells. There are other Treg cell populations, including CD8<sup>+</sup> Treg cells with the reported capacity both may inhibit T cell responses<sup>20,21</sup>. In addition, double negative (CD4<sup>-</sup>CD8<sup>-</sup>) TCR $\alpha\beta$ <sup>+</sup> Treg cells that mediate tolerance in several experimental autoimmune diseases<sup>22</sup> and TCR $\gamma\delta$  Treg cells which can play a role in the inhibition of immune responses to tumors<sup>23-26</sup> have been described. An immunoregulatory role for IL-10-secreting B cells and dendritic cells, (DC), which have regulatory/suppressor properties has been recently suggested<sup>27-29</sup>. In addition, natural killer (NK) cells, epithelial cells, macrophages and glial cells express suppressor cytokines such as IL-10 and TGF $\beta$ <sup>30,31</sup>. Although the role of many of these cell types has not been fully demonstrated as professional regulatory cells, so far we know little regarding their capacity and importance in modulating the allergic response and it is practically possible that some of these cells may efficiently contribute to the generation and maintenance of a regulatory/suppressor type of immune response. The discovery of new cytokines is still going on with full speed and novel cytokines help us to better understand functional T cell subsets<sup>32</sup>.

### **Mechanisms of immune suppression by Treg cells**

Several modes of action of Treg cells in suppressing other cells have been proposed, which include cell-contact dependent mechanisms, observed in most in vitro studies, as well as cytokine-dependent ones (Figure 1). Suppression and regulation may be targeted at effector T cells, B cells and/or APC to reduce the ability of APC to prime T cells via modulation of costimulation and cytokine production, or the increase of tryptophan metabolism. Cell-contact inhibitory mechanisms can involve delivery of negative costimulatory signals via CTLA-4, although this mechanism is not exclusive, since Treg cells isolated from mice with a deletion of the CTLA4 gene were still suppressive in vitro<sup>33,34</sup>. A role for cell surface TGF $\beta$  has also been proposed. CD4<sup>+</sup>CD25<sup>+</sup>Treg have been reported to directly kill T cell effectors in a perforin and granzyme dependant cytolysis<sup>35</sup>.

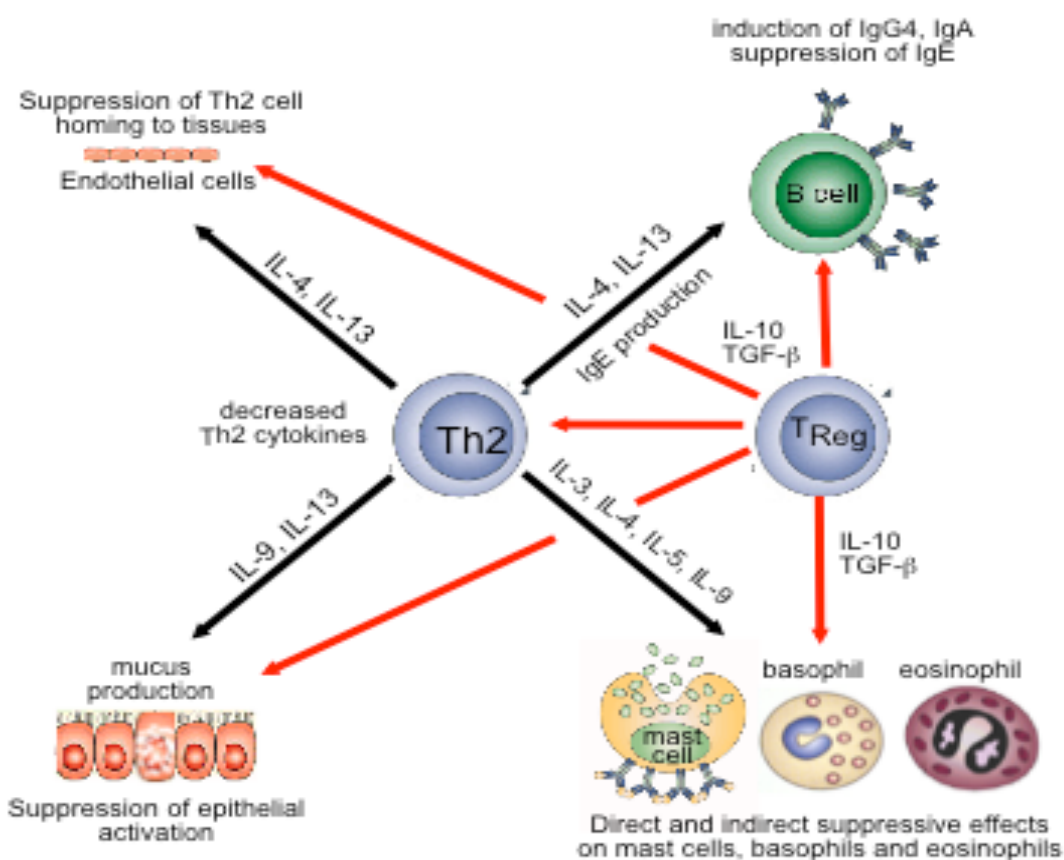


Figure 1. Immune deviation towards Treg cell response is an essential step in allergen-SIT and natural allergen exposure of nonallergic individuals. Treg cells utilize multiple suppressor factors, which influence the final outcome of SIT. Treg cells suppress proliferation, tissue infiltration, pro-inflammatory cytokine production and injury/apoptosis of epithelial cells by both Th1 and Th2 cells. IL-10 and TGF- $\beta$  induce IgG4 and IgA respectively from B cells as non-inflammatory Ig isotypes and suppress IgE production. These two cytokines directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils and eosinophils. Treg cells use IL-10, TGF- $\beta$ , CTLA-4, PD-1 and HR2 in these functions.

*In vitro* studies have suggested that human thymus-derived CD4+CD25+ Treg cells inhibit Th2 responses less efficiently than Th1 responses<sup>36</sup>. CD4+CD25+Treg cells may efficiently inhibit Th2 differentiation, but are less effective for inhibition of cytokine production and proliferation of established Th2 cells, requiring pre-activation *in vitro* for strong inhibition of Th2 responses<sup>37</sup>. Studies using peripheral blood T cells from healthy non-atopic donors show poor responses to allergen in culture for proliferative and Th2 cytokine responses, in comparison to atopic patients. If,

however, PBMC from non-atopic donors are depleted of the CD4+CD25+Treg compartment prior to stimulation with allergen, increased proliferative and Th2 cytokine responses are observed<sup>38</sup>. These studies imply that active control of the allergic response occurs in these individuals.

The mechanism of suppression *in vivo* appears to be highly dependent on the experimental system being studied and may vary according to the tissue, the type of inflammation and animal model under study. Several early studies demonstrated that naturally occurring CD25+Treg cells inhibit allergic airway disease in mice (reviewed and fully referenced in<sup>39,40</sup>). In a mechanistic study CD4+CD25+ T cells suppressed the Th2 cell-driven response to allergen *in vivo* by an IL-10-dependent mechanism whereby CD25+ Treg cells induced the expression of IL-10 by resident lung CD4+ T cells<sup>41</sup>, whilst a second suggested naturally occurring lung CD25+ T cell regulation of airway allergic responses was dependent on induction of TGF $\beta$  by IL-10<sup>42</sup>. Another study concluded that inhibition was mediated by CD4+CD25+ Treg cell suppression of DC activation and that the absence of this regulatory pathway contributed to disease susceptibility<sup>43</sup>. The maintenance of protective Treg activity depends on continuing allergen stimulation<sup>44</sup>. Whilst most studies to date have indicated at least some capacity to inhibit allergic airway inflammation, recent studies have also highlighted that some inhibition of airway hyper responsiveness occurred. Depending on the conditions of development, human Treg cells can express all of the secreted cytokines and surface molecules that may play a role in immune suppression. These findings suggest that inducible Treg cells have rather overlapping properties instead of major distinctions.

### **Mechanisms of allergen specific immunotherapy**

Allergen-specific immunotherapy (SIT) is highly effective in the treatment of IgE-mediated diseases such as allergic rhinitis, conjunctivitis and venom hypersensitivity. It is the only treatment which leads to a life-long tolerance against previously disease-causing allergens due to restoration of a normal immunity<sup>45-49</sup>. It is an important part of the complex treatment including anti-histamines, anti-leukotrienes,  $\beta$ 2 adrenergic receptor antagonists and corticosteroids aiming at suppression of mediators and immune cells. Immunotherapy also improves asthma and inhibits seasonal increases in bronchial hyperresponsiveness<sup>50</sup>. It has also been shown to prevent onset of new sensitisations<sup>51</sup> and reduce development of asthma in patients with rhinitis caused by inhalant allergens<sup>52,53</sup>.

The allergen specificity of immunotherapy is crucial in the understanding of its benefits and the underlying mechanisms, which are slowly being elucidated. In 1911, the original report of Noon<sup>54</sup> suggested that grass pollen extracts, used for

immunotherapy of hay fever, induced a toxin, causing allergic symptoms. It was suggested that in response to injection of pollen extract, antitoxins develop and prevent the development of disease. Indeed, generation of neutralizing antibodies was demonstrated during SIT<sup>55,56</sup>. Later on, it has been acknowledged that activated T cells and their products play a major role in the pathogenesis of allergic diseases and allergen-specific T cells were considered the major target for SIT<sup>48,57-64</sup>. SIT was earlier suggested to be associated with a decrease in IL-4 and IL-5 production by CD4<sup>+</sup> Th2 cells, and a shift towards increased IFN- $\gamma$  production by Th1 cells. A new light was shed when a further subtype of T cells, with immunosuppressive function and cytokine profiles distinct from either T helper (Th) 1 and Th2 cells, the role of Treg cells has been described<sup>39,65-68</sup>. The evidence for their existence in humans has been demonstrated<sup>39,59,68-70</sup>. Skewing of allergen-specific effector T cells to Treg cells appears as a crucial event in the control of healthy immune response to allergens and successful allergen-specific immunotherapy<sup>71,72</sup>.

### **T regulatory cells in allergen-specific immunotherapy**

Although in early studies a switch from Th2 to Th1 type cytokines have been reported<sup>64,73</sup>, recent studies have demonstrated that peripheral T cell tolerance is crucial for a healthy immune response and successful treatment of allergic disorders<sup>63,64,72,74</sup>. The tolerant state of specific cells results from increased IL-10 secretion (Figure 1)<sup>64</sup>. The cellular origin of IL-10 was demonstrated as being the antigen-specific T cell population and activated CD4<sup>+</sup>CD25<sup>+</sup> T cells as well as monocytes and B cells<sup>64</sup>. Consistently, the increase of IL-10 both during SIT and natural allergen exposure has been demonstrated<sup>63,64,72,74</sup>. A detailed study has been performed using IFN- $\gamma$ , IL-4- and IL-10-secreting allergen-specific CD4<sup>+</sup> T cells that resemble Th1, Th2 and Tr1-like cells, respectively. Healthy and allergic individuals exhibit all three subsets, but in different proportions. In healthy individuals IL-10-secreting Tr1 or IL-10-Treg cells represent the dominant subset for common environmental allergens, whereas a high frequency of allergen-specific IL-4 secreting T cells (Th2-like) is found in allergic individuals<sup>72</sup>. Hence, a change in the dominant subset may lead to either the development of allergy or recovery. Peripheral tolerance to allergens involved multiple suppressive factors such as IL-10, TGF- $\beta$ , cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1)<sup>72</sup>. Accordingly, allergen-specific peripheral T cell suppression mediated by IL-10 and TGF- $\beta$  and other suppressive factors, and a deviation towards a Treg cell response was observed in normal immunity as a key event for the healthy immune response to mucosal antigens. The analysis of other IL-10 family cytokines such as IL-19, IL-20, IL-22, IL-24 and IL-26 demonstrated that suppressor capacity for allergen/antigen-stimulated T cells is only a function of IL-10 in this family<sup>75</sup>.

Successfully treated patients develop specific T cell unresponsiveness against the entire allergen as well as T cell epitope-containing peptides. These decreased proliferative responses do not arise from deletion as they are restored by the addition of IL-2 and IL-15. However, unlike in mucosal allergies no increases in TGF- $\beta$  production during SIT were observed in venom allergy. Differences in the control mechanism, which regulate immune responses to venoms and to aeroallergens might be due to different routes of natural allergen exposure as well as the induction of chronic events of allergic inflammation leading to tissue injury and remodelling in the latter case. Apparently, T cells, which are becoming predominant during SIT and natural antigen exposure represent the Tr1 or IL-10-Treg cells in humans (Figure 1). CD4<sup>+</sup> Treg cells that specialize in the suppression of immune response are pivotal in maintaining peripheral tolerance<sup>76-79</sup>. Treg cells are enriched within the CD4<sup>+</sup>CD25<sup>+</sup> cells<sup>22,80-82</sup>. Increases in numbers of CD25<sup>+</sup> (possibly Treg) cells in the skin and nasal mucosa were also observed<sup>74,83</sup>. In humans, there is circumstantial evidence to suggest that Treg cells play a major role in the inhibition of allergic disorders. It has been reported that IL-10 levels in the bronchoalveolar-lavage fluid of asthmatic patients are lower than in healthy controls, and that T cells from children suffering from asthma also produce less IL-10 mRNA than T cells from control children<sup>84,85</sup>. Although some reports imply a role for TGF- $\beta$  in the pathogenesis of asthma, particularly in remodeling of injured lung tissue in humans<sup>86</sup>, a recent report indicated that the increased allergic inflammation observed after blocking of CTLA-4 is clearly associated with decreased TGF- $\beta$  levels in the bronchoalveolar-lavage fluid of mice<sup>87</sup>.

In the vast majority of the studies, the cultures of PBMCs were examined. The question whether this reflects the changes in the immune response in the mucosal tissues is of interest. T cell responses after grass pollen immunotherapy have been examined in nasal mucosal and skin tissue. Increased IL-10 mRNA-expressing cells after SIT with grass pollen during the pollen season was demonstrated. However, unlike the findings in the periphery, IL-10 was not increased in nonatopic subjects exposed during the pollen season. Increased Th1 activity was demonstrated both in the skin and nasal mucosa<sup>83,88,89</sup>. In addition, reduced accumulation of T cells in skin and nose after allergen challenge, but no decrease in T cell numbers during pollen season were shown. Increases in IFN- $\gamma$  observed after allergen challenge outside the pollen season correlated with the clinical improvement<sup>90</sup>. During the summer pollen season increases of both IFN- $\gamma$  and IL-5 with the ratio in favour of IFN- $\gamma$  were observed<sup>91</sup>. It seems however that the demonstration of the modulation of peripheral immune responses is pivotal for the effects of allergen-SIT. Local tissue responses do not necessarily reflect peripheral tolerance and are dependent upon a number of mechanisms like cell apoptosis, migration, homing and survival signals, which are very much dependent upon natural allergen exposure and environmental factors<sup>92</sup>.



### **Allergen-SIT and Treg cells influence allergen-specific antibody responses**

Specific IgE in serum and on effector cells in tissues of allergic patients is a hallmark of atopic disease. Although peripheral T cell tolerance is rapidly induced during SIT, there is no evidence for B cell tolerance in the early course<sup>57</sup>. Natural exposure to a relevant allergen is often associated with an increase in the IgE synthesis. Similarly, SIT frequently induces a transient increase in serum specific IgE, however followed by gradual decrease over months or years of treatment<sup>93-95</sup> (Figure 2). In pollen-sensitive patients, desensitization prevents elevation of the serum specific IgE titer during the pollen season<sup>96,97</sup>. However, the changes in IgE levels can hardly explain the diminished responsiveness to specific allergen due to SIT, since the decrease in serum IgE is late, relatively small, and is poorly correlated with clinical improvement after SIT.

The induction of blocking antibodies by SIT was suggested as early as in the 1930s by Cooke et al.<sup>56</sup>. Lichtenstein et al<sup>55</sup> assigned these blocking antibodies to IgG. Research focused on the subclasses of IgG antibodies, especially IgG4, believed to capture the allergen before reaching the effector cell-bound IgE, and thus to prevent the activation of mast cells and basophils. In fact, a substantial number of studies demonstrated increases in specific IgG4 levels together with clinical improvement<sup>91,98</sup>. In the case of venom allergy, the rise of anti-venom IgG correlates, at least at the onset of desensitization, with protection achieved by the treatment<sup>99,100</sup>. The concept of blocking antibodies has recently been reevaluated. Blocking Abs seem not only to inhibit allergen induced release of inflammatory mediators from basophils and mast cells, but also inhibit IgE-facilitated allergen presentation to T cells as well as prevent allergen-induced boost of memory IgE production during high allergen exposure in pollen season. It has been demonstrated that that grass pollen immunotherapy induced allergen-specific, IL-10-associated “protective” IgG4 responses<sup>101</sup>. The data established an absolute association between IgG4-dependent blocking of IgE binding to B cells in patients, who underwent immunotherapy and a trend towards a correlation with clinical efficacy. It seems to be relevant rather to measure the blocking activity of allergen-specific IgG than the crude levels in sera. This can explain the lack of correlation between antibody concentration and degree of clinical improvement. However, IgG4 antibodies can be viewed as having the ability to modulate the immune response to allergen and thus the potential to influence the clinical response to allergen. In a study using well defined recombinant allergen mixtures all treated subjects developed strong allergen specific IgG1 and IgG4 antibody responses<sup>102</sup>. Some patients were not sensitized to Phl p 5, but nevertheless developed strong IgG antibody responses to that allergen. It has been suggested that subjects without specific IgE against a particular allergen fail to mount

a significant IgG4 response<sup>103</sup>, but recent studies do not support this view and are consistent with induction of a tolerant immune response<sup>102</sup>.

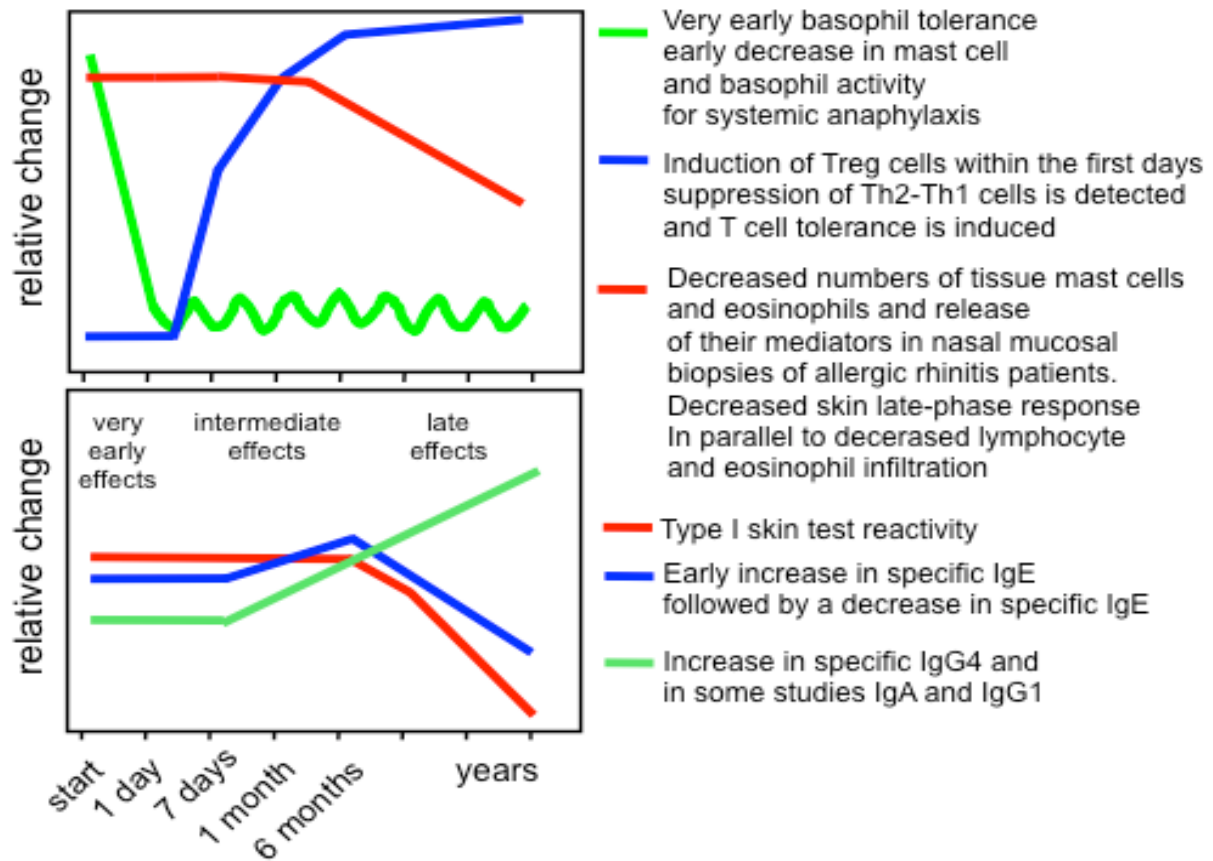
IL-10 that is induced and increasingly secreted by SIT, appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis<sup>59</sup>. IL-10 is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production<sup>59,104</sup>. Thus, IL-10 not only generates tolerance in T cells; it also regulates specific isotype formation and skews the specific response from an IgE to an IgG4 dominated phenotype. The healthy immune response to Der p1 demonstrated increased specific IgA and IgG4, small amounts of IgG1 and almost undetectable IgE antibodies in serum<sup>63</sup>. House dust mite-SIT did not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1 and IgG4 was observed<sup>63</sup>. The increase of specific IgA and IgG4 in serum coincides with increased TGF- $\beta$  and IL-10 respectively. This may account for the role of IgA and TGF- $\beta$  as well as IgG4 and IL-10 in peripheral mucosal immune responses to allergens in healthy individuals<sup>59,105</sup>.

#### **Treg cells are involved in the suppression of effector cells and inflammatory responses during SIT**

Long-term SIT is associated with significant reduction of not only the immediate response to allergen provocation, but also the late phase reaction (LPR) in the nasal and bronchial mucosa or in the skin. The mechanism of LPR is different from mast cell-mediated immediate reaction and involves the recruitment, activation and persistence of eosinophils, and activated T cells at the sites of allergen exposure. The immunopathologic changes in the mucosal tissues of subjects chronically exposed to inhalant allergens resemble those seen during the late phase. Since LPR is associated with increased bronchial and nasal hyperresponsiveness and mimics the pathologic condition of chronic allergic inflammation, it has been postulated that the effect of SIT on the LPR is relevant to its clinical efficacy<sup>106</sup>.

Successful SIT results not only in the increase of allergen concentration necessary to induce immediate or LPR in the target tissue, but also in the decreased responses to nonspecific stimulation (Figure 2). Bronchial, nasal, and conjunctival hyperreactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SIT and correlates with clinical improvement<sup>107, 108</sup>. During birch pollen SIT, reduced plasma levels of eosinophil cationic protein (ECP), a marker of eosinophil activation, as well as chemotactic factors for eosinophils and neutrophils correlated with decreased bronchial hyperreactivity and clinical improvement<sup>107,109</sup>. Inhibition by SIT of the seasonal increase in eosinophil priming has also been demonstrated<sup>110</sup>. In biopsies taken during grass pollen SIT decreased

eosinophil and mast cell infiltration in nasa1 and bronchial mucosa after SIT correlated with the anti-inflammatory effect. In addition, plasma concentrations and in vitro production of endothelin-1 (a bronchoconstrictor and proinflammatory peptide) were significantly decreased in asthmatic children after 2 years of immunotherapy with mite extract <sup>111,112</sup>.



**Figure 2.** Immunologic changes during the course of allergen-SIT. Although there is significant variation between donors and protocols, starting on from the first injection an early decrease in mast cell and basophil activity and degranulation and decreased tendency for systemic anaphylaxis is observed. This is followed by generation of allergen-specific Treg cells and suppression of both allergen-specific Th1 and Th2 cells. An early increase of specific IgE and late decrease is observed. This is in parallel to an increase of particularly IgG4 and in some studies IgG1 and IgA. A significant decrease in allergen-specific IgE/IgG4 ratio occurs after several months. A significant decrease in type I skin test reactivity is also observed relatively

late in the course. Decrease in tissue mast cells and eosinophils and release of their mediators and decrease in late phase response is observed after a few months.

The cardinal difference between true atopic diseases like allergic rhinitis, asthma or atopic dermatitis and venom allergy is the lack of many chronic events of allergic inflammation leading to tissue injury and remodelling in anaphylactoid monoallergies<sup>92</sup>. Despite the fact that definite decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires several years of SIT, most patients are protected against bee stings already at an early stage of BV-SIT. An important observation starting on from the first injection is an early decrease in mast cell and basophil activity for degranulation and systemic anaphylaxis. The mechanism of this desensitization effect is yet unknown. It has been shown that mediators of anaphylaxis (histamine and leukotrienes) are released during SIT without inducing a systemic anaphylactic response. Particularly, ultrarush protocols induce significantly increased release of these mediators to circulation. Their piecemeal release may affect the threshold of activation of mast cells and basophils. Although there are fluctuations and risk for developing systemic anaphylaxis during the course of allergen-SIT, the suppression of mast cells and basophils continues to be affected by changes in other immune parameters such as generation of allergen-specific Treg cells and decreased specific IgE. This is particularly because they require T cell cytokines for priming, survival and activity, which are not efficiently provided by suppressed Th2 cells and activated Treg cells<sup>113 114</sup>. Peripheral T cell tolerance to allergens, which is characterized by functional inactivation of the cell to antigen encounter can overcome both acute and chronic events in allergic reactions. SIT efficiently modulates the thresholds for mast cell and basophil activation and decreases immunoglobulin E-mediated histamine release<sup>115,116</sup>. In addition, IL-10 was shown to reduce proinflammatory cytokine release from mast cells<sup>117</sup>. Furthermore, IL-10 down regulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells<sup>118</sup>. Moreover, IL-10 inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death<sup>119</sup>.

### **Histamine receptor 2 as a major player in peripheral tolerance**

As a small molecular weight monoamine that binds to 4 different G-protein-coupled receptors, histamine has recently been demonstrated to regulate several essential events in the immune response<sup>92,120</sup>. Histamine receptor (HR) 2 is coupled to adenylate cyclase and studies in different species and several human cells demonstrated that inhibition of characteristic features of the cells by primarily cAMP formation dominates in HR2-dependent effects of histamine<sup>121</sup>. Histamine released

from mast cells and basophils by high allergen doses during SIT interferes with the peripheral tolerance induced during SIT in several pathways. Histamine enhances Th1-type responses by triggering the histamine receptor HR1, whereas both Th1 and Th2-type responses are negatively regulated by HR2. Human CD4+Th1 cells predominantly express HR1 and CD4+Th2 cells HR2, which results in their differential regulation by histamine<sup>122</sup>. Histamine induces the production of IL-10 by DC<sup>123</sup>. In addition, histamine induces IL-10 production by Th2 cells<sup>124</sup>, and enhances the suppressive activity of TGF- $\beta$  on T cells<sup>125</sup>. All three of these effects are mediated via HR2, which is relatively highly expressed on Th2 cells and suppresses IL-4 and IL-13 production and T cell proliferation<sup>122</sup>. Apparently, these recent findings suggest that HR2 may represent an essential receptor that participates in peripheral tolerance or active suppression of inflammatory/immune responses. Histamine also regulates antibody isotypes including IgE<sup>122</sup>. High amount of allergen-specific IgE is induced in HR1-deleted mice. In contrast, deletion of HR2 leads to a significantly less amounts of allergen-specific IgE production, probably due to direct effect on B cells and indirect effect via T cells.

The long-term protection from honeybee stings by H1 anti-histamine premedication during rush immunotherapy with honeybee venom in a double-blind, placebocontrolled trial was analysed<sup>126</sup>. After an average of 3 years, 41 patients were re-exposed to honeybee stings. Surprisingly, none of 20 patients who had been given HR1-antihistamine premedication, but 6 of 21 given placebo, had a systemic allergic reaction to the re-exposure by either a field sting or a sting challenge. This highly significant difference suggests that antihistamine premedication during the initial dose-increase phase may have enhanced the long-term efficacy of immunotherapy. Expression of HR1 on T lymphocytes is strongly reduced during ultrarush immunotherapy, which may lead to a dominant expression and function of tolerance-inducing HR2<sup>127</sup>. Administration of antihistamines decreases the HR1/H2R expression ratio, which may enhance the suppressive effect of histamine on T cells.



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