INTRODUCTION

Immunological research within the area of tolerance and anergy has become more and more popular throughout the last decade, leading to an arising number of suggestions that the same molecular events may be involved forming a link between the mechanisms of anergy, tolerance and active suppression. Induction of T cell unresponsiveness when subjected to antigen encounter provides the major characteristic of T cell tolerance. The occurrence of antigen exposure prior to the development of the immune response, specifically abrogates the response to antigen and therefore results in immunological tolerance. Anergy describes a process by which antigen is presented to T cell clones without the aid of professional antigen-presenting cells (APC) and results in the induction of a hyporesponsive state, which affects IL-2 production and proliferation upon restimulation. Reversible functional limitations characterize anergic T cells, including cell division, cell differentiation, and cytokine production. It is important to note that in earlier studies, which provide a basis for the definition of anergy/tolerance, functional unresponsiveness was analysed by non-sophisticated assays such as antigen-induced
[\textsuperscript{3}H] thymidine incorporation, IL-2 and total IgG production. Furthermore, until recent years, the antigens used within mouse models contained high amounts of impurities, such as LPS and other innate immune response stimulating substances, which may have influenced experimental outcomes.

Figure 1. The four sequential processes characterizing allergic inflammation. Various antigens or yet unidentified factors activate T cells; these cells then undergo organ-selective homing according to the influence of organ-related chemokine networks. T cells within subepithelial tissues show increased survival and are continuously stimulated. These activated T cells then trigger effector functions, including apoptosis, hyper IgE and eosinophilia. These four events can be summarized as shown.

The combination of four sequential processes characterizes allergic inflammation (Figure 1):

- **Activation:** memory/effector T cells and other effector cells (mast cells, eosinophils and basophils) are activated by aeroallergens, food antigens, auto-antigens and bacterial superantigens\textsuperscript{6,7}.
- **Organ-selective homing:** cells are influenced by the network of chemokines in the skin, nose and lung\textsuperscript{8,9}.
- **Survival and reactivation:** prolonged survival of inflammatory cells, which interact with resident cells and become reactivated within the allergic organs\textsuperscript{10,11}.
- **Effector Functions:** induction of hyper IgE, eosinophil survival and mucus hyperproduction and remodelling\textsuperscript{10,7,12}, all of which are important factors in atopic dermatitis and asthma. Furthermore, activated T cells induce apoptosis of bronchial epithelial cells and keratinocytes as main/essential tissue injury events\textsuperscript{13,14}.

As all of these pathological events require activated T cells. Peripheral T cell tolerance is seen to be vital for a healthy immune response and represents a mechanism to overcome allergic inflammation and successfully treat allergic disorders\textsuperscript{15-18}.

Allergen exposure causes some, but not all individuals, to suffer from atopic diseases due to the interaction of environmental and genetic factors. The generation of allergen-specific CD\textsuperscript{4}\textsuperscript{+} T helper cells provides the initial event responsible for the development of allergic diseases. The current view is that interleukin-4 (IL-4) -influenced naive T cells differentiate into Th2 cells when activated by APC\textsuperscript{19,20}. These effector Th2 cells produce IL-4, IL-5 and IL-13, which mediate several regulatory and effector functions (Figure 2). These functions include allergen-specific IgE production by B cells, eosinophil development and recruitment, mucus production and smooth muscle contraction, as well as tissue homing of Th2 cells\textsuperscript{19-23}. Type I hypersensitivity, involves IgE-mediated cross-linking of receptors, leading to the degranulation of mast cells and basophils, and may result in chronic allergic inflammation. Th1 cells, on the other hand, may contribute to chronicity and the effector phase in allergic disease\textsuperscript{24,25,14}. These two distinct T cell subpopulations are able to counter-regulate each other\textsuperscript{20}. A further subset of T cells termed regulatory/suppressor T (T\textsubscript{Reg}) cells, with a distinct immunosuppressive function and
cytokine profile to that of Th1 or Th2 cells, has been described. Like Th1 cells, TReg cells are able to inhibit the development of allergic Th2 responses and play an important role in allergen-specific immunotherapy (SIT)

Figure 2. Induction of immune deviation towards TReg cell response leads to peripheral tolerance in allergen-specific immunotherapy and healthy immune response. TReg cells utilize multiple suppressor factors to regulate undesired activity of effector cells. IL-10 and TGF-β suppress IgE production and induce non-inflammatory Ig isotypes IgG4 and IgA, respectively. Furthermore, these two cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils and eosinophils. In addition, TReg cells inhibit Th2 cells, which can no longer provide cytokines such as IL-3, IL-4 and IL-5. These cytokines are required for the differentiation, survival and activation of effector cells (mast cells, basophils and eosinophils). (Grey line: suppression, black line: stimulation).

Types of regulatory cells
T cells capable of suppressing the immune response were first described in the early 1970s. However, the mechanisms behind this suppression failed to be clarified and therefore this area of research became desolate by the 1980s. In the mid 1990s investigation into T cell-mediated immune suppression strengthened once more. At present, the concept of TReg cells suppressing immune responses via cell-to-cell interactions and/or the production of suppressor cytokines is well established. However, many aspects behind these mechanisms remain to be elucidated.

Type 1 T regulatory cells (Tr1) (Table 1)
Tr1 cells, also known as inducible TReg cells, are defined by their ability to produce high levels of IL-10 and TGF-β. Antigen-specific Tr1 cells arise in vivo, but may also differentiate from naive CD4+ T cells. Reports have shown that a Tr1 cell subset can be generated in vitro by stimulating naive CD4+ T cells in the presence of IL-10, IFN-α or a combination of IL-4 and IL-10. By combining vitamin D3 and dexamethasone, human and mouse naive CD4+ T cells can be induced in vitro to differentiate into Tr1 cells. However, in contrast to the previously described in vitro derived CD4+ T cells, these cells only produce IL-10, but not IL-5 and interferon (IFN)γ. It is now clear that during the early course of allergen-SIT, IL-10- and/or TGF-β-producing Tr1 cells in humans are generated in vivo, which implies that Tr1 cells are induced by high and increasing doses of allergens. Allergen-specific Th1 and Th2 responses are down-regulated by these Tr1 cells. Tr1 cells could be used as a promising target for the development of new therapeutic agents, as well as in cellular therapy for peripheral tolerance modulation in allergy and autoimmunity.

Th3 cells
There are similarities between Tr1 cells and Th3 cells. When activated with an appropriate antigen or anti-CD3 antibody, Th3 cells produce high levels of TGF-β, with variable amounts of IL-4 and IL-10. In vivo, these
cells have been found to suppress encephalitis induction with myelin basic protein. TGF-β and IL-10 seem to play a crucial role, as neutralizing antibodies abrogate the disease-protective effects of these cells. Furthermore, Th3 cells have been shown to exert bystander immune suppression in vitro.

CD4+CD25+TReg cells

Besides the expression of CD4 and CD25, these cells are also associated with the transcription factor FoxP3. This distinct subset of TReg cells, which are also called constitutive TReg cells, account for 5-10% of peripheral CD4+ T cells and has been shown to inhibit the activation of effector T cells in the periphery. The suppressive mechanism of CD4+CD25+ TReg cells has been shown to function via the inhibition of IL-2Rα-chain in target T cells, which is induced by the combined activity of CTLA-4 and membrane-bound TGF-β1.

Peripheral blood mononuclear cells (PBMC) from both atopic and non-atopic donors give a proliferative response to allergen in culture, but differ in Th2 cytokine production. Regulatory CD4+CD25+ T cells normally inhibit Th2 cytokine expression and proliferation of PBMC from non-atopic donors, in response to allergen. This suppression has been shown to be associated with the control of allergic disease.

IL-10 and TGF-β have been shown to induce T cell suppression during SIT and in normal immunity to mucosal allergens. SIT induced the antigen-specific suppressive activity of CD4+CD25+ T cells from allergic individuals. This suppression can be partially blocked by the neutralization of secreted or membrane bound IL-10 and TGF-β1. It is tempting to speculate that CD4+CD25+ T cells in humans may represent a whole repertoire of single antigen/allergen-specific Tr1 cells.

Various autoimmune diseases such as arthritis, diabetes and X-linked immune dysregulation, polyendocrinopathy and enteropathy syndrome (IPEX) may develop spontaneously when CD4+CD25+ T cells are eliminated, indicating the presence of a population of professional TReg cells.

Other TReg cells

It has been proposed that in addition to CD4+ T cells, CD8+ TReg cells may also have a role in oral tolerance. Furthermore, CD8+ TReg cells have been described to control proliferation of CD4+ T cells in a Qa-1 dependent (HLA-E in humans) manner. CD8+CD28 FOXP3+ T suppressor cells have recently been reported to induce tolerogenic endothelial cells, by inducing inhibitory receptors and down-regulating co-stimulatory and adhesion molecules.

Several studies on autoimmune disease describe double negative (CD4CD8) TCRγδ+ TReg cells.

### Table 1. Subsets of T regulatory cells

<table>
<thead>
<tr>
<th>T regulatory cells</th>
<th>Suppressor mechanism</th>
<th>References</th>
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<tbody>
<tr>
<td>Tr1</td>
<td>IL-10, TGF-β</td>
<td>26, 27, 16, 17, 32, 34, 36, 41, 33, 18</td>
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<tr>
<td>Th3</td>
<td>TGF-β</td>
<td>26, 47, 37</td>
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<td>CD4+CD25+TReg</td>
<td>IL-10, TGF-β, CTLA-4, PD-1, GITR</td>
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<td>CD8+CD25-CD28- TReg</td>
<td>Same as CD4+CD25+</td>
<td>48, 49, 51</td>
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<tr>
<td>Qa-1 dependent-CD8+</td>
<td>Qa-1-specific TCR</td>
<td>50</td>
</tr>
<tr>
<td>CD4+CD8 TReg</td>
<td>Induction of apoptosis</td>
<td>52, 53</td>
</tr>
<tr>
<td>TCRγδ TReg</td>
<td>IL-10, TGF-β</td>
<td>56, 57, 54, 55</td>
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Pathogenesis of autoimmune diseases...
TReg cells that mediate tolerance. This effect was mainly attributed to Fas-mediated apoptosis of alloreactive T cells.

A population of tumour-infiltrating γδTReg cells, with a cytokine profile reminiscent of Tr1 cell clones, may play a role in the inhibition of immune responses against tumours. Furthermore, they have been shown to play a role in the induction of tolerance against aerosol-delivered protein antigen and ovalbumin (OVA), but are not essential in IgE unresponsiveness.

Other cell types with regulatory function

IL-10-secreting B cells have recently been proposed to prevent the development of arthritis. It is generally accepted that immature dendritic cells (DC), which can not appropriately activate T cells, may induce tolerance. In normal immunity, DC receive sufficient signals from the surroundings of the antigen, T cells and other tissue cells, innate immune response stimulating (i.e. Toll-like receptor triggering) substances and via co-stimulatory ligands and cytokines, and therefore should not have any restriction in maturation. However, the full maturation of DC can be inhibited by IL-10, which induces a state of anergy in alloantigen-specific CD4+ T cells. Furthermore, there are indications that mature DC can induce peripheral T cell tolerance. Pulmonary DC from mice transiently produce IL-10 when exposed to respiratory antigens. These phenotypically mature pulmonary DC, which are B7+, stimulate the development of CD4+ Tr1-like cells. Adoptive transfer of IL-10− mice, but not IL-10+ pulmonary DC of mice exposed to respiratory antigen induced antigen-specific unresponsiveness in recipient mice. This study shows that under certain circumstances, phenotypically mature DCReg may exist.

It has been clearly demonstrated that natural killer cells, epithelial cells, macrophages, glial cells etc. express suppressor cytokines such as IL-10 and TGF-β. Although they have not been classified as professional regulatory cells, they may have an involvement in generating and suppressing an immune response.

Role of Tr1 cell-mediated peripheral T cell tolerance in the healthy immune response to allergens and allergen-specific immunotherapy (SIT)

During the last century, allergen-SIT has been most successful in the treatment of insect venom allergy and allergic rhinitis in clinical practice. Rather than a temporary suppression of mediators and immune cells, which is provided by anti-histamines, anti-leukotrienes, β2 adrenergic receptor antagonists and corticosteroids, a more long-term solution/treatment can be provided by allergen-SIT, specifically restoring normal immunity against allergens. An increase in allergen blocking IgG antibodies (mainly IgG4) is associated with successful allergen-SIT, the generation of IgE-modulating CD8+ T cells and a decline in the number of mast cells and eosinophils as well as the release of mediators. Furthermore, an affiliation with a shift in Th2 cytokine profile toward decreased levels of IL-4 and IL-5 production and an increase in IFN-γ production in allergen-SIT of allergy to bee venom, wasp venom, grass pollen and house dust mite (HDM) has been demonstrated. The induction of peripheral T cell tolerance plays a crucial role in allergen-SIT (Figure 2). T cell tolerance is initiated by the autocrine action of IL-10 and TGF-β, which are increasingly produced by antigen-specific T cells. Reactivation of tolerized T cells can result in the distinct production of either Th1 or Th2 cytokine profiles depending on cytokines present in the tissue microenvironment, and can therefore direct allergen-SIT towards either successful or unsuccessful treatment (Figure 2).
Another elegant approach in immunotherapy for the investigation of peripheral T cell tolerance in humans involves the use of T cell epitope containing, but non-IgE binding, peptides (PIT). Two types of allergy have been targeted by PIT in clinical trials at present. For the major cat allergen Fel d 1, studies with relatively long peptides of 27 and 35 amino acids or a mixture of peptides spanning the whole protein sequence resulted in the induction of tolerance in IL-4-producing cells in the late phase response. The observed effect was closely associated with an up-regulation of IL-10 production. In bee venom allergy, a study using a mixture of short peptides directly representing the T cell epitopes of the major bee venom allergen, phospholipase A2 induced specific T cell tolerance and a decline in the specific IgE:IgG4 ratio demonstrating a modulation of the immune response against the whole allergen.

Analysis of responses to various food and inhalant antigens has shown the healthy immune response to mucosal antigens to have a similar mechanism of active regulation to that, which maintains immunological unresponsiveness. IL-10-secreting allergen-specific T cells are predominant over IL-4-secreting T cells in healthy individuals. In contrast, this is reversed in allergic individuals.

A recent study has been performed using IFN-γ, IL-4 and IL-10 secreting allergen-specific CD4+ 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, Tr1 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. Therefore, a change in the dominant subset may lead to either allergy development or recovery.

Tr1 cells have many suppressive mechanisms, including secretion of suppressive cytokines IL-10 and TGF-β, as well as the surface molecules cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD1). It has been shown ex vivo, that allergen-specific Th2 cell activation is enhanced when these Tr1 suppressor activities are blocked or when the Th2 cell frequency is enhanced.

Association of allergen-specific peripheral T cell tolerance with antibody isotype regulation and effector cell suppression

Although peripheral tolerance has been demonstrated in specific T cells, the ability of B cells to produce specific IgE antibodies is not eliminated during SIT. In fact, the specific serum levels of both IgE and IgG4 antibodies increase during the early phase of treatment. However, the increase in antigen-specific IgG4 is more striking and the ratio of specific IgE to IgG4 decreases by 10- to 100-fold. Also the in vitro production of PLA-specific IgE and IgG4 antibodies by PBMC changes in parallel to the serum levels of specific isotypes. A similar change in specific isotype ratio has been observed in SIT of various allergies. Moreover, IL-10 produced and progressively secreted during SIT, appears to counter-regulate synthesis of antigen-specific IgE and IgG4 antibodies. IL-10 potently suppresses both total and allergen-specific IgE, whereas it simultaneously increases IgG4 production. Therefore, IL-10 not only generates T cell tolerance, it also regulates specific isotype formation and skews the specific IgE response towards an IgG4 dominated phenotype (Figure 2).

High levels of specific IgA and IgG4, low amounts of IgG1 and trace amounts of IgE antibodies in serum were demonstrated in the healthy immune response to Der p. Specific IgE levels did not significantly change after 70 days of HDM-SIT treatment of allergic
patients; however, a significant increase in specific IgA, IgG1 and IgG4 was observed. The increase of specific IgA and IgG4 in serum coincides with increased TGF-β and IL-10 respectively. This may account for the role of IgA and TGF-β as well as IgG4 and IL-10 in mucosal immune responses to allergens in healthy individuals.

Several years of SIT are normally required for an obvious decrease in IgE antibody levels and IgE-mediated skin sensitivity to be observed, however, most patients are already protected against bee stings at an early stage of BV-SIT. The reason behind this could be that the effector cells (mast cells, basophils and eosinophils) require T cell cytokines for priming, survival and activity, which can not be sufficiently provided by suppressed Th2 and T_{Reg} cells (Figure 2). SIT efficiently modulates the activation thresholds for mast cells and basophils, and decreases immunoglobulin E-mediated histamine release. In addition, IL-10 has been shown to reduce pro-inflammatory cytokine release from mast cells, down-regulate eosinophil function and activity, and suppress IL-5 production from resting human Th0 and Th2 cells.

Since, IL-13 and IL-4 have been shown to induce the upregulation of vascular adhesion molecule-1 (VCAM-1) expression on human endothelial cells, suppression of Th2 cells may prevent the recruitment of inflammatory cells (eosinophils, macrophages and T cells) to the site of allergic inflammation (Figure 2). CD8^+CD28^−FOXP3^+^ T cells upregulate the inhibitory receptors, immunoglobulin-like transcripts (ILT)3 and ILT4 and downregulate co-stimulatory molecules, which results in the suppression of T cell activation. Furthermore, these cells can prevent the adhesion and transmigration of Th2 cells.

Clinical aspects of regulatory T cells
There is circumstantial evidence that T_{Reg} cells play a major role in the inhibition of allergic disorders in humans. Studies have shown that IL-10 is present in lower levels in the bronchoalveolar-lavage fluid of asthmatic patients than in healthy controls, and that T cells of asthmatic children produce less IL-10 mRNA than those of healthy children. These data suggest that a decrease in allergic reactions is associated with an increase in IL-10 production. T_{Reg} cells are hypothesized to play a role in the suppression of allergic Th2 responses in humans, as they are a major source of IL-10. This hypothesis is supported by several human allergen-SIT studies.

A recent report indicated an association between the increased allergic inflammation seen after blocking CTLA-4 and decreased TGF-β levels within the bronchoalveolar-lavage fluid. Furthermore, experimental tracheal eosinophilia was inhibited by the induction of CD4^+ T cells secreting TGF-β. Currently, in regards to development and activation, the relationship between the different T_{Reg} cell populations remains unclear. However, as seen in numerous animal (in vivo) studies, by suppressing both Th1 and Th2 responses, T_{Reg} cells can actively suppress the development of autoimmune and allergic responses. In mice, protection against the development of allergen-induced Th2 responses has been shown, after the application of in vitro engineered allergen-specific T_{Reg} cells.

CD4^+CD25^+ T cells have been found to inhibit lethal graft versus host disease in rodents. In vitro, CD4^+CD25^+ T cells act antigen-non-specifically, but, when isolated from naive mice and activated by allogenic APC long term tolerance to bone marrow grafts is induced. Protection of target bone marrow occurred simultaneously to rejection of third-party bone marrow, providing evidence that CD4^+CD25^+ T cells work antigen-specifically in vivo.

Maternal CD4^+CD25^+ T cells suppress allogenic responses against the fetus, in
addition to autoimmune responses, during pregnancy. In order to analyze the in vivo existence of human T_{Reg} cells, lymphocyte populations in human lymph nodes have been investigated with emphasis on CD4^+ CD25^+ T_{Reg} cells\textsuperscript{101}. T_{Reg} cells from the lymph nodes are anergic and efficiently inhibit proliferation of other CD4^+ and CD8^+ lymphocytes in vitro, as previously found in T_{Reg} cells from peripheral blood\textsuperscript{101}.

Depletion of T_{Reg} cells should result in an enhanced immune response against tumour antigens, an important group of auto-antigens. Antibody-mediated depletion of CD25^+ T cells has been demonstrated to facilitate the induction of tumour immunity\textsuperscript{102, 103}.

Ongoing clinical studies are required to determine, whether in vivo generation or adoptive transfer of T_{Reg} cells and/or their suppressive cytokines may alter the progression of allergy and asthma. Generation of T_{Reg} cells or increasing their suppressive properties by small molecular weight compounds, provide important targets for use in allergy and asthma, as well as in transplantation and autoimmunity.

Although suppression of an immune response may be beneficial in the case of such hypersensitivity reactions, it may be harmful in the event of cancer, chronic infection or tissue remodelling\textsuperscript{104-109}. Suppression of effector T cells could aggravate the disease due to the uncontrolled growth of potentially harmful target cells. Therefore, care should be taken with long term induction of T_{Reg} cells by SIT.

**Conclusion**

Peripheral T cell tolerance is the key mechanism in healthy immune responses to non-infectious, non-self antigens. This hypothesis is clinically well documented in allergy, transplantation, tumour and infection. Substantial and increasing evidence supports the role of T_{Reg} cells and/or immunosuppressive cytokines in mediating allergen-SIT and healthy immune responses (Figure 2). This development of prophylactic vaccines to induce allergen-specific T_{Reg} cells should be considered along with the treatment of established allergy. Successful SIT has been shown to rely on the balancing of Th2 responses with the aid of T_{Reg} cells. Furthermore, increased activity could be an adequate measure for the suppression of allograft rejection, graft versus host disease and autoimmunity. However, caution should be taken concerning the over-activation of T_{Reg} cells, since several studies show that they may be responsible not only for healthy responses, but also for the chronicity of infections and tumour tolerance. Furthermore, the strong fibrogenic capacity of the regulatory cytokine TGF-\beta, may be responsible for incorrect remodelling in the lungs\textsuperscript{110}.

It has been proven possible to grow, expand and clone T_{Reg} cell populations in vitro, without losing their suppressor function. Considering this, recent advances in the knowledge of peripheral tolerance mechanisms may lead to the future development of safer treatment of immune mediated diseases.

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