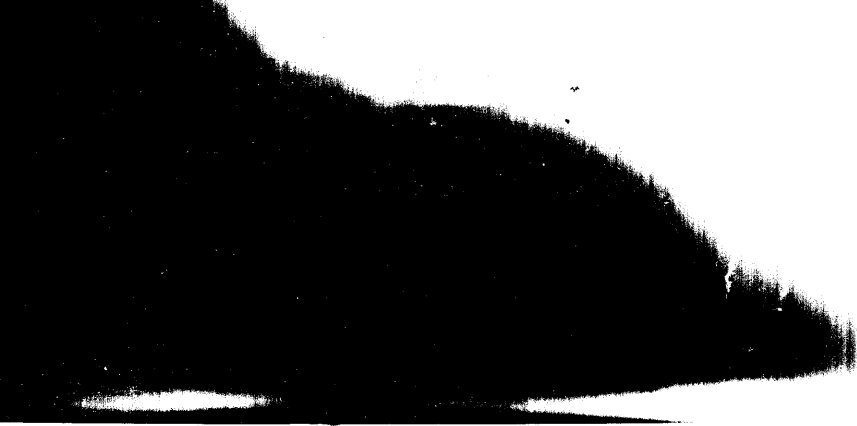




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Mechanisms of Specific Immunotherapy: Current Knowledge

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Abstract

Induction of specific unresponsiveness (tolerance) in peripheral T cells by IL-10 and/or TGF- β and recovery by cytokines from the tissue microenvironment represent two key steps in specific immunotherapy of allergy and natural exposure to allergens in healthy individuals. IL-10 and TGF- β elicit tolerance in T cells and thereby control the suppression and development of antigen-specific immunity. Both cytokines also play an important role on the generation of a non-inflammatory IgG4 and IgA type of allergen-specific antibodies during the course of specific immunotherapy. Histamine plays an important role in upper and lower airway inflammation. In addition to its well-characterized effects in the acute inflammatory and allergic responses, histamine regulates several aspects of antigen-specific immune response development. Histamine affects the maturation of dendritic cells and alters their T cell polarizing capacity. Histamine regulates antigen specific Th1 and Th2 cells as well as related antibody isotype responses. Histamine and four different known histamine receptors (HR) display a very complex system and their expression changes according to the stage of cell differentiation as well as microenvironmental influences.

Introduction

Allergic diseases basically are immunological disorders related to the development of distinct T cell cytokine patterns, including increased secretion of allergic inflammatory cytokines, in particular of IL-4, IL-5 and/or IL-13. Whereas the symptoms of immediate and late type allergic reactions can be ameliorated by various pharmacological treatments, the allergen-specific immunotherapy (SIT) represents the only curative approach for specific Type I allergy. Although SIT is most efficiently used in allergy to insect venoms and allergic rhinitis, the mechanism why SIT achieves clinical

making IgG antibodies of IgE-modulating eosinophils, including the BV-SIT. Furthermore, production by CD4⁺ T cells. However, it is unclear if the steps in the mechanism and production of tolerance develops towards a

molecular mechanisms, the immune response represents the major step with short PLA peptide immunotherapy; PLA-multiple bee stings in allergen exposure in

Environmental

SIT (5,7) and further enhancing the immunosuppression in both BV-SIT and PLA-unresponsiveness containing peptides. The release and secretion of cytokines IL-2 and IFN- γ induced by these treatments is specific to the allergen.

Modulation of anergic capacity for IL-2 and IL-4. In contrast, specific stimulation therefore recovered environmental tissue (7). They can generate a normal allergic response. In established polyspecific stage of disease.

A regulatory/suppressor T cell profile during SIT and natural high dose of antigen exposure: High IL-10 and TGF- β production and decreased proliferation and Th1 and Th2 cytokines

Tolerized T cells showed suppressed PLA-specific T cell proliferative and cytokine responses that could be reconstituted by *ex vivo* neutralization of endogenous IL-10. This indicates that IL-10 is actively involved in development of tolerance in specific T cells. Whereas in both BV-SIT and PLA-PIT the antigen- and peptide-induced proliferative responses and Th1 and Th2 cytokine production decreased, the IL-10 production simultaneously increased and reached maximal levels after four weeks, when the specific tolerance was fully established. The cellular origin of IL-10 was demonstrated by intracytoplasmic IL-10-staining in PBMC and coexpression of cellular surface markers (5). Intracellular IL-10 significantly increased after seven days of SIT in the antigen-specific T cell population and activated CD4⁺ T lymphocytes.

Interestingly, the same features of peripheral T cell tolerance were found in T cells of healthy bee keepers, who were previously stung by high numbers of bees. These naturally anergized individuals show increased numbers of IL-10-producing CD4⁺CD25⁺ T cells and monocytes similar to allergic patients after BV-SIT. Neutralization of endogenous IL-10 in PBMC cultures from these individuals fully reconstituted the proliferative T cell response and cytokine production (5). Besides SIT and PIT of BV allergy, evidence for induction of peripheral T cell tolerance was obtained in SIT of wasp venom allergy, grass pollen asthma, conjunctivitis and rhinitis. Furthermore, the importance of T cell targeting and modulation of T cell responses were reported in PIT of cat allergy (9). Moreover, SIT-induced IL-10 increase was demonstrated also in wasp allergy, grass pollen asthma and nasal immunotherapy of weed-induced allergic rhinitis (reviewed in 1,2).

IL-10 is a major regulatory cytokine of inflammatory responses. It was originally described as a mouse Th2 cell factor, inhibiting cytokine synthesis by Th1 cells. However, several studies demonstrated that IL-10 acts as a general inhibitor of proliferative and cytokine responses of both Th1 and Th2 cells *in vitro* and *in vivo*. IL-10 is released by mononuclear phagocytes, natural killer cells and by both Th1 and Th2 type lymphocytes. In mice IL-10 administration before allergen treatment induced antigen-specific T cell tolerance and demonstrated the pivotal role of IL-10 in establishment of peripheral T cell tolerance. Moreover, inhibition of graft versus host disease by IL-10 and allograft rejection in HLA-mismatched bone marrow-transplanted SCID patients gives further evidence for a key role of this cytokine in the induction and maintenance of an anergic state. Similarly, inappropriate stimulation of tumor reactive human T cells was shown to result from increased endogenous IL-10 production by these cells, indicating also a role for IL-10 in tumor specific T cell tolerance. Recently, IL-10-derived regulatory CD4⁺ T cells producing IL-10 but not IL-2 and IL-4, which suppressed antigen-specific T cell response *in vitro* and prevented antigen-induced murine colitis were identified in human and in mice. Apparently, T cells observed during SIT and natural antigen exposure represent the so-called T regulatory 1 cells in humans.

Successful SIT with aeroallergens increases patient's tolerance to natural exposure to a specific aeroallergen. SIT was demonstrated to reduce specific cutaneous,

bronchial, nasal or ocular reactivity assessed by skin and provocation tests with both seasonal and perennial inhalant allergens. The altered allergen-specific T cell function due to SIT is critical for its clinical efficacy. Diminished end organ responsiveness to specific stimulation results from the modulation of T-cell functions. Moreover, the changes in T cell function may explain the specificity of the immunotherapy response. Indeed, studies have shown that allergen-specific T cell proliferation is diminished after allergic desensitization. We have demonstrated that HDM-SIT suppressed specific T cell proliferative and cytokine responses, which could be recovered by neutralization of IL-10 and TGF- β in the cultures. Furthermore, IL-10 and TGF- β production is increased during HDM-SIT in parallel with suppression of other cytokines.

IL-10 plays an inhibitory role on IgE and effector cells of allergic inflammation

The serum levels of specific IgE and IgG4 antibodies delineate allergic and normal immunity to allergen. Whereas peripheral tolerance was demonstrated in specific T cells, the capacity by B cells to produce specific IgE and IgG4 antibodies was not abolished. In fact, specific serum levels of both isotypes increased during the early phase of treatment. However, the increase in specific IgG4 was more pronounced and the ratio of specific IgE to IgG4 decreased by 10 to 100 fold. Also, the *in vitro* production of PLA-specific IgE and IgG4 antibodies by PBMC changed in parallel to the serum levels of specific isotypes. A similar change in specific isotype ratio was observed in SIT of various allergies. Moreover, IL-10 that was induced and increasingly secreted by SIT appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis. It is a potent suppressor of both total and allergen-specific IgE, while simultaneously IgG4 formation is increased (5). 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.

Despite the fact that definite decrease in IgE antibody levels and IgE mediated skin sensitivity normally requires several years of treatment, most patients are protected against bee stings already at an early stage of BV-SIT. Increase of allergen-specific IgG4 antibodies, blocking IgE binding to the allergen, may explain only the late phase of protection by SIT. At the early phase of SIT, however, a decrease in histamine and sulfidoleukotriene release from basophils may be of more relevance. This decreased basophilic mediator releasability can be attributed to suppression of cytokines in anergic T cells. There is clear evidence that effector cells of the allergic inflammation (mast cells, basophils and eosinophils) require T cell cytokines for priming, survival and activity. In addition, IL-10 was shown to reduce TNF- α , GM-CSF and IL-6 generation from mouse bone marrow and rat peritoneal mast cells. Moreover, IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 clones. Furthermore, IL-10 inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death.

Molecular basis IL-10 inhibits T cell

The molecular mechanism of allergen-specific PBMC inhibition of the proliferative response of T cells to allergen staphylococcal protein A is mediated by the inhibition of the anti-CD3/CD28 induced proliferation of T cells. IL-10 inhibited the anti-CD3/CD28 induced proliferation of T cells and its effect was reversed by the addition of anti-IL-10 antibodies. T cells which were stimulated with a constant amount of anti-CD3/CD28 costimulation. The inhibition was triggered and while

IL-10 inhibits tyrosine phosphorylation of phosphatidylinositol

Stimulation of CD28. Ligation of CD28 with its ligand B7-1 induces tyrosine phosphorylation of CD28 and IL-10 on CD28 appears to affect ZAP-70 tyrosine phosphorylation, a key event for signaling. IL-10 inhibits the interaction of CD28 with its ligand B7-1, leading to its receptor. The 85 kDa regulatory subunit of the kinase and lipid kinase is a two src-homology domain protein. The interaction between SH-PTase and the cytoplasmic part of CD28 is required for its activity. However, the mechanism of IL-10 demonstrated to p

Previous studies have shown that IL-10 is a potent inhibitor of macrophage function. IL-10 does not inhibit the activation of JAK-STAT pathway and the kinases family and has been shown to induce expression of genes that may play a role in the immune response. However, the effect of IL-10 on the

ation tests with both specific T cell function and responsiveness to antigens. Moreover, the anergy response is diminished after suppression of specific T cell function by neutralization of IL-10. IL-10 production is suppressed by cytokines.

IL-10 and allergic

IL-10 in allergic and normal mice was demonstrated in specific antibody synthesis was not affected during the early phase of the response. IL-10 was more pronounced and inhibited the *in vitro* production of IgE in parallel to the decrease in the ratio was observed. IL-10 increasingly secreted by Th2 cells inhibited antibody synthesis. IL-10 while simultaneously suppresses Th1 tolerance in T cells; it suppresses the response from an IgE-

IL-10 mediated skin reactions. IL-10 protects mice from allergic sensitization. IL-10 of allergen-specific Th2 cells only the late phase of the response in histamine and IgE production. This decreased production of cytokines in anergy. IL-10 inflammation (mast cell survival and activation). IL-10 generation from Th2 cells. IL-10 downregulates human resting Th0 cells. IL-10 IFN- γ production and Th1 cell death.

Molecular basis of T cell suppression by IL-10: IL-10 inhibits T cells only if costimulation is required

The molecular mechanisms of T cell suppression by IL-10 was investigated in antigen-specific PBMC cultures, purified CD45RO⁺ T cells and T cell clones. IL-10 inhibited the proliferative T cell response in PBMC to various antigens and the superantigen staphylococcal enterotoxin B (6). However, IL-10 did not affect the proliferative responses of T cells that were stimulated by anti-CD3. In contrast, IL-10 significantly inhibited the anti-CD28-stimulated proliferation. The analysis of T cell receptor (TCR) numbers on T cells demonstrated the essential requirement for costimulation in T cell activation and its relation to the number of triggered TCRs. IL-10 inhibited the T cell proliferation within a certain range of triggered TCRs that T cells require costimulation. T cells which were stimulated by different concentrations of anti-CD3, and a constant amount of anti-CD28 showed that low numbers of triggered TCRs required CD28 costimulation. Thus, IL-10 suppressed only those T cells that had low numbers of TCRs triggered and which required CD28 for proliferation.

IL-10 inhibits tyrosine phosphorylation of CD28 and phosphatidylinositol 3-kinase binding

Stimulation of CD28 by B7 surface molecules leads to tyrosine phosphorylation of CD28. Ligation of IL-10 receptor (IL-10R) at the time of CD28 stimulation inhibits tyrosine phosphorylation of CD28 as detected after 10 min (6). The inhibitory effect of IL-10 on CD28 appeared to be specific for the CD28 pathway, because IL-10 did not affect ZAP-70 tyrosine phosphorylation stimulated by CD3 cross-linking. As a consecutive event for signal transduction, phosphatidylinositol 3-kinase (PI3-K) should bind to CD28 by its p85 subunit. The association of CD28 with the PI3-K p85 molecule was inhibited by IL-10. This inhibition can be specifically blocked by preventing IL-10 binding to its receptor with an anti-IL-10R mAb. PI3-K is a heterodimer that comprises an 85 kDa regulatory and a 110 kDa catalytic subunit possessing both protein serine-kinase and lipid-kinase activity. The p85 subunit contains a p110 binding site as well as two src-homology-2 (SH2) domains. Binding of PI3-K to CD28 occurs by direct interaction between SH2 domain motifs of p85 PI3-K and a (p)YXXM motif in the cytoplasmic part. This requires CD28 tyrosine phosphorylation. From the primary protein sequence there is no indication that the CD28 cytoplasmic tail may display enzymatic activity. However, intervening protein tyrosine kinases, such as p56^{lck} and p59^{lyn} were demonstrated to phosphorylate CD28 at its ¹⁷³Y position.

Previous studies demonstrated that IL-10 does not only inhibit T cells, it is also a potent inhibitor of activated monocytes and macrophages. Since monocytes and macrophages do not express CD28, the inhibitory impact of IL-10 is likely to occur through other mechanisms in non-T cells. IL-10 exerts its biological functions through the activation of Jak1 and Tyk2, the members of the receptor-associated Janus tyrosine kinases family and Stat1 and Stat3 and in certain cells Stat5. In monocytes, IL-10 was shown to induce expression of the suppressor of the cytokine signaling 3 (SOCS3) gene that may play a role in inhibition of IFN-induced tyrosine phosphorylation of Stat1. However, the effect of IL-10 on the inhibition of CD28 tyrosine phosphorylation is vis-

ible already after 10 min. Obviously this does not require new protein synthesis and the mechanism of inhibition of CD28 tyrosine phosphorylation remains to be elucidated. Moreover, the identification of the second chain of the IL-10R, termed IL-10R2 or IL-10R β as an essential molecule for the action of IL-10 receptor complex will further help to characterize IL-10 signaling.

Immune regulation by TGF- β

TGF- β is also indicated in the control of specific immune responses to high antigen doses in mucosal tissues. It was shown *in vitro* that TGF- β can inhibit B cell proliferation, antibody secretion, and expression of surface molecules, including antigen receptors. TGF- β has been implicated in induction of class switch and secretion of IgA and acts as an important player in mucosal immune responses. In human lungs, TGF- β is produced by various cell types and in very high amounts by bronchial epithelial cells. TGF- β might exert a regulatory function in human airways, preventing deleterious inflammation in response to aero-antigens or allergens, maintaining a harmonious bronchial architecture. It also plays a crucial role in the induction of oral tolerance through activation of antigen-specific TGF- β -producing T cells, which induce and maintain unresponsiveness to antigen in a TGF- β -dependent manner. It has been shown that oral administration of antigens generates antigen-specific TGF- β -secreting Th3 regulatory T cells, which localize to target organs and suppress inflammation in the local microenvironment.

An important pathogenetic aspect of specific immunotherapy and allergic inflammation: Immune regulation by histamine

The pharmacological effects of histamine are mediated through four types of membrane receptors, H1R, H2R, H3R, and H4R, which are heptahelical G protein-coupled receptors (GPCRs) (10,11). Histamine receptors possess all structural features of GPCRs including seven putative transmembrane domains, NH₂-terminal glycosylation sites, and phosphorylation sites for protein kinase A and protein kinase C. Stimulation of H1R and H2R activate Gq and Gs, respectively, while both H3R and H4R are coupled to Gi/o. H1R and H2R are expressed on many cell types including nerve cells, airway and vascular smooth muscles, hepatocytes, chondrocytes, endothelial cells, neutrophils, eosinophils, monocytes, dendritic cells, T and B cells. Activation of H1R produces signals via the Gq/11 family of G proteins, which are capable of communicating with T cell receptor-mediated signaling. The primary signal from H1R is delivered through calcium-dependent activation of phospholipase C with generation of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol. H2Rs are both coupled to adenylate cyclase and phosphoinositide systems, however stimulation through H2R results primarily in cAMP formation. Remarkably, in most systems histamine can exert both suppressive properties through H2R and enhancing effects through H1R activation. H3R is expressed on histaminergic neurons in brain, and on some peripheral tissues, however the density of this receptor is rather low as compared to H1R or H2R. H4R is expressed mainly on hematopoietic cells including neutrophils, eosinophils, and T helper cells as well as on basophils and mast cells. The expression of HR may be affected during nor-

mal immune responses or allergic rhinitis.

The cells which include mast cells, eosinophils, and dendritic cells are capable to produce histamine and MHC II on membrane. Histamine on all four types of cells causes actin polymerization. The loss of these receptors enhances intracellular production of IL-4 and IL-13 transients, actin polymerization of H1R and H2R involves the H2R.

During specific immunotherapy, basophils may occur. Histamine receptor expression is enhanced by amine stimulation, while Th2 cells suppress triggering the H1R. Histamine is regulated by H2R, due to deletion of H1R, cytokines (IL-4 and IL-13) are produced. In addition, but not on Th2 cells, basophil development may account for negative regulation (10,11).

In mice, histamine is involved in H1R-dependent antigen-independent antigen signaling in response to LP. Antibody response is different pattern. Receptor mice was reported. Histamine levels in comparison decreased serum histamine and H1R knock-out mice. IL-4 and IL-13. Cytokines IFN- γ . Thus H1R is involved in humoral immune response.

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The cells which are critical in the regulation of immune response, i.e. T cells, monocytes, and dendritic cells (DC), all possess histamine receptors on their surface and are capable to produce and secrete histamine. Histamine induces increases in CD80, CD86, and MHC II on monocytes and DC. Immature and mature DC express the mRNA for all four types of histamine receptors. Histamine induces intracellular Ca^{2+} transients, actin polymerization, and chemotaxis in immature DC. Maturation of DC results in the loss of these responses. In maturing DC, however, histamine dose-dependently enhances intracellular cAMP levels and stimulates IL-10 secretion while inhibiting production of IL-12. Specific histamine receptor agonists or antagonists reveal that Ca^{2+} transients, actin polymerization, and chemotaxis of immature DC are due to stimulation of H1R and H3R subtypes. Modulation of IL-12 and IL-10 secretion by histamine involves the H2R and H3R exclusively.

During specific immunotherapy, a controlled histamine release by peripheral basophils may occur. It has been demonstrated that differential patterns of histamine receptor expression on Th1 and Th2 cells determine reciprocal responses following histamine stimulation. Th1 cells show predominant, but not exclusive expression of H1R, while Th2 cells show upregulation of H2R. Histamine enhances Th1-type responses by triggering the H1R, whereas both Th1- and Th2-type responses are negatively regulated by H2R, due to activation of different biochemical intracellular signals. In mice, deletion of H1R results in suppression of IFN- γ and dominant secretion of Th2 cytokines (IL-4 and IL-13). H2R-deleted mice show upregulation of both Th1 and Th2 cytokines. In addition, IL-3 stimulation significantly increases H1R expression on Th1, but not on Th2 cells. IL-3 plays an important role in T cell regulation of mast cell and basophil development. The increase in H1R expression on Th1 cells by IL-3 may account for negative feed back regulation due to the anti-allergic role of Th1 cells (10,11).

In mice, histamine enhances anti-IgM-induced proliferation of B cells, which is abolished in H1R-deleted mice. In H1R-deleted mice antibody production against a T cell-independent antigen-TNP-Ficoll is decreased, suggesting an important role of H1R signaling in responses triggered by B cell receptors. Interestingly, B cell proliferative responses to LPS, IL-4 or CD40 were apparently normal in H1R-deleted mice. Antibody responses to T cell-dependent antigens like ovalbumin (OVA) show a different pattern. Recently, OVA-specific antibody production in H1R- and H2R-deleted mice was reported (10). H1R-deleted mice produced high OVA-specific IgG1 and IgE levels in comparison to wild type mice. In contrast, H2R-deleted mice showed decreased serum levels of OVA-specific IgG3 and IgE in comparison to wild type mice and H1R knock-out mice. Although T cells of H2R knock-out mice secreted increased IL-4 and IL-13, OVA-specific IgE was suppressed in the presence of highly increased IFN- γ . Thus H1R and related Th1 response may play a dominant role in the suppression of humoral immune response.

Conclusion

IL-10- and TGF- β -induced peripheral T cell tolerance during specific immunotherapy as well as natural antigen exposure represents a key event in the control of specific immune responses to high antigen doses. In particular, a distinct immune response profile occurs, which promotes IgA and IgG4 isotype antibody formation against allergens with suppressed T cell proliferation and decreased Th2 cytokines (Figure 1). The reactivation and modulation of distinct cytokine patterns in anergic T cells suggests a pivotal role of microenvironmental cytokines in the development of SIT. T cell-secreted cytokines are essential for priming, survival and activity of inflammatory effector cells. Therefore, specific T cell reactivity is directly involved in the pathogenesis of allergic inflammation. IL-10 and TGF- β do not only induce tolerance in T cells; they may also inhibit the activation of inflammatory reactions by mast cells and eosinophils. In this context, by the application of the recent knowledge in the immunological mechanisms of allergen-specific immunotherapy, more rational and safer approaches are awaiting the future of vaccine development (12).

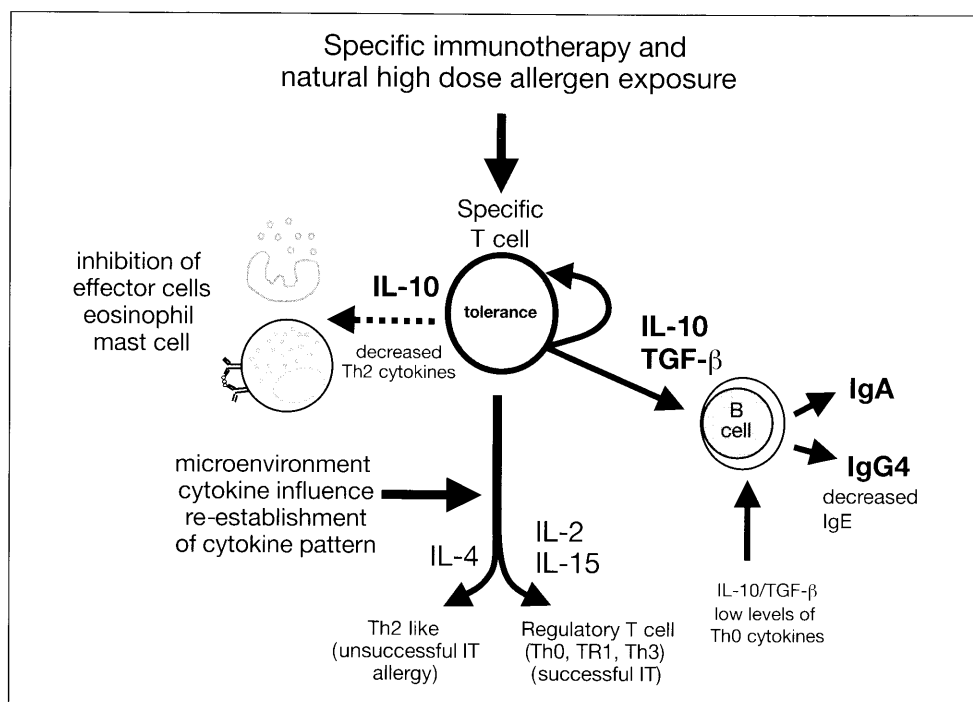


Fig. 1. Immunological mechanisms of SIT. Continuous treatment with high doses of allergen establishes specific tolerance in peripheral T cells. This state is characterized by suppressed proliferative and T cell cytokine responses and simultaneous increases in IL-10 and TGF- β -production. IL-10 and TGF- β suppress specific T cells in an autocrine fashion. IL-10 suppresses specific IgE and enhances IgG4 production. In parallel, TGF- β suppresses IgE and promotes IgA production by B cells. In consequence, activation, priming and survival of allergic inflammatory effector cells, such as eosinophils, mast cells and basophils are downregulated. The anergic T cells can be reactivated by cytokines from the tissue microenvironment. In successful SIT, anergic T cells recover by the influence of microenvironmental IL-2 and/or IL-15 to produce Th0/Th1 cytokines. In an atopic individual, IL-4 may reconstitute a Th2 cytokine pattern and reactivate an allergic response.

Acknowledgements

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References

1. Akdis CA. Biological mechanisms of allergic disease: environmental cytokines and the immune system. *Immunol Rev* 2001; 103: 131-47.
2. Akdis CA. Biological mechanisms of allergic disease: environmental cytokines and the immune system. *Immunol Rev* 1998; 165: 1-10.
3. Durham SR. The role of the immune system in allergic disease. *Immunol Rev* 2001; 103: 131-47.
4. Blaser K. Cytokines and the immune system. *Immunol Rev* 1998; 165: 1-10.
5. Akdis CA. Biological mechanisms of allergic disease: environmental cytokines and the immune system. *Immunol Rev* 2001; 103: 131-47.
6. Akdis CA. T cell subsets and the CD28-associated co-stimulatory molecule. *Immunol Rev* 2001; 103: 131-47.
7. Akdis CA. Allergic disease: tolerance to pathogens. *Immunol Rev* 2001; 103: 131-47.
8. Müller UR. Allergic disease: tolerance to pathogens. *Immunol Rev* 2001; 103: 131-47.
9. Haselden BM. Allergic disease: tolerance to pathogens. *Immunol Rev* 2001; 103: 131-47.
10. Jutel M, Watanabe T, Kobayashi T, Blaser K. Expression of HLA-DR and HLA-DQ in allergic disease. *Immunol Rev* 2001; 103: 131-47.
11. Jutel M, Watanabe T, Kobayashi T, Blaser K. Expression of HLA-DR and HLA-DQ in allergic disease. *Immunol Rev* 2001; 103: 131-47.
12. Akdis CA, Blaser K. Trends in Immunology. *Immunol Rev* 2001; 103: 131-47.

Discussion

Malling: I think... be that you should...

Akdis: I would... are just at the beginning... nary results and I... in clinical studies... therapy. Data do... that you should u...

Chapman: Do yo... could be used rou...

Acknowledgements:

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References

1. Akdis CA, Blaser K. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *Faseb J* 1999; 13: 603-609.
2. Akdis CA, Blaser K. Mechanisms of interleukin-10-mediated immune suppression. *Immunology* 2001; 103: 131-136.
3. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998; 102: 157-164.
4. Blaser K, Carballido JM, Faith A, Crameri R, Akdis CA. Determinants and mechanisms of human immune response to bee venom phospholipase A₂. *Int Arch Allergy Immunol* 1998; 117: 1-10.
5. Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K. Role of IL-10 in specific immunotherapy. *J Clin Invest* 1998; 102: 98-106.
6. Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *Faseb J* 2000; 14: 1666-1669.
7. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Müller U, Blaser K. Epitope specific T cell tolerance to phospholipase A₂ in bee venom immunotherapy and recovery by IL-2 and IL-15 *in vitro*. *J Clin Invest* 1996; 98: 1676-1683.
8. Müller UR, Akdis CA, Fricker M, Akdis M, Bettens F, Blesken T, Blaser K. Successful immunotherapy with T cell epitope peptides of bee venom phospholipase A₂ induces specific T cell anergy in bee sting allergic patients. *J Allergy Clin Immunol* 1998; 101: 747-754.
9. Haselden BM, Kay AB, Larché M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999; 189: 1885-1894.
10. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OAR, Malolepszy J, Zak-Nejmark T, Koga R, Kobayashi T, Blaser K, Akdis CA. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* 2001; 413: 420-425.
11. Jutel M, Watanabe T, Akdis M, Blaser K and Akdis CA. Immune regulation by histamine. *Curr Opin Immunol* 2002; 14: 735-740.
12. Akdis CA, Blaser K. Bypassing IgE and targeting T cells for specific immunotherapy of allergy. *Trends in Immunol* 2001; 22: 175-178.

Discussion

Malling: I think, these aspects of histamine are very interesting. The implication must be that you should not use anti-histamine during allergen-specific immunotherapy.

Akdis: I would not say it like that because you have four different receptors and we are just at the beginning of this line of research. I just wanted to present these preliminary results and I do not have any main conclusions. However, Ulrich Müller has shown in clinical studies that anti-histamine usage is also successful in bee venom immunotherapy. Data do not only show that you should not use anti-histamines, they also show that you should use anti-histamines. We do not yet fully understand this at all.

Chapman: Do you think that the IL-10 and the regulatory T cells provide a marker that could be used routinely during immunotherapy?

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