

IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy

CEZMI A. AKDIS¹ AND KURT BLASER

Swiss Institute of Allergy and Asthma Research, CH-7270 Davos, Switzerland

ABSTRACT Specific immunotherapy (SIT) is widely used for treatment of allergic diseases and could potentially be applied in other immunological disorders. Induction of specific unresponsiveness (anergy) in peripheral T cells and recovery by cytokines from the tissue microenvironment represent two key steps in SIT with whole allergen or antigenic T cell peptides (PIT). The anergy is directed against the T cell epitopes of the respective antigen and characterized by suppressed proliferative and cytokine responses. It is initiated by autocrine action of IL-10, which is increasingly produced by the antigen-specific T cells. Later in therapy, B cells and monocytes also produce IL-10. The anergic T cells can be reactivated by different cytokines. Whereas IL-15 and IL-2 generate Th1 cytokine profile and an IgG4 antibody response, IL-4 reactivates a Th2 cytokine pattern and IgE antibodies. Increased IL-10 suppresses IgE and enhances IgG4 synthesis, resulting in a decreased antigen-specific IgE:IgG4 ratio, as observed normally in patients after SIT or PIT. The same state of anergy against the major bee venom allergen, phospholipase A₂, can be observed in subjects naturally anergized after multiple bee stings. Together, these data demonstrate the pivotal role of autocrine IL-10 in induction of specific T cell anergy and the important participation of the cytokine microenvironment in SIT. Furthermore, knowledge of the mechanisms explaining reasons for success or failure of SIT may enable possible predictive measures of the treatment.—Akdis, C. A., Blaser, K. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J.* 13, 603–609 (1999)

Key Words: interleukin-10 · T cell epitopes · microenvironments · bee venom

BACKGROUND

SPECIFIC IMMUNOTHERAPY (SIT)² is an efficient treatment for allergic diseases to defined allergens and is used most effectively in allergic reactions to insect venom and allergic rhinitis (1–3). The mechanisms by which SIT achieves clinical im-

provement has been proposed in a number of reports. A rise in allergen-blocking immunoglobulin G (IgG) antibodies, particularly of IgG4 class (4–6), the generation of CD8⁺ T cells modulating the IgE response (7), a reduction in numbers of mast cells and eosinophils, and inhibition of mediator release (8–10) were found to be associated with successful SIT. Moreover, successful SIT of allergic diseases was found to correlate directly with decreased interleukin 4 (IL-4) and IL-5 production by CD4⁺ T cells (11, 12). A shift from T helper cell type 2 (Th2) cytokine production toward a pattern with increased interferon gamma (IFN-γ) in SIT of allergy to bee venom (BV), wasp venom, grass pollen, and house dust mites was also observed (11–13). It appears, however, that the induction of a state of specific unresponsiveness or anergy in peripheral T cells represents a key step in the mechanism of SIT (11, 14–17). The specific anergy is characterized mainly by suppressed proliferative and cytokine responses against the major allergen(s) and its T cell recognition sites (18). Anergized T cells can be reactivated to produce either a distinct Th1 or Th2 cytokine pattern, depending on the cytokines in the tissue microenvironment, thus directing SIT toward successful or unsuccessful clinical outcome (14, 19).

BV phospholipase A₂ (PLA) represents the major antigen and allergen of BV (20, 21). The response to PLA provides a model especially suited with which to study the cellular and molecular mechanisms of specific immune response regulation in humans (14–16, 20–23). SIT with whole BV (BV-SIT) and with short peptides bearing immunodominant T cell epitopes of PLA (PIT) was

¹ Correspondence: Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse, 22, CH-7270, Davos, Switzerland. E-mail: akdisac@siaf.unizh.ch

² Abbreviations: BV, bee venom; BV-SIT, SIT with whole BV; Ig, immunoglobulin; IFN, interferon; IL, interleukin; PBMC, peripheral blood mononuclear cells; PI 3, phosphatidylinositol 3; PIT, immunotherapy with peptides bearing immunodominant T cell epitopes; PLA, phospholipase A₂; SIT, specific immunotherapy; TcR, T cell receptor; Th2, T helper cell type 2.

applied successfully and enabled us to study the immunological mechanisms of SIT (14–16).

RECENT RESULTS

Successful SIT with whole allergen or T cell epitope-containing peptides induces specific anergy in peripheral T cells

The immunological mechanism of SIT was investigated in BV-SIT and further elucidated in PIT using a mixture of three peptides representing the immunodominant T cell epitopes PLA45–62, PLA82–92, and PLA113–124 (24). In both BV-SIT and PIT, patients who were successfully treated showed specific T cell unresponsiveness against the entire PLA allergen as well as to the three T cell epitope-containing peptides (14–16). The specific proliferative T cell responses as well as the secretion of both the Th1 cytokines IL-2 and IFN- γ and the Th2 cytokines IL-4, IL-5, and IL-13 were suppressed. The PPD or TT control responses were not affected by either treatment, indicating that the suppressive effect of SIT and PIT was specifically directed against the respective allergen (14–16). Decreased T cell proliferative responses in SIT have been demonstrated so far in allergy to ragweed, cat dander, grass pollen, and BV (11, 12, 25, 26). In mice, antigenic peptides of house dust mite and cat allergen were shown to induce anergy in T cells (27, 28). Recent studies with T cell peptides of Fel *d* 1 indicated suppression of specific T cell reactivity by PIT in cat allergy (17, 29).

Recently we have shown that the induction of an anergic state in Th2 cells represents an active process that is associated with increased levels of basal tyrosine kinase activity, cytokine production, and CD25 up-regulation (30). It appears to be related to alterations in the signaling pathways mediated through the T cell receptor (TcR). Anergized Th2 cells failed to respond to anti-CD3 stimulation with either increased tyrosine kinase activity or increased levels of tyrosine phosphorylation of p56^{lck} and ZAP70. In addition, intracellular calcium flux, observed in untreated Th2 cells in response to anti-CD3 monoclonal antibody, was absent in anergic Th2 cells (30).

T cell anergy in SIT results from initial IL-10 production by specific T cells.

In BV-SIT and PIT, the proliferative responses and Th1 and Th2 cytokine production against the entire antigen and the antigenic peptides decreased within 4 wk (14–16). At the same time, antigen- and peptide-induced secretion of IL-10

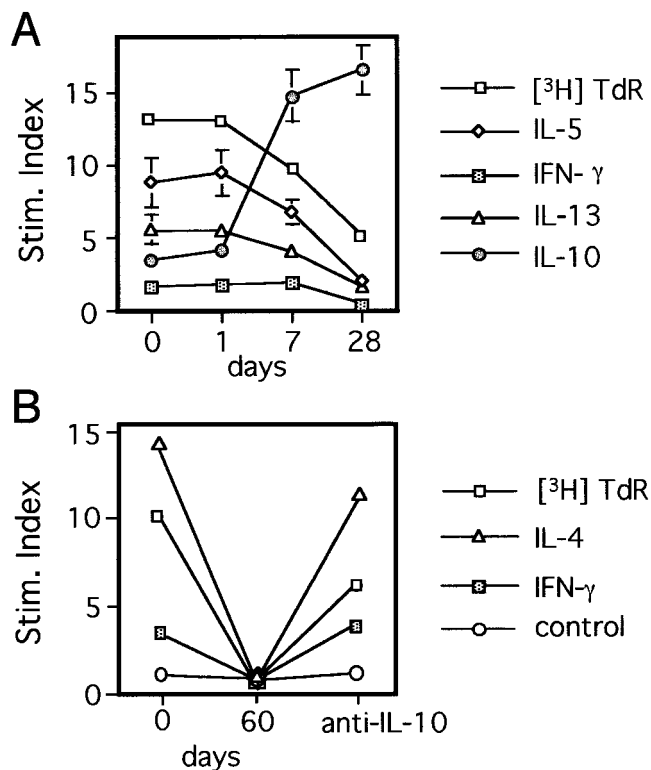


Figure 1. A) Changes of cytokine production and proliferation in PBMC cultures during BV-SIT. PBMC were stimulated with PLA before and after 1, 7, and 28 days of BV-SIT. Cytokines and thymidine incorporation were determined after 5 days of culture. T cell proliferation, IL-5, IL-13, and IFN- γ decreased, whereas IL-10 increased simultaneously. B) T cell proliferation and cytokine response were not suppressed in IL-10-neutralized PBMC cultures. Abolished PLA-specific proliferation and IL-4 and IFN- γ were reconstituted by neutralization of endogenously produced IL-10.

increased simultaneously after 7 days and reached maximal levels after 28 days of treatment, when the specific anergy was fully established (15) (Fig. 1A). The suppressed PLA-specific T cell proliferative and cytokine responses could be reconstituted by *ex vivo* neutralization of endogenous IL-10, indicating that this cytokine is actively involved in development of anergy in specific T cells (15) (Fig. 1B). The cellular origin of IL-10 was demonstrated by intracytoplasmic IL-10 staining in peripheral blood mononuclear cells (PBMC) and coexpression of cellular surface markers (15). As shown in Fig. 2, intracellular IL-10 had already increased significantly after 7 days of SIT in the antigen-specific T cell population and in activated CD4⁺ T lymphocytes. After 4 wk of SIT, increased intracytoplasmic IL-10 was also observed in monocytes and B cells (15). Along with the facts that anti-IL-10 inhibited the induction of anergy in T cells (15) and recovered T cells from the anergic state (Fig. 1), this suggests that an autocrine action of IL-10 on the T cells induces the state of peripheral

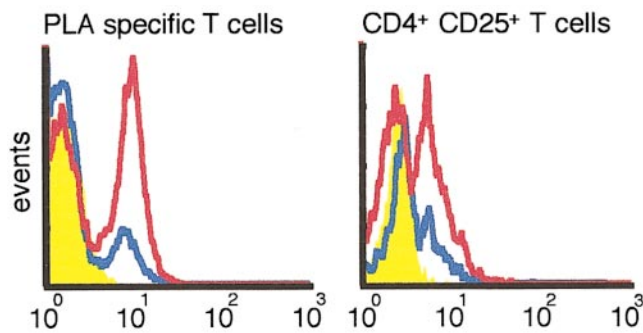


Figure 2. Changes in intracytoplasmic IL-10 content of T cells during SIT. Immediately after isolation, PBMC were stained for CD4 and CD25 (activated CD4⁺ T cells). After fixing and permeabilizing, the cells were counterstained for intracytoplasmic IL-10. PLA-specific T cells were derived from PLA-stimulated PBMC that were cultured for 10 days and T cells were restimulated with an anti-CD3/anti-CD28 mixture in the presence of monensin for 5 h. The yellow area represents the isotype control Ab, blue curve before SIT, and red curve 7 days after SIT.

anergy. This state is further maintained by IL-10 produced by monocytes, B cells, and nonspecific bystander T cells.

The same features of specific anergy were found in healthy beekeepers who were multiply stung by bees. Similar to allergic patients after BV-SIT, these naturally anergized individuals showed a substantial increase in IL-10-producing CD4⁺CD25⁺ T cells and monocytes. Neutralization of endogenous IL-10 in cell cultures of naturally anergized individuals fully reconstituted the proliferative T cell and cytokine responses (15).

IL-10 is a major regulatory cytokine of inflammatory responses. It was originally described as a Th2 cell factor in the mouse, inhibiting cytokine synthesis by Th1 cells (31). However, increasing evidence showed that IL-10 acts as a general inhibitor of proliferative and cytokine responses of both Th1 and Th2 cells *in vitro* and *in vivo* (32–38). IL-10 is released by mononuclear phagocytes (32, 33), natural killer cells (34), and both Th1 and Th2 lymphocytes (35). The inhibitory effect of IL-10 in T cell clones was observed exclusively in APC-dependent culture systems, but not in T cells stimulated by solid-phase bound anti-CD3 (33, 36, 37, 39). The reason for this may be that IL-10 blocks the CD28-B7.1 interaction and subsequent costimulatory signaling pathways in T cells (38). Thus, inhibition of accessory molecule signaling may explain peptide-ligand-induced specific anergy in certain T cell systems (30, 40). In addition, IL-10 action at the level of cytokine gene transcription (41) and inhibition of cytokine mRNA accumulation has been demonstrated (42).

In mice, IL-10 administration before allergen treatment induced antigen-specific T cell tolerance and demonstrated the pivotal role of IL-10 in estab-

lishment of peripheral T cell anergy. (43). Moreover, inhibition of graft-vs.-host disease by IL-10 and allograft rejection in HLA mismatched, bone marrow transplanted SCID patients provide further evidence for a key role of this cytokine in the induction and maintenance of an anergic state (44). Similarly, inappropriate stimulation of tumor reactive human T cells was shown to result from increased endogenous IL-10 production by these cells (45), indicating a role for IL-10 in tumor-specific anergy. Recently, IL-10-derived regulatory CD4⁺ T cells producing IL-10, but not IL-2 and IL-4, that suppressed antigen-specific T cell response *in vitro* and prevented antigen-induced murine colitis were identified in humans and mice (46).

Additional evidence for IL-10-induced anergy in specific immunotherapy

Induction of peripheral T cell anergy resulting from IL-10 released by specific immunotherapy was clearly demonstrated in allergy to honey bee and wasp venom (11, 13–15). Furthermore, induction of T cell nonresponsiveness was observed in specific T cell peptide treatment of cat allergy (17), similar to the PLA-peptide immunotherapy of bee venom allergy (16). Moreover, down-regulated T cell proliferative responses were found in specific immunotherapy of atopic patients with allergic rhinitis, conjunctivitis, and asthma (25, 26). Evidence for increased IL-10 in nasal allergy and asthma immunotherapy has been shown in two recent studies. In successful ragweed nasal immunotherapy, the group receiving nasal weed extract had elevated IL-10 levels in nasal lavage at the peak of ragweed season compared with those treated with placebo (47). In addition, increased plasma IL-10 levels have been found in grass pollen immunotherapy of asthma after 24 h of initiation (48). Together, these studies demonstrate the pivotal role of IL-10 in suppressing the T cell responses in specific immunotherapy and implicate the generation of an anergic state in T lymphocytes as a general regulatory mechanism of specific immune responses.

Specific responses can be reestablished in anergic T cells by cytokines from the tissue microenvironment

The abrogated proliferative response after SIT was almost fully recovered by stimulation of anergic cells in the presence of antigen and IL-2 or IL-15 (14). The full capacity for IFN- γ secretion was reestablished by both cytokine treatments (**Fig. 3**). In contrast, specific stimulation in the presence of IL-4 induced IL-4, IL-5, and IL-13, and therefore recovered a Th2 cytokine pattern typical for an allergic

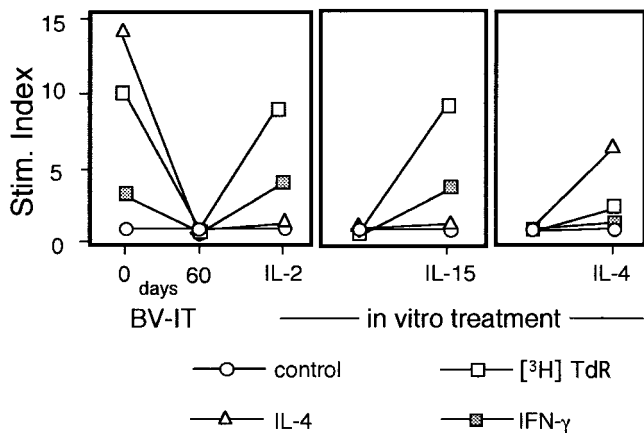


Figure 3. Induction of anergy in peripheral T cells by SIT and reactivation of anergic T cells *in vitro*. After 2 months of SIT, PLA-induced proliferative and cytokine responses were fully suppressed. Anergic T cells were cultured with PLA in the presence of IL-2, IL-15, or IL-4 for 12 days and restimulated with the same antigen in the presence of autologous irradiated PBMC. Proliferation and IFN- γ production but not IL-4 were recovered by IL-2 and IL-15, whereas IL-4 treatment partially recovered the production of IL-4 but not of IFN- γ .

state (14). IL-2 is produced mainly by activated T cells. However, basically the same immunological properties are displayed by IL-15 secreted by most antigen-presenting cell types and tissue cells, but not by T cells (49). Thus, IL-15, but also IL-2, may act as important microenvironmental cytokines that regulate and recover T cells from SIT-induced anergy. Certain cytokines from the tissue microenvironment can control the secondary induction of distinct Th1 or Th2 cytokine patterns associated with either normal immunity and successful therapy or further persistence of allergy. In particular, IL-15, being released by professional antigen-presenting cells and various tissue cells in normal immune defense, may direct anergic T cells toward normal immunity. In contrast, long-lasting success of SIT to single allergen may be difficult to achieve in an already established polyallergic and atopic individual.

Regulation of specific IgE and IgG4 antibodies in SIT and PIT

The serum levels of specific IgE and IgG4 antibodies delineate allergic and normal immunity to allergen (6, 20, 24, 50, 51). Whereas peripheral anergy was demonstrated in specific T cells, the capacity by B cells to produce specific IgE and IgG4 antibodies was not abolished. Specific serum levels of both isotypes increased during the early phase of treatment, but the increase in specific IgG4 was more pronounced and the ratio of specific IgE to IgG4 was decreased by 10- to 100-fold (14, 16, 24, 52). Also, the *in vitro* production of PLA-specific IgE and IgG4 antibodies by PBMC changed in parallel with the serum levels of

specific isotypes (14). Similar changes in specific isotype ratio have been observed in SIT of various allergies (53–55). IL-10 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 PLA-specific IgE, while IgG4 formation is simultaneously increased (15, 56). Thus, in SIT, IL-10 not only generates anergy in T cells, but also counter-regulates specific isotype formation and changes the response from an IgE- to an IgG4-dominated phenotype.

The effect of IL-10 on the effector cells of allergic inflammation

At a very early stage of BV-SIT, most patients are already protected against bee stings even though a definite decrease in IgE antibodies and IgE-mediated skin sensitivity normally requires several years of treatment (1, 57). Increase of allergen-specific IgG4 antibodies blocking IgE binding could explain only the late phase protection of SIT (48, 57). At the early phase, however, a decrease in the histamine and sulfidoleukotrienes release from basophils may be more relevant (58). This decreased basophil 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 (59–63). Moreover, IL-10 was shown to reduce generation of tumor necrosis factor α , granulocyte-macrophage colony-stimulating factor, and IL-6 from mouse bone marrow and rat peritoneal mast cells in response to specific IgE cross-linking (64, 65). In addition, IL-10 down-regulates eosinophil function and activity (66). IL-10 suppresses IL-5 production by human resting T cells and in Th0 and Th2 clones (37, 38). The inhibitory action of IL-10 on IL-5 synthesis and eosinophil recruitment has been confirmed in a murine model of allergic eosinophilic peritonitis and airway eosinophilia (36, 37). Furthermore, IL-10 inhibits endogenous granulocyte-macrophage colony-stimulating factor production and CD40 expression by activated eosinophils and enhances eosinophil death (67, 68).

CONCLUDING REMARKS

Induction of specific anergy in peripheral T cells by autocrine IL-10 production and subsequent reactivation of distinct cytokine patterns by different cytokines from the microenvironment are pivotal key steps in the immunological mechanism of specific allergy treatment (Fig. 4). This mechanism, however, has implications that may reach beyond allergy treat-

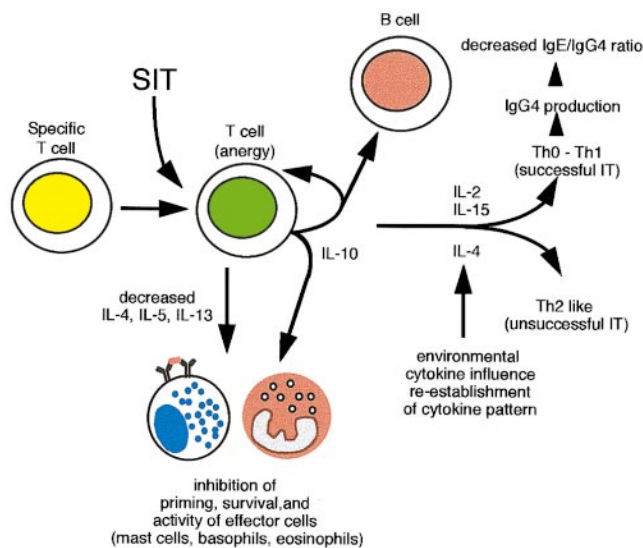


Figure 4. Immunological mechanisms of SIT. Continuous treatment with allergen establishes a state of peripheral anergy in specific T cells, which is characterized by suppressed proliferative and T cell cytokine responses and a simultaneous increase in IL-10 production. As a consequence, activation, priming and survival of allergic inflammatory effector cells are down-regulated. The anergic T cells can be recovered by cytokines from the tissue microenvironment. In successful SIT, anergic T cells recover by the influence of IL-2 and/or IL-15 to produce Th0-Th1 cytokines. In an atopic or polyallergic microenvironment, IL-4 reconstitutes a Th2 cytokine pattern and may reactivate allergic responses.

ment and IgE antibody regulation. Induction and maintenance of specific anergy may be important steps in transplantation and treatment of autoimmunity. Furthermore, it may lead to a better understanding of tumor growth, parasite infection, and development of AIDS (18, 44, 45, 69, 70). Both SIT and PIT generate high amounts of IL-10, which, in turn, induces specific anergy in peripheral T cells, apparently without directly inhibiting B cell function. As a result of these treatments, the antigen-specific IgE:IgG4 ratio in peripheral blood decreases toward normal. Moreover, specific Th2 cytokine reactivity is directly involved in the pathogenesis of allergic inflammation, since these cytokines are essential for priming, survival, and activity of inflammatory effector cells. IL-10, initially produced by the specific T cells themselves not only induces anergy, but also inhibits the inflammatory reaction by inactivating mast cells, basophils, and eosinophils.

Many studies affirm that SIT is not always successful (55, 71). The reactivation of anergic T cells and the modulation of cytokine patterns suggest a decisive role of microenvironmental cytokines in directing SIT toward success or failure. High success rates of more than 90% are observed mainly in mono- or oligo-specific allergies, occurring mostly in venom-allergic individuals (55, 71). They display a normal immune microenvironment, reactivating a Th0-Th1

cytokine pattern and normal IgG4 antibody response. In contrast, highly polyallergic or atopic states are characterized by increased IL-4 contents, which may reestablish a Th2 allergic response. Moreover, increased IL-10 contents in T cells during SIT may represent a predictive measure, avoiding long-term treatment over several years in unsuccessful cases.

Finally, the biochemical pathways of anergy induction and maintenance remain to be determined. The inhibitory effect of IL-10 in T cells was observed exclusively in APC-dependent culture systems and antigen-specific responses, and not in T cells stimulated by solid-phase bound anti-CD3. The objective for this is that IL-10 blocks the CD28 stimulation and subsequent costimulatory signaling pathways in T cells (72). Lack of signaling through CD28, even in the presence of an optimal TcR signal, can render the cell anergic (73). Although CD28 can display its signaling potential through an array of intermediates, the phosphatidylinositol 3 (PI 3) kinase has the greatest affinity for CD28. The PI 3 kinase appears to deliver, in synergy with TcR signals, an activation signal for the AP-1 component, c-jun. The activation of PI 3-kinase depends on membrane localization through binding by its SH2 domain to a conserved motif in the cytoplasmic domain of CD28 (74). Indeed, in results not yet published, we found that IL-10 is active on T cells that require CD28 costimulation for proliferation and cytokine production. IL-10 ligation to its receptor blocks the tyrosine phosphorylation of CD28. This leads to an inhibition of CD28 association to p85 PI 3-kinase and inhibition of CD28 signaling pathway. These results also support the relevance of the experimental model of anergy induction in human T cells lacking a costimulatory signal (19, 30, 40). Accordingly, SIT and PIT are clearly based on immunological and biochemical mechanisms, also explaining the resulting clinical effect of the treatment. This knowledge may help to improve SIT, and further defining biochemical signaling pathways may open new strategies of immune suppression by drug interaction. **[F]**

This work was supported by the Swiss National Science Foundation no. 31.39.177.93 and 31-50590.97/1.

REFERENCES

1. Müller, U. R., Helbling, A., and Berchtold, E. (1992) Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. *J. Allergy Clin. Immunol.* **89**, 529–535
2. Walker, S. M., Varney, V. A., Gaga, M., Jacobson, M. R., and Durham, S. R. (1995) Grass pollen immunotherapy: efficacy and safety during a 4 year follow-up study. *Allergy* **50**, 405–413
3. Varney, V. A., Gaga, M., Frew, A. J., Aber, V. R., Kay, A. B., and Durham, S. R. (1991) Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by anti-allergic drugs. *Br. Med. J.* **302**, 489–500

4. Hussain, R., Poindexter, R. W., and Ottesen, E. A. (1992) Control of allergic reactivity in human filariasis. Predominant localization of blocking antibody to the IgG4 subclass. *J. Immunol.* **148**, 2731–2739
5. Reid, M. J., Moss, R. B., Hsu, Y. P., Kwasnicki, J. M., Commerford, T. M., and Nelson, B. L. (1986) Seasonal asthma in northern California: allergic causes and efficacy of immunotherapy. *J. Allergy Clin. Immunol.* **78**, 590–600
6. Wetterwald, A., Skvaril, F., Müller, U., and Blaser, K. (1985) Isotypic and idiotypic characterization of anti-bee venom phospholipase A₂ antibodies. *Arch. Allergy Appl. Immunol.* **77**, 195–197
7. Rocklin, R. E., Sheffer, A., Greineder, D. K., and Melmon, K. L. (1980) Generation of antigen-specific suppressor cells during allergy desensitization. *N. Engl. J. Med.* **302**, 1213–1219
8. Creticos, P. S., Adkinson, N. F., Jr., Kagey-Sabotka, A., Proud, D., Meier, H. L., Naclerio, R. M., Lichtenstein, L. M., and Norman, P. S. (1983) Nasal challenge with ragweed in hay fever patients: effect of immunotherapy. *J. Clin. Invest.* **76**, 2247–2253
9. Rak, S., Rowhagen, O., and Venge, P. (1988) The effect of immunotherapy on bronchial hyper-responsiveness and eosinophil cationic protein in pollen allergic patients. *J. Allergy Clin. Immunol.* **82**, 470–480
10. Otsuka, H., Mezawa, A., Ohnishi, M., Okubo, K., Seki, H., and Okuda, M. (1991) Changes in nasal metachromatic cells during allergen immunotherapy. *Clin. Exp. Allergy* **21**, 115–120
11. Jutel, M., Pichler, W. J., Skrbic, D., Urwyler, A., Dahinden, C., and Müller, U. R. (1995) Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen stimulated T cell cultures. *J. Immunol.* **154**, 4178–4194
12. Secrist, H., Chelen, C. J., Wen, Y., Marshall, J. D., and Umetsu, D. T. (1993) Allergen immunotherapy decreases interleukin 4 production in CD4 T cells from allergic individuals. *J. Exp. Med.* **178**, 2123–2130
13. Bellinghousen, I., Metz, G., Enk, A. H., Christmann, S., Knop, J., and Saloga, J. (1997) Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. *Eur. J. Immunol.* **27**, 1131–1139
14. Akdis, C. A., Akdis, M., Blesken, T., Wymann, D., Alkan, S. S., Müller, U., and Blaser, K. (1996) 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.* **98**, 1676–1683
15. Akdis, C. A., Blesken, T., Akdis, M., Wüthrich, B., and Blaser, K. (1998) The role of IL-10 in specific immunotherapy. *J. Clin. Invest.* **102**, 98–106
16. Müller, U. R., Akdis, A. C., Fricker, M., Akdis, M., Bettens, F., Blesken, T., and Blaser, K. (1998) 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.* **101**, 747–754
17. Marcotte, G. V., Braun, C. M., Norman, P. S., Nicodemus, C. F., Kagey-Sabotka, A., Lichtenstein, L. M., and Essayan, D. M. (1998) Effects of peptide therapy on *ex vivo* T cell responses. *J. Allergy Clin. Immunol.* **101**, 506–513
18. Parijs, L. V., and Abbas, A. K. (1998) Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* **280**, 243–248
19. Essery, G., Feldmann, M., and Lamb, J. (1988) Interleukin-2 can prevent and reverse antigen-induced unresponsiveness in cloned human T lymphocytes. *Immunol.* **64**, 413–417
20. Kagey-Sabotka, A., Franklin, R. M., Adkinson, N. F., Jr., Valentine, M., Bauer, H., and Lichtenstein, L. M. (1976) Allergy to insect sting II. Phospholipase A: the major allergen in honey bee venom. *J. Allergy Clin. Immunol.* **57**, 29–40
21. Müller, U. R., Dudler, T., Schneider, T., Cramer, R., Fischer, H., Skrbic, D., Maibach, R., Blaser, K., and Suter, M. (1995) Type 1 skin reactivity to native and recombinant phospholipase A₂ from honey bee venom is similar. *J. Allergy Clin. Immunol.* **96**, 395–402
22. Akdis, C. A., Blesken, T., Akdis, M., Alkan, S. S., Wüthrich, B., Heusser, C. H., and Blaser, K. (1997) Induction and differential regulation of bee venom phospholipase A₂-specific human IgE and IgG4 antibodies *in vitro* requires allergen-specific and non-specific activation of T and B cells. *J. Allergy Clin. Immunol.* **99**, 345–352
23. Akdis, C. A., Blesken, T., Akdis, M., Alkan, S. S., Heusser, C. H., and Blaser, K. (1997) Glucocorticoids inhibit human antigen-specific and enhance total IgE and IgG4 production due to differential effects on T and B cells *in vitro*. *Eur. J. Immunol.* **27**, 2351–2357
24. Carballido, J. M., Carballido-Perrig, N., Kägi, M. K., Meloen, R. H., Wüthrich, B., Heusser, C. H., and Blaser, K. (1993) T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A₂. *J. Immunol.* **150**, 3582–3591
25. Creticos, P. S. (1992) Immunological changes associated with immunotherapy. In *Immunotherapy of IgE Mediated Disorders* (Greenberger, P. A., ed) pp. 13–37, W. B. Saunders, Philadelphia
26. Greenstein, J. L., Morgenstern, J. P., LaRaia, J., Counsell, C. M., Goodwin, W. H., Lussier, A., Creticos, P. S., Norman, P. S., and Garman, R. D. (1992) Ragweed immunotherapy decreases T cell reactivity to recombinant Amb a I.1. *J. Allergy Clin. Immunol.* **89**, 322
27. Hoyne, G. F., O'Hehir, R., Wraith, D. C., Thomas, W. R., and Lamb, J. R. (1993) Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J. Exp. Med.* **178**, 1783–1788
28. Briner, T., Kou, M., Keating, K., Rogers, B., and Greenstein, J. (1993) Peripheral T cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 7608–7612
29. Norman, P., Ohmann Jr, J. L., Long, A. A., Creticos, P. S., Geffer, M. A., Shaked, Z., Wood, R., Eggleston, P., Lichtenstein, L. M., and Jones, N. (1996) Treatment of cat allergy with T cell reactive peptides. *Am. J. Resp. Crit. Care Med.* **154**, 1623–1628
30. Faith, A., Akdis, C. A., Akdis, M., Simon, H-U., and Blaser, K. (1997) Defective TCR stimulation in anergized type 2 T helper cells correlates with abrogated p56^{lck} and ZAP-70 tyrosine kinase activities. *J. Immunol.* **159**, 53–60
31. Fiorentino, D. F., Bond, M. W., and Mosmann, T. R. (1989) Two types of mouse T helper cell IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* **170**, 2081–2095
32. Fiorentino, D. F., Zlotnik, A., Mosmann, T. R., Howard, M., and O'Garra, A. (1991) IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* **147**, 3815–3822
33. De Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G., and De Vries, J. E. (1991) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* **174**, 1209–1220
34. Hsu, D. H., Moore, K. W., and Spits, H. (1992) Differential effects of interleukin-4 and -10 on interleukin-2 induced interferon- γ synthesis and lymphokine activated killer activity. *Int. Immunol.* **4**, 563–569
35. Del Prete, G., De Carli, M., Almerigogna, F., Giudizi, M. G., Biagiotti, R., and Romagnani, S. (1993) Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J. Immunol.* **150**, 353–356
36. Zuany-Amorim, C., Haile, S., Leduc, D., Dumarey, C., Huerre, M., Vergaftig, B. B., and Pretolani, M. (1995) Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J. Clin. Invest.* **95**, 2644–2651
37. Zuany-Amorim, C., Creminon, C., Nevers, M. C., Nahori, M. -A., Vergaftig, B. B., and Pretolani, M. (1996) Modulation by IL-10 of antigen-induced IL-5 generation, and CD4+ T lymphocyte and eosinophil infiltration into the mouse peritoneal cavity. *J. Immunol.* **157**, 377–384
38. Schandane, L., Alonso-Vega, C., Willems, F., Gerard, C., Delvaux, A., Velu, T., Devos, R., de Boer, M., and Goldman, M. (1994) B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. *J. Immunol.* **152**, 4368–4374
39. Ding, L., and Shevach, E. M. (1992) IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage co-stimulatory function. *J. Immunol.* **148**, 3133–3139
40. Lamb, J. R., Skidmore, B. J., Green, N., Chiller, J. M., and Feldman, M. (1983) Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J. Exp. Med.* **157**, 1434–1447

41. Wang, P., Wu, P., Siegel, M. I., Egan, R. W., and Billah, M. M. (1994) IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. *J. Immunol.* **153**, 811–816
42. Bogdan, C., Paik, J., Vodovotz, Y., and Nathan, C. (1992) Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor- β and interleukin-10. *J. Biol. Chem.* **267**, 23301–23310
43. Enk, A. H., Saloga, J., Becker, D., Mohamadadeh, M., and Knop, J. (1994) Induction of hapten-specific tolerance by interleukin 10 *in vivo*. *J. Exp. Med.* **179**, 1397–1402
44. Bacetta, R., Bigler, M., Touraine, J. -T., Parkman, R., Tovo, P. -A., Abrams, J., and De Waal Malefyt, R. (1994) High levels of interleukin 10 production *in vivo* are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J. Exp. Med.* **179**, 493–502
45. Becker, J., Czerny, C. C., and Bröcker, E. -B. (1994) Maintenance of clonal anergy by endogenously produced IL-10. *Int. Immunol.* **6**, 1605–1612
46. Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., De Vries, J. E., and Roncarolo, M. G. (1997) A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature (London)* **389**, 737–742
47. Gaglani, B., Borish, L., Bartelson, B. L., Bucheimer, A., Keller, L., Nelson, H. S. (1997) Nasal immunotherapy in weed-induced allergic rhinitis. *Annals of Allergy, Asthma, and Immunology* **79**, 259–265
48. Ippoliti, F., Ragno, V., Del Nero, A., McEwen, N. M., McEwen, H., and Businco, L. (1997) Effect of preseasonal enzyme potentiated desensitization (EPD) on plasma IL-6 and IL-10 of grass pollen-sensitive asthmatic children. *Allerg. Immunol. (Paris)* **29**, 123–125
49. Grabstein, K. H., Eisenman, J., Shanebeck, K., Rauch, C., Srinivasan, S., Fung, V., Beers, C., Richardson, J., Schoenborn, M. A., Ahdieh, M., Johnson, L., Anderson, M. R., Watson, J. D., Anderson, D. M., and Giri, J. G. (1994) Cloning of a T cell growth factor which interacts with the γ chain of the interleukin-2 receptor. *Science* **264**, 965–968
50. Aalberse, R. C., Van der Gaag, R., and van Leuwen, J. (1983) Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J. Immunol.* **130**, 722–726
51. Lucas, A. H. (1990) IgG subclass restricted immune responses to allergens. *Springer Semin. Immunopathol.* **12**, 385–420
52. Wyss, M., Scheitlin, T., Stadler, B. M., and Wüthrich, B. (1993) Immunotherapy with aluminium hydroxide adsorbed insect venom extracts (Alutard SQ): immunologic and clinical results of a prospective study over 3 years. *Allergy* **48**, 81–86
53. Ortolani, C., Pastorello, E. A., Incorvaia, C., Ispano, M., Farioli, L., Zara, C., Pravettoni, V., and Zanussi, C. (1994) A double-blind, placebo-controlled study of immunotherapy with an alginate-conjugated extract of *Parietaria judaica* in patients with *Parietaria* hay fever. *Allergy* **49**, 13–21
54. Tsai, L. C., Tang, R. B., Hung, M. W., and Chang, Z. N. (1991) Changes in levels of house dust mite specific IgG4 during immunotherapy in asthmatic children. *Clin. Exp. Allergy* **21**, 367–372
55. Bertelsen, A., Andersen, J. B., Christensen, J., Ingemann, L., Kristensen, T., and Ostergaard, P. A. (1989) Immunotherapy with dog and cat extracts in children. *Allergy* **44**, 330–335
56. Punnonen, J., De Waal Malefyt, R., Van Vlasselaer, P., Gauchat, J.-F., and De Vries, J. E. (1993) IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. *J. Immunol.* **151**, 1280–1289
57. Müller, U. R., and Mosbech, H. (1993) Position paper: Immunotherapy with hymenoptera venoms. *Allergy* **48**, 36–46
58. Jutel, M., Müller, U. M., Fricker, M., Rihs, S., Pichler, W., and Dahinden, C. (1996) Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. *Clin. Exp. Allergy* **26**, 112–1118
59. Akdis, C. A. (1997) Glucocorticoids. In *Strategies for Immunointervention in Dermatology* (Burg, G., and Dummer, R., eds) pp. 131–146, Springer-Verlag, Heidelberg
60. Hamaguchi, Y., Kanakura, Y., Fujita, T., Takeda, S., Nakano, T., Tauri, S., Honjo, T., and Kitamura, Y. (1987) Interleukin 4 is an essential factor for *in vitro* clonal growth of murine connective tissue-type mast cells. *J. Exp. Med.* **165**, 268–273
61. Kuna, P., Reddigari, S. R., Schall, T. J., Rucinski, D., Sadick, M., and Kaplan, A. P. (1993) Characterization of the human basophil response to cytokines, growth factors, and histamine releasing factors of the intercrine/chemokine family. *J. Immunol.* **150**, 1932–1943
62. Schleimer, R. P., Derse, C. P., Friedman, B., Gillis, S., Plaut, M., Lichtenstein, L. M., and MacGlashan, D. W., Jr. (1989) Regulation of human basophil mediator release by cytokines. I. Interaction with anti-inflammatory steroids. *J. Immunol.* **143**, 1310–1327
63. Walker, C., Virchow, J.-C., Bruijnzeel, P. L. B., and Blaser, K. (1991) T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. *J. Immunol.* **146**, 1829–1835
64. Arock, M., Zuany-Amorim, C., Singer, M., Benhamou, M., and Pretolani, M. (1996) Interleukin-10 inhibits cytokine generation from mast cells. *Eur. J. Immunol.* **26**, 166–170
65. Marshall, J. S., Leal-Berumen, I., Nielsen, L., Glibetic, M., and Jordana, M. (1996) Interleukin (IL)-10 inhibits long-term IL-6 production but not preformed mediator release from rat peritoneal mast cells. *J. Clin. Invest.* **97**, 1122–1128
66. Pretolani, M., and Goldman, M. (1997) IL-10: a potential therapy for allergic inflammation? *Immunol. Today* **18**, 277–280
67. Takanashi, S., Nonaka, R., Xing, Z., O'Byrne, P., Dolovich, J., and Jordana, M. (1994) Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. *J. Exp. Med.* **180**, 711–715
68. Ohkawara, Y., Lim, K. G., Glibetic, M., Nakano, K., Dolovich, J., Croitoru, K., Weller, P. F., and Jordana, M. (1996) CD40 expression by human peripheral blood eosinophils. *J. Clin. Invest.* **97**, 1761–1766
69. Dadaglio, G., Garcia, S., Montagnier, L., and Gougeon, M.-L. (1994) Selective anergy of V β 8⁺ T cells in human immunodeficiency virus-infected individuals. *J. Exp. Med.* **179**, 413–424
70. Mahanty, S., Mollis, S. N., Ravichandran, M., Abrams, J. S., Kumaraswami, V., Jayaraman, K., Ottesen, E. A., and Nutman, T. B. (1996) High levels of spontaneous and parasite antigen-driven interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. *J. Infect. Dis.* **173**, 769–773
71. Müller, U., Helbling, A., and Berchtold, E. (1992) Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. *J. Allergy Clin. Immunol.* **89**, 529–535
72. Schandene, L., Alonso-Vega, C., Willems, F., Gerard, C., Delvaux, A., Velu, T., Devos, R., de Boer, M., and Goldman, M. (1994) B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. *J. Immunol.* **152**, 4368–4374
73. Rudd, C. E. (1996) Upstream-Downstream CD28 cosignaling pathways and T cell function. *Immunity* **4**, 527–534
74. Ward, S. G., Westwick, J., Hall, N. D., Sansom, D. M. (1993) Ligation of CD28 by B7 induces formation of D-3 phosphoinositides in T lymphocytes independently of T cell receptor/CD3 activation. *Eur. J. Immunol.* **23**, 2572–2577