T cell responses during allergen-specific immunotherapy of Type I allergy

Barbara Bohle[1]

[1]*Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria*

TABLE OF CONTENTS

1. ABSTRACT

Although allergen-specific immunotherapy (SIT) has been performed in humans for already a century, the immune mechanisms underlying this treatment are still not entirely solved. Allergen-specific $CD4^+$ T lymphocytes are considered as pivotal for the induction and maintenance of allergic disorders. Consequently, their role for allergy treatment has been - and still is – of great interest. Whereas two decades ago immune deviation, i.e. the switch from the allergic Th2 response to a Th1-like response, was described as the most important alteration induced by SIT, more recently the induction of allergen-specific regulatory T cells producing IL-10 has been considered as a main event causing peripheral T cell tolerance. In view of very recent data indicating that both mechanisms may occur consecutively during allergy treatment this review summarizes the current understanding of the immunological mechanisms involved in allergy vaccination.

2. INTRODUCTION

Immunoglobulin (Ig)E-mediated allergic disorders are hypersensitivity reactions of the immune system that manifest in certain organs, e.g. the nose and eyes (rhinoconjunctivitis), the lung (asthma), the skin (urticaria, atopic dermatitis) and the gastrointestinal tract. At present, approximately 25% of the population in the western world suffer from these disorders. This high prevalence demands efficient therapeutic interventions that reconstitute a physiological immune reaction to allergens in allergic individuals. Drug treatment of allergic symptoms is symptomatic and has minimal effects on the underlying immunological dysfunction. The disease process can, however, be modified by allergy vaccination, which is traditionally applied by injecting increasing amounts of the offending allergens (1). This so-called allergen-specific immunotherapy (SIT) is the only causative treatment for allergic disorders and has been performed in humans for almost one century with the aim to induce clinical tolerance

(or "desensitization") to the disease-eliciting allergens in allergic individuals (2, 3). Since approximately 70 years, allergologists are interested to discover the immune mechanisms underlying successful SIT. The revelation of these processes not only increases the insights into the pathophysiology of allergic disorders but also instigates novel approaches of SIT with improved efficacy and safety.

First studies in the 1930s have focussed on possible changes of the humoral arm of the immune response during SIT (4). An increase of allergen-specific IgG antibodies was detected and interpreted as "blocking antibodies" on the presumption that they may block the access of allergenic proteins to their targets, e.g. specific IgE antibodies bound on the surface of effector cells. Later it was discovered that many of the SIT-induced allergen-specific antibodies belong to the IgG4 subclass and that their production is promoted by interleukin (IL)-10 (5-11). Moreover, it was shown that allergen-specific IgG4 antibodies can block IgE-mediated histamine release (12, 13) and can suppress allergen-specific T cell responses *in vitro* by inhibiting binding of IgE-allergen complexes to antigen-presenting cells (10, 14, 15). However, it is still a controversial issue whether increased IgG4 levels during SIT are an epiphenomenon or of clinical relevance. Frequently, increased IgG4 levels do not correlate with the clinical outcome of SIT (16, 17). Thus, more detailed analyses of the *in vivo* blocking capacity of allergen-specific IgG4 antibodies are needed.

3. T CELL RESPONSE IN TYPE I ALLERGY

The development of an IgE-mediated response to allergens requires a series of molecular and cellular interactions involving antigen-presenting cells (APC), T cells and B cells. After uptake and processing of the allergen, APC (e.g. dendritic cells, monocytes) present small peptide fragments (T cell epitopes) in conjunction with major histocompatibility complex (MHC) class II molecules on their cell surface to T lymphocytes. $CD4^+$ T helper (Th) lymphocytes bearing the appropriate T cell receptor bind the peptide-MHC complex which leads to T cell proliferation and cytokine production. In contrast to non-allergic individuals, allergen-specific T cells of allergic patients are predominantly $CD4^+$ T lymphocytes synthesizing high amounts of IL-4, IL-13 and IL-5 but little or no interferon (IFN)-γ (18-20). This T cell subset has been designated T helper (Th)2 cells. IL-4 and IL-13 are cytokines that stimulate B cells to produce IgE antibodies. Allergen-specific Th2 cells have been identified in the peripheral blood and the nasal mucosa of patients suffering from allergic rhinitis, in skin lesions of patients with atopic dermatitis and in the bronchial alveolar lavage (BAL) fluid of asthma patients, confirming their important role in the pathophysiology of Type I allergies (19, 21-24). In contrast to Th2 cells, T cells belonging to the Th1 subset synthesize high levels of IFN-γ, a potent antagonist of IL-4. In addition to Th1 and Th2 cells, allergen-specific $CD4^+$ T cells producing a more heterogeneous cytokine profile have also been detected, e.g. Th0 characterized by the synthesis of high amounts of both, IL-4 and IFN-γ. Nevertheless, the so-called "Th1/Th2 paradigm" has provided indispensable

help to understand basic immunological mechanisms underlying allergic disease.

Lately, a heterogenous family of $CD4^+$ regulatory T (Treg) cells able to suppress effector immune responses was defined. Treg cells comprise naturally occurring thymus-derived $CD4^+CD25^+$ cells and adaptive Treg phenotypes that can be triggered by antigen in the periphery (25). The main subsets of induced Treg cells are Th3 cells producing transforming growth factor (TGF)-β that are linked to mucosal/oral tolerance induction (26) and Type 1 regulatory (Tr1) cells producing high levels of IL-10 and TGF-β (27). Moreover, additional subsets have been described according to their cytokine production: Treg cells that only produce IL-10 but no TGF-β (28) or Treg cells that produce IL-10 and IFN-γ (29). Compared to non-allergic individuals $CD4^+CD25^+$ Treg cells from atopic individuals have been shown to be less effective in suppressing the proliferation of $CD4^+CD25^-$ cells (30, 31). Furthermore, non-allergic individuals possessed a higher proportion of peripheral allergen-specific IL-10-producing Treg cells than Th2 cells as compared to allergic patients (32). Children who had outgrown their food allergy (tolerant children) displayed higher numbers of $CD4^+CD25^+$ cells with suppressive capacities as compared to children with active food allergy (33). Together, these data have emphasized that Treg cells play an important role in the protection from developing allergic disorders.

Only recently, another subset of $CD4^+$ T cells, namely TH17 cells, were considered to be involved in allergic diseases (34). TH17 cells are characterized by IL-17 (or IL-17A), IL-17F, IL-6, TNF-α, and IL-22 expression. The functions of IL-17 clearly indicate a pro-inflammatory role and therefore, TH17 cells were considered as possible participants in autoimmunity. Experimental models suggested that TH17 cells may be important for neutrophilic inflammation in acute airway inflammation (35). Moreover, they might be important for inflammatory skin responses such as contact hypersensitivity and atopic dermatitis (36). Nevertheless, the role of TH17 cells in allergy is still largely unclear.

For a long period the Th1/Th2 paradigm has dominated allergy research. This bivalent model gave rise to the hygiene hypothesis suggesting that increased hygiene conditions limit Th1-like responses which in turn promotes the induction of Th2 responses (37). In view of the recent achievements in T cell immunology the Th1/Th2 paradigm needs to be modified and newly discovered subsets of $CD4^+$ T cells need to be integrated in the "big picture" of allergen-specific T cell responses and further studies are necessary to investigate the role of these subsets in the pathogenesis and treatment of Type I allergies.

4. T CELL RESPONSE DURING ALLERGEN-SPECIFIC IMMUNOTHERAPY

4.1. Subcutaneous allergen-specific immunotherapy (SCIT)

Several studies have addressed the alterations of peripheral T cell responses during conventional SIT, i.e.

subcutaneous application of allergen extracts (SCIT). The majority reported a clear reduction in proliferative responses to the administered allergen (38-43). This *in vitro* phenomenon has been interpreted as the induction of allergen-specific peripheral T cell tolerance and is also reflected by reduced T cell-mediated clinical reactions *in vivo*, e.g. suppressed late phase responses (44, 45). Applying the Th1/Th2 paradigm to allergic diseases it became tempting to investigate whether SIT had an impact on the cytokine patterns produced by allergen-specific T cells. Indeed, a switch from the disease-eliciting allergen-specific Th2 response towards a more Th1-like response - either due to an increase of IFN-γ production or the reduction of IL-4 production - was observed (38, 39, 41, 45). In addition to peripheral T cells increased synthesis of Th1 cytokines was also detected at the sites of allergic inflammation, e.g. the nasal mucosa or the skin (46, 47). Consequently, immune deviation away from the disease-eliciting Th2-like towards a Th0/1-like immune response was considered as a main mechanism induced by SIT. In the end of the 1990s, first evidence arouse that SIT with insect venom induced IL-10-production (48, 49). Later, similar data were obtained for inhalant allergies and confirmed that SIT with house dust mite or grass pollen extract induces $CD4^+CD25^+$ T cells that produce IL-10 and show suppressive capacity (43, 50, 51). This suppression could be partially blocked by the addition of anti-IL-10 or anti-TGF-β antibodies (43). IL-10 was also shown to be important for the induction of specific unresponsiveness (anergy) in peripheral T cells (52, 53). As a consequence, the upregulation of Treg cells is now considered as the cardinal event of SCIT.

4.2. Sublingual allergen-specific immunotherapy (SLIT)

The potential risk of side effects upon subcutaneous administration and the inconvenience for patients due to the continuously repeated injections has led to the exploration of alternative routes for allergen administration. There is now increasing evidence that sublingual administration of allergen extracts (SLIT) is also effective for the treatment of allergies (54, 55). However, information on the modulation of the allergen-specific T cell response upon sublingual administration of allergens is still scarce. SLIT was shown to cause a reduction of allergen-induced proliferation in PBMC from adult patients (56-58). Recent studies reported an increased synthesis of IL-10 and IFN-γ in patients undergoing SLIT (57, 59, 60). These observations indicated that immune deviation and up-regulation of IL-10-producing Treg cells may also occur upon administration of allergens *via* the oral mucosa.

Being interested to investigate the allergen-specific T cell responses during sublingual allergy treatment in more detail we recently monitored allergen-specific T cell responses during SLIT with birch pollen extract. We considered the major birch pollen allergen, Bet v 1, as ideal model allergen because it has been well characterized at the T cell level (20, 61-63). Furthermore, Bet v 1 belongs to the family 10 of pathogenesis-related (PR) proteins and shows high IgE cross-reactivity with other members of this protein family, e.g. allergens in stone-fruits, hazelnuts and certain vegetables (64-67).

These dietary proteins and Bet v 1 also cross-react at the T cell level and Bet v 1-specific T cells proliferate and produce cytokines in response to stimulation with Bet v 1-related food allergens (62, 68-70). We took advantage of this cross-reactivity and elucidated the specificity of SLIT-induced alterations of the T cell response by including Mal d 1, the Bet v 1-homologue in apple, in our study (68). Phenotypic and functional characteristics of T cells reactive with Bet v 1 and Mal d 1 were analysed before, after 4 and 52 weeks of SLIT in patients with improved nasal provocation scores to birch pollen after one year of SLIT with birch pollen extract (58, 71). Our analyses provided evidence for the consecutive occurrence of Treg cell induction, immune deviation and highly allergen-specific peripheral T cell tolerance.

4.3. Early and late phase T cell responses during SIT

After 4 weeks of SLIT we found evidence for the presence of IL-10-producing Treg cells in the periphery of treated individuals (71). This evidence was provided by increased numbers of $CD4^+CD25^+$ cells, increased mRNA expression of IL-10 and FoxP3, a transcriptional regulator in Treg cells (72), but reduced IL-4 and IFN-γ synthesis in purified, allergen-stimulated T lymphocytes. The significantly reduced T cell proliferation to both allergens, Bet v 1 and Mal d 1, was significantly enhanced by either the depletion of $CD25^+$ cells or the addition of anti-IL-10 antibodies. In line, other longitudinal studies monitoring the individual T cell response have reported enhanced numbers of $CD4^+CD25^+$ cells within the first 70 days of SCIT (43, 73, 74). The observation that only 7 days after oral challenge with milk increased numbers of $CD4^+CD25^+$ cells were found in PBMC of milk-tolerant children further supports that after allergen exposition Treg cells are rapidly up-regulated (33). In the same study, the depletion of $CD25^+$ cells restored suppressed allergen-induced proliferative responses *in vitro*. We conclude that the induction of IL-10-producing Treg cells represents an early event during SIT and is responsible for the early suppression of allergen-specific T cell responses.

However, after one year of birch pollen SLIT no evidence for Treg cells was found. The numbers of $CD4^+CD25^+$ cells as well as Foxp3 and IL-10-specific mRNA levels had declined (71). Neither the depletion of $CD25^+$ cells nor the addition of anti-IL-10 antibodies increased the abolished Bet v 1-specific proliferation. In contrast to Bet v 1, Mal d 1-induced proliferative responses had returned to levels observed before therapy which further confirmed the lack of Treg cells with suppressive capacity. In addition, the observation that birch pollen SLIT did not induce tolerance in Mal d 1-reactive T cells indicated that the long-term tolerance of birch pollen-specific T cells was highly allergen-specific. The apple allergen shares 64% of amino acid sequence similarity with the major birch pollen allergen. Together, these findings suggested that during the later phase of SLIT IL-10-producing Treg cells were no longer involved in the suppression of the allergen-specific T cell proliferation. At this time point other more specific forms of T cell tolerance had evolved. At present, we cannot discern whether clonal deletion of allergen-reactive T lymphocytes has reduced the
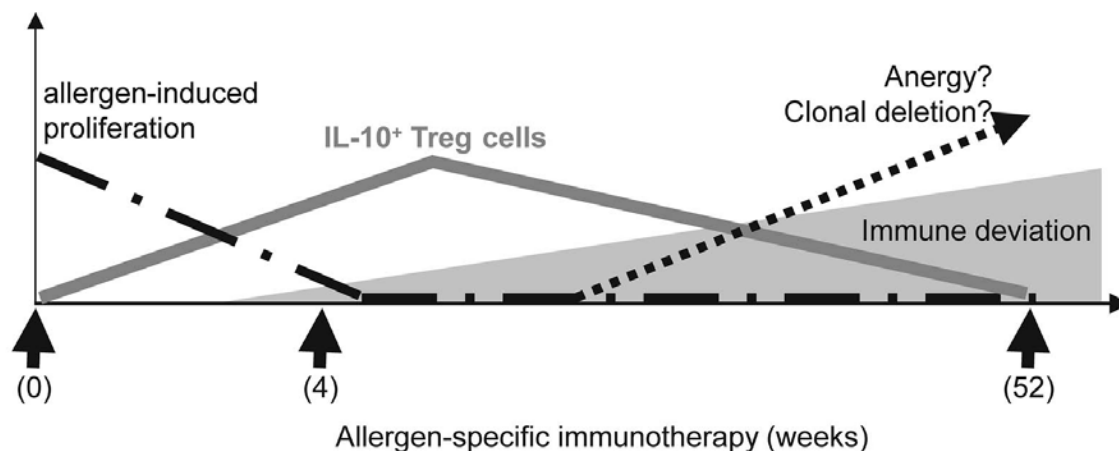
Figure 1. Schematic overview on sequential immune mechanisms during the course of specific immunotherapy. In an early phase, IL-10-producing Treg cells appear in the periphery that suppress allergen-induced proliferative and cytokine responses. Treg cells fade over time and other mechanisms, e.g. clonal deletion or anergy of allergen-specific T cells, are responsible for the long-term suppression of allergen-specific peripheral tolerance. In parallel, during the treatment remaining allergen-specific T cells shift towards a more Th0/1 like phenotype (immune deviation).

number of Bet v 1-specific T cells *in vivo* or whether the increased synthesis of IL-10 during the early phase of SLIT has induced anergic Bet v 1-specific T lymphocytes. We could expand Bet v 1-reactive T cells in T cell lines generated after one year of SLIT indicating that they had not been totally removed from the periphery (58). Of note, the remaining Bet v 1-reactive T cells produced significantly higher levels of IFN-γ as compared to the time points before and after 4 weeks of SLIT. This finding may be interpreted as immune deviation. In line, most data reporting the shift of allergen-induced T cell responses in favour of Th1 cytokine synthesis were gained after at least 1 year of treatment (38, 41, 42, 45, 47).

5. CONCLUSION

Deduced from monitoring the T cell responses in individuals undergoing SLIT and supported from other longitudinal studies analysing T cell changes during SCIT we propose that different immune mechanisms are operative during subsequent phases of specific allergy treatment (Figure 1). The induction of IL-10-producing Treg cells represents an early event to actively suppress allergen-specific T cell proliferation and cytokine production. In addition, the early increase of IL-10-producing Treg cells also results in increased IL-10 levels which support the early production of allergen-specific IgG4. Antigen-induced Treg cells have been shown to have a short life span and to be rapidly removed from the human periphery (75) supporting our observation that the presence of Treg cells faded over time during SIT. This removal of Treg cells may represent an important regulatory feature of the human immune system: In response to pathogens we need to quickly mount a defending immune response. However, after some time this response needs to be turned off again in order to avoid chronic and potentially self-destroying immune responses. Later in SLIT immune deviation is paralleled by suppressed allergen-specific T cell proliferation due to other highly allergen-specific

forms of tolerance. These tolerance mechanisms may be clonal deletion and/or anergy of allergen-specific T lymphocytes. We are aware that our hypothesis is based on the observation of PBMC and that these cultures may provide only a crude reflection of immune interactions and responses in lymphoid tissues, mucosal tissues or both. Nevertheless, our recent data on sequential immune mechanisms during birch pollen SLIT are a first step to explain the previously obtained conflicting results on the mechanisms responsible for SIT-induced changes of the allergic T cell response. Still, further studies are necessary to evaluate which mechanism is responsible for the durable and highly allergen-specific tolerance induction during the late phase of treatment. Is it clonal deletion, anergy or probably, both?

6. ACKNOWLEDGEMENTS

7. REFERENCES

1. J. Bousquet, R. Lockey and H. Malling: World Health Organization Position Paper. Allergen immunotherapy: therapeutical vaccines for allergic diseases. *Allergy* 53, 20-22 (1998)

2. L. Noon: Prophylactic inoculation against hay fever. *Lancet* 2(1572-73 (1911)

3. J. Freemann: Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. *Lancet* 2, 814-817 (1911)

4. R. Cooke, J. Barnard, S. Hebald and A. Stull: Serological evidence of immunity with coexisting stensitization in a type of human allergy. *J Exp Med* 62, 733-750 (1935)

5. M. E. Devey, D. V. Wilson and A. W. Wheeler: The IgG subclasses of antibodies to grass pollen allergens produced

in hay fever patients during hyposensitization. *Clin Allergy* 6, 227-236 (1976)

6. R. Djurup: The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 40, 469-486 (1985)

7. P. Jeannin, S. Lecoanet, Y. Delneste, J. F. Gauchat and J. Y. Bonnefoy: IgE versus IgG4 production can be differentially regulated by IL-10. *J Immuno.* 160, 3555-3561 (1998)

8. F. M. Lu, C. C. Chou, B. L. Chiang and K. H. Hsieh: Immunologic changes during immunotherapy in asthmatic children: increased IL-13 and allergen-specific IgG4 antibody levels. *Ann Allergy Asthma Immuno.* 80, 419-423 (1998)

9. K. Gehlhar, M. Schlaak, W. Becker and A. Bufe: Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 29, 497-506 (1999)

10. R. J. van Neerven, M. Arvidsson, H. Ipsen, S. H. Sparholt, S. Rak and P. A. Wurtzen: A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation. *Clin Exp Allergy* 34, 420-428 (2004)

11. J. S. Satoguina, E. Weyand, J. Larbi and A. Hoerauf: T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* 174, 4718-4726 (2005)

12. B. E. Garcia, M. L. Sanz, J. J. Gato, J. Fernandez and A. Oehling: IgG4 blocking effect on the release of antigen-specific histamine. *J Investig Allergol Clin Immunol* 3, 26-33 (1993)

13. A. M. Witteman, S. O. Stapel, D. H. Sjamsoedin, H. M. Jansen, R. C. Aalberse and J. S. van der Zee: Fel d 1-specific IgG antibodies induced by natural exposure have blocking activity in skin tests. *Int Arch Allergy Immunol* 109, 369-375 (1996)

14. R. J. van Neerven, T. Wikborg, G. Lund, B. Jacobsen, A. Brinch-Nielsen, J. Arnved and H. Ipsen: Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 163, 2944-2952 (1999)

15. P. A. Wachholz, N. K. Soni, S. J. Till and S. R. Durham: Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 112, 915-922 (2003)

16. T. Birkner, H. Rumpold, E. Jarolim, H. Ebner, M. Breitenbach, F. Skvaril, O. Scheiner and D. Kraft: Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. Correlation with clinical response. *Allergy* 45, 418-426 (1990)

17. U. Bodtger, A. M. Ejrnaes, L. Hummelshoj, H. H. Jacobi, L. K. Poulsen and M. Svenson: Is immunotherapy-induced birch-pollen-specific IgG4 a marker for decreased allergen-specific sensitivity? *Int Arch Allergy Immunol* 136, 340-346 (2005)

18. E. A. Wierenga, M. Snoek, J. D. Bos, H. M. Jansen and M. L. Kapsenberg: Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. *Eur J Immunol* 20, 1519-1526 (1990)

19. P. Parronchi, D. Macchia, M. P. Piccinni, P. Biswas, C. Simonelli, E. Maggi, M. Ricci, A. A. Ansari and S. Romagnani: Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci U S A* 88, 4538-4542 (1991)

20. C. Ebner, S. Schenk, N. Najafian, U. Siemann, R. Steiner, G. W. Fischer, K. Hoffmann, Z. Szepfalusi, O. Scheiner and D. Kraft: Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J Immunol* 154, 1932-1940 (1995)

21. S. R. Durham, S. Ying, V. A. Varney, M. R. Jacobson, R. M. Sudderick, I. S. Mackay, A. B. Kay and Q. A. Hamid: Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 148, 2390-2394 (1992)

22. Q. Hamid, M. Boguniewicz and D. Y. Leung: Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 94, 870-876 (1994)

23. G. F. Del Prete, M. De Carli, M. M. D'Elios, P. Maestrelli, M. Ricci, L. Fabbri and S. Romagnani: Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. *Eur J Immunol* 23, 1445-1449 (1993)

24. K. J. Bodey, A. E. Semper, A. E. Redington, J. Madden, L. M. Teran, S. T. Holgate and A. J. Frew: Cytokine profiles of BAL T cells and T-cell clones obtained from human asthmatic airways after local allergen challenge. *Allergy* 54, 1083-1093 (1999)

25. J. A. Bluestone and A. K. Abbas: Natural versus adaptive regulatory T cells. *Nat Rev Immunol* 3, 253-257 (2003)

26. H. L. Weiner: Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* 182, 207-214 (2001)

27. M. K. Levings and M. G. Roncarolo: Phenotypic and functional differences between human CD4+CD25+ and type 1 regulatory T cells. *Curr Top Microbiol Immunol* 293, 303-326 (2005)

28. I. Bellinghausen, J. Knop and J. Saloga: The role of interleukin 10 in the regulation of allergic immune responses. *Int Arch Allergy Immunol* 126, 97-101 (2001)

29. P. Stock, O. Akbari, G. Berry, G. J. Freeman, R. H. Dekruyff and D. T. Umetsu: Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol* 5, 1149-1156 (2004)

30. E. M. Ling, T. Smith, X. D. Nguyen, C. Pridgeon, M. Dallman, J. Arbery, V. A. Carr and D. S. Robinson: Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 363, 608-615 (2004)

31. I. Bellinghausen, B. Konig, I. Bottcher, J. Knop and J. Saloga: Regulatory activity of human CD4 CD25 T cells depends on allergen concentration, type of allergen and atopy status of the donor. *Immunology* 116, 103-111 (2005)

32. M. Akdis, J. Verhagen, A. Taylor, F. Karamloo, C. Karagiannidis, R. Crameri, S. Thunberg, G. Deniz, R.

Valenta, H. Fiebig, C. Kegel, R. Disch, C. B. Schmidt-Weber, K. Blaser and C. A. Akdis: Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 199, 1567-1575 (2004)

33. M. R. Karlsson, J. Rugtveit and P. Brandtzaeg: Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 199, 1679-1688 (2004)

34. S. L. Reiner: Development in motion: helper T cells at work. *Cell.* 129, 33-6 (2007)

35. C. B. Schmidt-Weber, M. Akdis and C. A. Akdis: TH17 cells in the big picture of immunology. *J Allergy Clin Immunol* 120, 247-254 (2007)

36. A. J. van Beelen, M. B. Teunissen, M. L. Kapsenberg and E. C. de Jong: Interleukin-17 in inflammatory skin disorders. *Curr Opin Allergy Clin Immunol* 7, 374-81 (2007)

37. H. Garn and H. Renz: Epidemiological and immunological evidence for the hygiene hypothesis. *Immunobiology* 212, 441-452 (2007)

38. H. Secrist, C. J. Chelen, Y. Wen, J. D. Marshall and D. T. Umetsu: Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J Exp Med* 178, 2123-2130 (1993)

39. M. Jutel, W. J. Pichler, D. Skrbic, A. Urwyler, C. Dahinden and U. R. Muller: Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 154, 4187-4194 (1995)

40. C. A. Akdis, M. Akdis, T. Blesken, D. Wymann, S. S. Alkan, U. Muller and K. Blaser: 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 (1996)

41. C. Ebner, U. Siemann, B. Bohle, M. Willheim, U. Wiedermann, S. Schenk, F. Klotz, H. Ebner, D. Kraft and O. Scheiner: Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 27, 1007-1015 (1997)

42. N. P. Eusebius, L. Papalia, C. Suphioglu, S. C. McLellan, M. Varney, J. M. Rolland and R. E. O'Hehir: Oligoclonal analysis of the atopic T cell response to the group 1 allergen of Cynodon dactylon (bermuda grass) pollen: pre- and post-allergen-specific immunotherapy. *Int Arch Allergy Immunol* 127, 234-244 (2002)

43. M. Jutel, M. Akdis, F. Budak, C. Aebischer-Casaulta, M. Wrzyszcz, K. Blaser and C. A. Akdis: IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 33, 1205-1214 (2003)

44. M. M. Pienkowski, N. F. Adkinson, Jr., M. Plaut, P. S. Norman and L. M. Lichtenstein: Prostaglandin D2 and histamine during the immediate and the late-phase components of allergic cutaneous responses. *J Allergy Clin Immunol* 82, 95-100 (1988)

45. V. A. Varney, Q. A. Hamid, M. Gaga, S. Ying, M. Jacobson, A. J. Frew, A. B. Kay and S. R. Durham: Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 92, 644-651 (1993)

46. S. R. Durham, S. Ying, V. A. Varney, M. R. Jacobson, R. M. Sudderick, I. S. Mackay, A. B. Kay and Q. A. Hamid: Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 97, 1356-1365 (1996)

47. Q. A. Hamid, E. Schotman, M. R. Jacobson, S. M. Walker and S. R. Durham: Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 99, 254-260 (1997)

48. I. Bellinghausen, G. Metz, A. H. Enk, S. Christmann, J. Knop and J. Saloga: 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 (1997)

49. C. A. Akdis, T. Blesken, M. Akdis, B. Wuthrich and K. Blaser: Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 102, 98-106 (1998)

50. J. N. Francis, S. J. Till and S. R. Durham: Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 111, 1255-1261 (2003)

51. L. M. Gardner, F. C. Thien, J. A. Douglass, J. M. Rolland and R. E. O'Hehir: Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 34, 1209-1219 (2004)

52. C. A. Akdis and K. Blaser: 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)

53. C. A. Akdis, A. Joss, M. Akdis, A. Faith and K. Blaser: 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. 14(12), 1666-8 (2000)

54. D. R. Wilson, M. T. Lima and S. R. Durham: Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 60, 4-12 (2005)

55. Z. Calamita, H. Saconato, A. B. Pela and A. N. Atallah: Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. Allergy. 61, 1162-1172 (2006)

56. C. Fanta, B. Bohle, W. Hirt, U. Siemann, F. Horak, D. Kraft, H. Ebner and C. Ebner: Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol* 120, 218-224 (1999)

57. L. Cosmi, V. Santarlasci, R. Angeli, F. Liotta, L. Maggi, F. Frosali, O. Rossi, P. Falagiani, G. Riva, S. Romagnani, F. Annunziato and E. Maggi: Sublingual immunotherapy with Dermatophagoides monomeric

allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy*. 36, 261-272 (2006)

58. T. Kinaciyan, B. Jahn-Schmid, A. Radakovics, B. Zwolfer, C. Schreiber, J. N. Francis, C. Ebner and B. Bohle: Successful sublingual immunotherapy with birch pollen has limited effects on concomitant food allergy to apple and the immune response to the Bet v 1 homolog Mal d 1. *J Allergy Clin Immunol* 119, 937-943 (2007)

59. E. Enrique, F. Pineda, T. Malek, J. Bartra, M. Basagana, R. Tella, J. V. Castello, R. Alonso, J. A. de Mateo, T. Cerda-Trias, M. San Miguel-Moncin Mdel, S. Monzon, M. Garcia, R. Palacios and A. Cistero-Bahima: Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-controlled study with a standardized hazelnut extract. *J Allergy Clin Immunol* 116, 1073-1079 (2005)

60. G. Ciprandi, D. Fenoglio, I. Cirillo, A. Vizzaccaro, A. Ferrera, M. A. Tosca and F. Puppo: Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 95, 38-44 (2005)

61. C. Ebner, Z. Szepfalusi, F. Ferreira, A. Jilek, R. Valenta, P. Parronchi, E. Maggi, S. Romagnani, O. Scheiner and D. Kraft: Identification of multiple T cell epitopes on Bet v I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 150, 1047-1054 (1993)

62. B. Jahn-Schmid, A. Radakovics, D. Luttkopf, S. Scheurer, S. Vieths, C. Ebner and B. Bohle: Bet v 1142-156 is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1-related food allergens. *J Allergy Clin Immunol* 116, 213-219 (2005)

63. T. Nagato, H. Kobayashi, M. Yanai, K. Sato, N. Aoki, K. Oikawa, S. Kimura, Y. Abe, E. Celis, Y. Harabuchi and M. Tateno: Functional analysis of birch pollen allergen Bet v 1-specific regulatory T cells. *J Immunol* 178, 1189-1198 (2007)

64. M. Vanek-Krebitz, K. Hoffmann-Sommergruber, M. Laimer da Camara Machado, M. Susani, C. Ebner, D. Kraft, O. Scheiner and H. Breiteneder: Cloning and sequencing of Mal d 1, the major allergen from apple (Malus domestica), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun* 214, 538-551 (1995)

65. D. Luttkopf, U. Muller, P. S. Skov, B. K. Ballmer-Weber, B. Wuthrich, K. Skamstrup Hansen, L. K. Poulsen, M. Kastner, D. Haustein and S. Vieths: Comparison of four variants of a major allergen in hazelnut (Corylus avellana) Cor a 1.0.4 with the major hazel pollen allergen Cor a 1.0.1. *Mol Immunol* 38, 515-525 (2002)

66. H. Breiteneder, K. Hoffmann-Sommergruber, G. O'Riordain, M. Susani, H. Ahorn, C. Ebner, D. Kraft and O. Scheiner: Molecular characterization of Api g 1, the major allergen of celery (Apium graveolens), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 233, 484-489 (1995)

67. K. Hoffmann-Sommergruber, G. O'Riordain, H. Ahorn, C. Ebner, M. Laimer Da Camara Machado, H. Puhringer, O. Scheiner and H. Breiteneder: Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 29, 840-847 (1999)

68. R. Fritsch, B. Bohle, U. Vollmann, U. Wiedermann, B. Jahn-Schmid, M. Krebitz, H. Breiteneder, D. Kraft and C. Ebner: Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. *J Allergy Clin Immunol* 102, 679-686 (1998)

69. B. Bohle, A. Radakovics, B. Jahn-Schmid, K. Hoffmann-Sommergruber, G. F. Fischer and C. Ebner: Bet v 1, the major birch pollen allergen, initiates sensitization to Api g 1, the major allergen in celery: evidence at the T cell level. *Eur J Immunol* 33, 3303-3310 (2003)

70. B. Bohle, A. Radakovics, D. Luttkopf, B. Jahn-Schmid, S. Vieths and C. Ebner: Characterization of the T cell response to the major hazelnut allergen, Cor a 1.0.4: evidence for a relevant T cell epitope not cross-reactive with homologous pollen allergens. *Clin Exp Allergy* 35, 1392-1399 (2005)

71. B. Bohle, T. Kinaciyan, M. Gerstmayr, A. Radakovics, B. Jahn-Schmid and C. Ebner: Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 120, 707-713 (2007)

72. S. Hori and S. Sakaguchi: Foxp3: a critical regulator of the development and function of regulatory T cells. *Microbes Infect* 6, 745-751 (2004)

73. E. Mamessier, J. Birnbaum, P. Dupuy, D. Vervloet and A. Magnan: Ultra-rush venom immunotherapy induces differential T cell activation and regulatory patterns according to the severity of allergy. *Clin Exp Allergy* 36, 704-713 (2006)

74. S. M. Nasser, S. Ying, Q. Meng, A. B. Kay and P. W. Ewan: Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur J Immunol* 31, 3704-3713 (2001)

75. M. Vukmanovic-Stejic, Y. Zhang, J. E. Cook, J. M. Fletcher, A. McQuaid, J. E. Masters, M. H. Rustin, L. S. Taams, P. C. Beverley, D. C. Macallan and A. N. Akbar: Human CD4+ CD25hi Foxp3+ regulatory T cells are derived by rapid turnover of memory populations in vivo. *J Clin Invest* 116, 2423-2433 (2006)

Send correspondence to: Barbara Bohle, Department of Pathophysiology, Center for Physiology, Pathophysiology and Immunology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria, Tel: 43-1-40400-5114, Fax: 43-1-40400-5130, E-mail: barbara.bohle@meduniwien.ac.at