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

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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
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(or “desensitization”) to the disease-eliciting allergens in allergic individuals (2, 3). Since approximately 70 years, allergologists are interested in discovering 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 described 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 IL-10 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 aroused that SIT with birch pollen extract induces CD4+CD25+ T cells that produce IL-10 and Foxp3, a transcriptional regulator of IL-10 and Foxp3, a transcriptional regulator of Treg cells (20, 61-63). Furthermore, 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).

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 pollen 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
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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?

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Abbreviations: APC: antigen-presenting cell; IFN, interferon; Ig: immunoglobulin; IL: Interleukin; SIT: specific immunotherapy; SLIT: sublingual immunotherapy; SCIT: subcutaneous immunotherapy; Th: T helper cell; Treg: regulatory T cell; TGF: transforming growth factor

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