

Grass allergen-specific T-cells of atopic patients

Review article

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The prevalence of atopic diseases has increased dramatically in the Western world over the last 20 years. Further insight into the mechanisms of the allergic reaction may help in the development of new more efficient protocols for the specific treatment of allergic diseases. Until a decade ago the major focus in allergy research was on the IgE reactivity of atopic patients and the description of major allergens responsible for the allergic reactions. More recently, the important role of CD4⁺ T-cells in orchestrating the production of IgE and the maturation of eosinophils through the production of IL-4 (or IL-13) and IL-5 has been clearly established. This has led to the characterization of T-cell responsiveness to numerous major allergens, demonstrating the importance of T-cells for the allergic response of atopic patients and for the specific treatment of allergic diseases. One of the major objectives of allergy research is the development of improved treatment protocols for specific therapy. In this review our results on the T-cell reactivity of grass allergic patients to the group 5 major allergen of the grass *Phleum pratense* (Phl p 5) are discussed in relation to the literature on allergen-specific T-cell reactivity and the specific treatment of allergic disease. Our investigations focused on three subjects: 1) Grass allergen-specific T-cell reactivity of atopic patients in freshly isolated PBMC cultures, 2) Phl p 5 isoallergen recognition of T-cell lines (TCL) and clones (TCC) established from PBMC of grass allergic patients, and 3) Identification of T-cell epitopes on Phl p 5.

Key words: Atopic patients; allergen-specific T-cells; grass allergy.

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Atopic allergy is an increasing problem in the industrial part of the world where approximately 20% of the population are troubled by allergic reactions. The allergens of house dust mites, pollens, and animal dander are the most frequent causes of atopic reactions in the Western world (1).

Type 1 allergic diseases are characterized by high serum levels of allergen-specific IgE and tissue eosinophilia. Cross-linking of IgE bound to high-affinity IgE-receptors on the surface of mast cells by the allergen causes the immediate allergic response (2), and the recruitment and

activation of eosinophils and T-lymphocytes is crucial for the late-phase allergic reaction (3).

CD4⁺ T-cells deliver the signals necessary for the induction of IgE production by B-cells (IL-4 or IL-13 production and CD40L expression) (4, 5) and the differentiation and activation of eosinophils (IL-5) (3), clearly demonstrating the involvement of allergen-specific T-helper cells in the pathophysiology of allergic diseases (6, 7). In addition, the shift in cytokine production profiles of allergen-specific T-cells observed after specific immunotherapy (SIT) (8–12) indicates that T-lymphocytes may be the principal target cells of this specific allergy treatment. In addition, it was recently shown that the increased level of allergen-specific non-IgE antibodies observed after SIT compete with IgE for allergen

Abbreviation:

Specific immunotherapy (SIT); T-cell clone (TCC); T-cell line (TCL).

binding and block the IgE mediated facilitation of allergen-specific T-cell activation (13). In this way the initial change in cytokine profiles after treatment is followed by a raised threshold for activation of allergen-specific T-cells. These changes in T-cell reactivity and antibody production may explain the reduction in both immediate and late-phase allergic reactions accomplished through SIT (10, 12). A further characterization of human allergen-specific T-cells and their co-operation with the allergen-specific antibodies may help to increase our understanding of the allergen-specific immune response and is a prerequisite for the development of new approaches to allergen immunotherapy.

Allergen extracts used for SIT are complex mixtures of naturally existing allergens that are difficult to standardize, which explains why recombinant allergens (14–16) or even allergen-derived peptides (17–19) have been suggested as useful alternatives for use in SIT. As an increasing number of the genes encoding major allergens have been cloned, sequenced and expressed as recombinant proteins (20, 21), it has been shown that in most cases they exist in several closely related versions leading to protein isoforms containing a variable number of amino acid substitutions (20). The extensive T-cell and IgE cross-reactivity to homologous allergens of related allergen sources found for most allergens (22) could indicate that the different combinations of isoallergens in related organisms do not markedly influence the immune recognition of homologous allergens from different species. However, IgE and T-cell clones from atopic patients and allergen-specific mAb have been shown to recognize differentially individual isoallergens of the major tree pollen allergen Bet v 1 (14, 23–25). This indicates that the investigation of immune responses might be biased by the use of single isoallergens instead of purified natural allergens and this has to be considered when new treatment strategies are developed exclusively on the basis of results obtained in such model systems.

Theoretically, group 1 and 5 allergens of the Poaceae grasses are good candidates for substitution with recombinant material for SIT. They are highly clinically relevant major allergens recognized by IgE from the vast majority of patients allergic to these grasses, and are present

in allergenic extracts of all temperate Poaceae grasses (26). Group 5 allergens are proteins with Mw in the 28–32 kD range (27, 28). The genes encoding group 5 allergens from several Poaceae grasses have been cloned and sequenced, and numerous grass group 5 isoallergen sequences have been described (29–33). Natural Phl p 5 can be subdivided into Phl p 5a (31 kD) and Phl p 5b (29 kD). The Mw difference between Phl p 5a and 5b is the result of several inserts or deletions, whereas almost complete sequence identity is found within the Phl p 5a and Phl p 5b isoallergen groups (30).

The availability of recombinant grass group 5 allergens has resulted in the determination of immunologically important sites on the allergens with respect to IgE binding (15, 30, 31, 34) and T-cell reactivity of atopic patients (35–40).

The main objective of our work was the characterization of the T-cell response of grass allergic patients to the major grass allergen of timothy (*Phleum pratense*) Phl p 5. This was accomplished in part by investigating the polyclonal grass-specific T-cell response of freshly isolated blood cell cultures (41), and in part by studying several aspects of T-cell activation (specificity, cytokine production, HLA restriction and TCR usage) in oligoclonal Phl p 5 specific T-cell lines and T-cell clones established from the peripheral blood of grass allergic patients (37, 38)

Aberrant allergen-specific T-cell responses of atopic patients

Allergen-specific Th2-like T-cell clones have been isolated from the peripheral blood (6, 7, 42) and the sites of allergic inflammation of atopic patients (43, 44), which points to the importance of allergen-specific T-cells for the pathophysiology of atopic disease. Studies on primary in vitro T-cell cultures have demonstrated that atopic patients exhibit an enhanced production of Th2-type cytokines (45–47) and increased T-cell proliferation in response to allergens, compared to non-atopic control individuals (41, 48–54). We found that T-cell proliferative responses of allergic donors to single grass group 5 allergens were significantly higher than responses of non-allergic control donors (41). In addition, the cytokine production of the atopic patients in response to purified Phl p 5 allergen was dominated by the production of

IL-5 (41). This cytokine is an excellent marker of Th2 activation in PBMC cultures (45, 55, 56) and the level of allergen-specific IL-5 production is highly increased in atopics compared with non-atopic controls (57). Interestingly, the IFN- γ -dominated response to the *Phleum pratense* allergen extract (41) indicates that this mixture of proteins favors the production of Th1-cytokines, thereby inducing a Th1-like T-cell response to grass allergens important for the effect of conventional SIT. An overall Th0 response was found when the cytokine production of Phl p 5-specific TCL and TCC (37) was investigated. A clear-cut Th2 profile, as described for TCC specific to indoor allergens (6, 58–60), was only observed for a few Phl p 5-specific TCC. Similar results were reported from other studies of pollen allergen-specific TCC derived from the peripheral blood (40, 61–65). This probably reflects the relatively mild symptoms of pollen allergic patients, as the level of Th2 cytokines produced has been shown to reflect the severity of the symptoms (45, 55, 56). Furthermore, allergen-specific T-cells of non-atopic donors (6) and T-cells of atopic patients specific to tuberculin (PPD) and tetanus toxoid (6, 7) show clear-cut Th1 profiles with very low IL-4 production, demonstrating that the pollen allergen-specific Th0 response of atopic patients is skewed towards the production of Th2 cytokines, which connects the allergen-specific T-cells to the atopic phenotype of the patients.

Cross-reactive recognition of allergens by serum IgE and T-lymphocytes

An extensive IgE cross-reactivity to homologous allergens of taxonomically related allergen sources has been described for most major allergens (21). As a result of this, allergic patients experience symptoms when they are exposed to allergens to which they have not been sensitized. The high degree of amino acid sequence identity described for homologous allergens from related allergen sources implies that a comparable cross-reactivity exists at the T-cell level. Few studies have investigated the cross-reactivity of allergen-specific T-cells (35, 61, 62, 64, 66). However, the importance of T-cells for the effect of allergen immunotherapy calls for more detailed studies on this subject, especially if single allergen extracts (e.g. birch extract for tree pollen allergy) or even single purified major

allergens are considered for specific treatment of allergy.

We addressed this question by investigating the T-cell reactivity to grass allergen extracts and purified grass group 5 allergens of *Phleum pratense* and related Poaceae grasses in PBMC cultures and Phl p 5-specific TCL and TCC of atopic patients (37, 41). The comparable T-cell proliferation observed in response to extracts of related grasses in PBMC cultures (41) suggests that treatment with one grass allergen extract may be sufficient to treat allergy to different related grass species. The demonstration of cross-reactivity of allergen-specific TCL and TCC (35, 37, 41, 61, 62, 64, 66) confirms that the simultaneous T-cell response in PBMC cultures to multiple related allergens is in part the direct result of cross-reactivity, and that T-cell epitopes present on related allergens are frequently recognized. However, the various cross-reactivity patterns observed for TCC specific to grass allergens (35, 37, 61, 62) as well as tree pollen allergens (64, 66) demonstrate that species-specific TCC do exist, and that multiple T-cell epitopes, unequally represented in related allergens, are recognized by allergen-specific T-cells of atopic patients. In addition, when T-cell responses to purified grass group 5 allergens were investigated, differential reactivity to related grass group 5 allergens was observed in TCL and PBMC cultures from some patients (37, 41). This probably reflects that a high percentage of grass group 5-specific TCC in these patients exhibit restricted cross-reactivity, and stresses the importance of species-specific diagnosis if purified or recombinant single allergens are considered for treatment.

Individual allergens contain multiple T-cell epitopes

The T-cell unresponsiveness induced in animal models (67) and in human T-cell cultures in vitro (68–71) by treatment with peptides containing T-cell epitopes indicates that peptide immunotherapy may efficiently downregulate allergen-specific T-cell responses. If allergen-specific T-cells of atopic patients were specific to a limited number of T-cell epitopes of individual major allergens, these T-cells could be targeted by allergenic peptides containing such immunodominant T-cell epitopes. In addition, the introduction of short synthetic allergenic peptides

with low IgE-binding capacity for SIT may reduce the risk of IgE-mediated side-effects compared to conventional treatment with allergen extracts. T-cell epitopes of allergens of tree pollen (64, 72–74), house dust mite (58, 75–77), cat (59, 78), grass (38, 40, 61, 62, 79) and bee venom allergens (80, 81) recognized by T-cells of allergic patients and non-atopic controls have been described during the last 5–10 years. The main conclusion of these studies is that allergens contain multiple T-cell epitopes scattered throughout the peptide backbones of the allergen molecules (22). T-cells of atopic and non-atopic donors recognize the same epitopes (73, 80, 82), the epitope recognition patterns generally differ considerably among patients, and only a low percentage of TCC isolated from individual atopic patients react to each epitope recognized (38, 40, 58, 59, 61, 62, 72, 77). We investigated the epitope specificity of 16 Phl p 5-specific TCC from three grass allergic patients. Several epitopes were identified in both isoallergens. T-cell epitopes shared between Phl p 5a and b, and Phl p 5a-specific epitopes were found in the N-terminal, the C-terminal, and the central part of the Phl p 5a molecule, and T-cell epitopes were identified in two of the three Phl p 5b unique sequence clusters (38). Immunodominant T-cell epitopes of Phl p 5 were not identified as none of the epitopes identified were recognized by more than 2 of the 16 TCC tested. Similar results on T-cell epitopes of Phl p 5 were described by Müller et al. (40) and other groups have demonstrated that grass group 1 allergens also contain multiple epitopes differentially recognized by individual patients (61, 62) (Fig. 1). In general, the diversity of allergen-specific T-cells with respect to epitope specificity indicates that the existing allergen-specific T-cell population from atopic patients cannot be targeted by small allergenic peptides. The limited success of the clinical trials to treat cat allergic patients with peptides of the major cat allergen Fel d 1 (18, 19) supports this conclusion. However, there are conceptual counter-arguments involving T-cell receptor promiscuity and linked suppression (83) and the results on treatment of five bee venom allergic patients with phospholipase A2 peptides (84) and other preliminary data (85) suggest a positive effect of peptide-based therapy. An alternative to synthetic peptides is truncated recombinant allergen fragments (16,

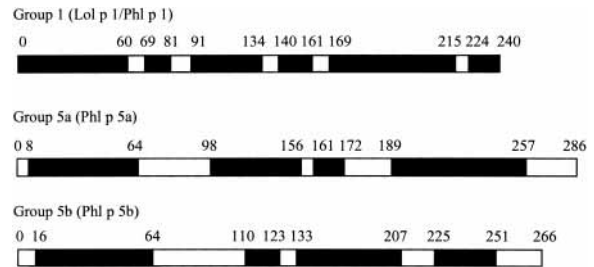


Fig. 1. Regions of grass group 1 and 5 allergen amino acid sequences containing T-cell epitopes recognized by grass allergic patients. Sequence areas containing T-cell epitopes are indicated in grey. T-cell epitopes of grass group 1 allergens were adapted from Spiegelberg et al. (61) (Lol p 1) and Schenck et al. (62) (Phl p 1). T-cell epitopes of grass group 5 allergens were adapted from Müller et al. (40) (Phl p 5a and b) and Würtzen et al. (38) (Phl p 5a and b).

39, 86, 87) with partly intact tertiary structure, lowered IgE binding capacity and retained T-cell-activating ability. These preparations may be efficient in the specific treatment of allergic disease, but raise the question of T-cell recognition of individual isoallergens discussed in the next paragraph.

Differential T-cell recognition of individual isoallergens

Recombinant allergens have been suggested as a simple alternative to allergen extracts for SIT (14–16) as they are single proteins which are easily produced and standardized. However, the fact that multiple isoallergens have been described for most major allergens may complicate this approach (20, 88). T-cells recognize linear epitopes and the amino acid substitutions found among different allergen isoforms may destroy important T-cell epitopes, and thereby reduce the efficacy of the treatment. This important question has only been addressed in a few studies (14, 24, 38, 40, 64), that demonstrated that isoallergenic variation influences the activation of Bet v 1- and Phl p 5-specific TCC. In addition, TCC recognizing purified natural allergen, but not the corresponding recombinant isoallergen, have been described in studies on different allergens (58, 61, 72, 79), suggesting that in general single isoallergens are differentially recognized by allergen-specific T-cells of atopic patients. Despite the fact that individual Bet v 1-specific TCC differentially recognized isoallergens of Bet v 1, and that all

isoallergens only activated a subpopulation of the TCC investigated, the overall T-cell recognition of the isoallergens did not differ among patients (14). In contrast, we (37, 38) and others (40) demonstrated that reactivity of Phl p 5-specific T-cells from individual grass allergic patients was preferentially directed to Phl p 5a, or Phl p 5b, or even other as yet unidentified Phl p 5 isoallergens. This possibly reflects the relatively high degree of variation between Phl p 5a and b isoforms and indicates that the use of recombinant Phl p 5 for SIT may be complicated by isoallergen variation as only a subpopulation of the patients treated may recognize the isoallergen used. In addition, most patients allergic to grass pollen respond to several major allergens, suggesting that preparations of recombinant grass allergens should include several recombinant major allergens and possibly several isoallergen variants of at least the group 5 allergen.

For allergen sources containing one major allergen that exhibits a high degree of identity between isoallergens, a single recombinant allergen may replace the natural allergen extracts for SIT. Furthermore, with selected recombinant isoallergens (14, 89) or genetically modified recombinant allergens (90, 91) with low IgE-binding capacity and retained T-cell-activating ability, one might treat patients with full-length allergens containing both T- and B-cell epitopes, and simultaneously reduce the risk of IgE-mediated side effects. Most importantly, the T-cell recognition of preparations of recombinant allergens intended for SIT has to be investigated in vitro on a large patient material before use in actual clinical trials to ensure that they retain a T-cell-activating capacity comparable to that of the natural allergens.

Novel approaches for specific treatment of allergy

Even though the exact mechanism behind the clinical effect of SIT is still unknown, several ways to improve the safety, standardization, and efficacy of this treatment strategy are currently under investigation (92). As discussed above, recombinant allergens and allergen-derived peptides or fragments have been suggested as simple alternatives to the complex allergen extracts conventionally used for SIT. Alternative routes of administration (93–97) as well as re-

duction of the duration of treatment (98) would make the treatment acceptable for a larger proportion of allergic patients. Interestingly, tolerance induction in ovalbumin (OVA)-sensitized mice with high concentrations of OVA administered orally or intranasally prevents the production of OVA-specific IgE (99) and eosinophilia (100), possibly through the induction of regulatory allergen-specific T-cells with production of TGF- β (101, 102) or expression of the inhibitory surface molecule Delta 1 (71). Recent reports of clinical trials investigating the effect of sublingual or nasal application of allergen extracts indicate that the immune system can be addressed through these mucosal surfaces and suggest that the clinical effect may be comparable to that of injection immunotherapy (94, 95, 97, 103). Studies on the formulation of the allergen preparation necessary to retain the potency of the allergens administered may improve the applicability and effect of oral and sublingual therapy (93).

Several new approaches to the specific treatment of allergic diseases have been suggested during the late 1990. DNA vaccination (104, 105), co-administration of IL-12 and antigen (106, 107), and IL-12 fusion allergens (108) have all been shown to enhance Th1 responses to allergens, while treatments with non-anaphylactic anti-IgE (109) may be a useful unspecific alternative. The Th1-favoring effect of gene therapy in mice treated with plasmid DNA-encoding allergens has been shown to depend on the presence of immunostimulatory nucleotide sequences ISS (104, 110–114). These CpG nucleotide sequences act as strong Th1-inducing adjuvants in mice through induction of IL-12, IL-18, and interferon production (112). The introduction of a strong Th1-inducing adjuvant may markedly improve the efficacy of SIT without changing the allergen preparations used, while the application of gene therapy in the treatment of allergy may lead to serious ethical considerations as well as many technical problems.

Concluding remarks

The characterization of CD4⁺ T-cells specific to well-characterized major allergens from an increasing number of allergen sources has resulted in detailed knowledge of the specificity and activation requirements of allergen-specific

T-cells and their involvement in the pathophysiology of allergic diseases. We and others have demonstrated that in general allergens contain multiple T-cell epitopes dispersed throughout the molecules, and that the responding T-cell populations of atopic patients are highly heterogeneous with respect to epitope specificity, HLA restriction and TCR usage. The diversity of allergen-specific T-cells with respect to epitope specificity suggests that novel approaches for specific treatment of allergy should be based on full-length allergens as opposed to allergenic peptides. Furthermore, the differential T-cell recognition in the case of Phl p 5 isoallergens suggests that several recombinant allergens have to be applied to target the full Phl p 5-specific T-cell response of grass allergic patients. In vitro studies investigating T-cell responses to preparations of recombinant allergens on a large patient material and clinical trials determining the in vivo effect of treatment with recombinant allergen preparations need to be performed in order to improve allergen immunotherapy. On the other hand, the heterogeneity of the allergen-specific T-cell response of atopic patients might rather indicate that improvements in specific immunotherapy could be more easily obtained by changing the adjuvants or the route of administration for the allergen extracts used for conventional SIT instead of reducing the number of components in the protein preparations. In addition, determination of the exact mechanisms involved in the beneficial effect of specific allergen immunotherapy is crucial for the development of new specific treatment approaches for allergic diseases.

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