

Differential Recognition of Recombinant Phl p 5 Isoallergens by Phl p 5-Specific T Cells

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Key Words

Phl p 5 · T cell · Isoallergen · Specific allergy vaccination

Abstract

Background: At present, recombinant allergens are considered for the diagnosis and treatment of atopic allergies. To evaluate the theoretical impact of isoallergen variation on the selection of isoallergens for specific allergy vaccination, we characterized the T-cell response of allergic patients to the *Phleum pratense* major allergen, Phl p 5, and five of its recombinant isoallergens. **Methods:** Phl p 5-specific T cell lines (TCLs) were isolated from the peripheral blood of 3 allergic rhinitis patients, and their reactivity patterns were studied in detail. **Results:** The TCLs were highly crossreactive with related grasses. The crossreactivity with *Poa pratensis* was more extensive than with *Lolium perenne*, directly reflecting the sequence identity between Phl p 5, Poa p 5, and Lol p 5. The TCLs produced IFN- γ and IL-4 simultaneously, resembling a Th0-like cytokine production profile. Interestingly, when T cell clones were tested with natural Phl p 5 and five rPhl p 5 isoallergens, a differential recognition pattern was found. One of the TCLs exclusively reacted with Phl p 5b, another reacted with all isoforms tested, and the third reacted strongly with native purified Phl p 5, but

only weakly with all five recombinant isoallergens. **Conclusion:** These results indicate that Phl p 5-specific T cells are highly heterogeneous, and that they differentially recognize isoallergens. This indicates that when recombinant Phl p 5 is considered for allergy vaccination, a mixture of isoallergens representing the different isoallergen groups may clinically prove to be more effective than single isoallergens.

Introduction

The role of allergen-specific T cells in the allergic reaction is well established [1]. The crucial importance of T cells in allergy via the induction of allergen-specific IgE by production of IL-4 and the maturation of eosinophils by production of IL-5 is strongly supported by the identification of IL-4 and IL-5 producing Th2-like cells at the sites of allergic inflammation [2, 3]. In addition, the shift in cytokine production profiles of allergen-specific T cells observed after specific allergy vaccination [4–6] indicates that T lymphocytes may be the principal target cells of this specific allergy treatment. As allergen extracts used for allergy vaccination are complex mixtures of naturally existing allergens that are difficult to standardize, recombinant allergens have been suggested as a useful alternative for use in allergy vaccination [7].



Fig. 1. Multiple alignment of the deduced amino acid sequences of Phl p 5 isoforms used for T-cell stimulation assays: 10200, 10170, 10064, and 10082 (all Phl p 5a isoallergens) [Wissenbach et al., manuscript in preparation], and Phl p 5b [9]. Amino acids identical with 10200 are indicated by dashes, gaps are represented by dots. The alignment was performed using the Wisconsin Package, version 9.1 (Genetics Computer Group, Madison, Wisc., USA).

However, natural allergen extracts have been shown to contain multiple isoallergenic forms of the major allergens, which has been confirmed by the cloning and sequencing of cDNAs encoding major allergens from many different allergen sources. Therefore we characterized the T-cell response of allergic patients to the *Phleum pratense* major allergen, Phl p 5 and five of its recombinant isoallergens (subdivided into Phl p 5a and Phl p 5b) to evaluate the theoretical impact of isoallergenic variation on the selection of isoallergens for specific immunotherapy.

Materials and Methods

Pollens from *P. pratense*, *Poa pratensis* and *Lolium perenne* were obtained from Greer Laboratories (N.C., USA) and extracted as described elsewhere [8]. The allergen extracts were fractionated on a phenyl Sepharose CL-4B column followed by purification by gel filtration on a S-75 Superdex column to obtain purified group-5 allergens. Recombinant isoform Phl p 5b (fig. 1) was produced as described elsewhere [9]. The recombinant Phl p 5a isoallergens expressed as Phl p 5-MBP fusion proteins and a control Bet v 1-MBP fusion protein were produced as described elsewhere [8]. T-cell lines

(TCLs) specific to Phl p 5 were established from PBMC isolated from freshly drawn heparinized blood by culturing with Phl p 5 for 5 days and expanded with rIL-2 until day 14, followed by restimulation with feeder cells.

Results and Discussion

Phl p 5-specific CD4⁺ TCLs were isolated from the peripheral blood of 3 allergic rhinitis patients. When tested with allergen extracts and purified group-5 allergens of related grasses, the Phl p 5-specific TCLs were shown to be highly crossreactive. The crossreactivity with *P. pratensis* was more extensive than with *L. perenne*, directly reflecting the sequence identity between Phl p 5, Poa p 5, and Lol p 5. The TCLs simultaneously produced IL-4 and IFN- γ upon allergen-specific activation, resembling a Th0 cytokine production profile. To address the question whether single recombinant Phl p 5 isoallergens can replace Phl p 5 for specific immunotherapy, the reactivity pattern of the Phl p 5-specific T cells derived from single individuals with recombinant Phl p 5a and b isoforms (sequences are shown in

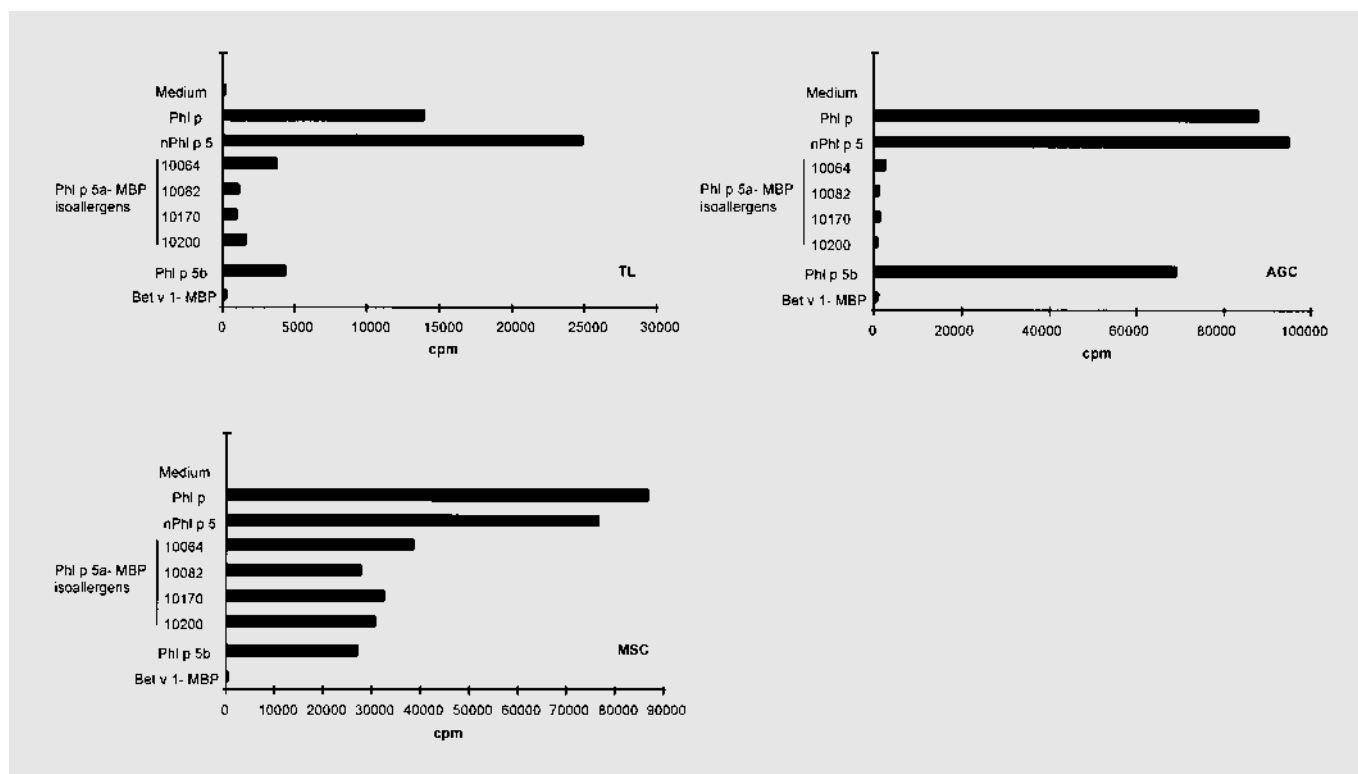


Fig. 2. Recognition of Phl p 5a and Phl p 5b isoallergens by Phl p 5-specific T cells. T cells ($10^5/\text{ml}$) were stimulated with Phl p allergen extract, purified native Phl p 5, recombinant Phl p 5a-MBP isoallergens, rPhl p 5b, rBet v 1-MBP as a control, or medium only in the presence of irradiated autologous PBMC ($5 \times 10^5/\text{ml}$). The cells were cultured for 48 h, followed by a 16-hour ^3H -TdR pulse, harvesting and liquid scintillation counting. Representative results of two separate experiments are shown. Values given represent mean cpm of triplicate cultures.

figure 1) was investigated. Interestingly, a differential recognition pattern was found (fig. 2). Patient AGC reacted exclusively with recombinant Phl p 5b, patient MSC reacted with all rec Phl p 5a and Phl p 5b isoforms, and patient TL reacted primarily with native Phl p 5. These data are complimentary to our finding that Phl p 5-specific T cell clones (TCCs) from the same patients differentially recognize recombinant Phl p 5 allergens [8]. In this study most TCCs (52.9%) did not react with any of the rPhl p 5 isoallergens, some were Phl p 5a isoform-specific (11.8%), or Phl p 5b-specific (26.5%), and some reacted with all isoforms (8.8%). TCCs derived from each of the patients had a reactivity similar to their polyclonal Phl p 5-specific TCLs shown here. Interestingly, the TCCs recognizing Phl p 5a isoallergens generally reacted with all Phl p 5a isoforms simultaneously. This indicates that the amino acid substitutions within the Phl p 5a subgroup do not have a large impact on T-cell activation, whereas the large difference between the Phl p 5a and Phl p 5b isoallergen groups does

have a large impact on T-cell reactivity. Taken together these results indicate that Phl p 5-specific T cells are highly heterogeneous, and that they can differentially recognize isoallergens, which may argue against immunotherapy with single recombinant Phl p 5 isoallergens, but rather indicates that at least two or three isoallergens must be included to represent all Phl p 5 isoallergen subgroups when vaccination with recombinant Phl p 5 allergens is considered.

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