

Implications of the Grass Group I Allergens on the Sensitization and Provocation Process

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Key Words

Group I allergens · Type I allergy · Posttranslational modifications · Epitope mapping · Protease

Abstract

Grass pollen allergens of group I are particularly important because of their high IgE prevalence and occurrence in all grass species. Four independent IgE-binding regions and one continuous epitope were identified. The posttranslational modifications on the molecule increased allergenicity. Phl p 1 is a cysteine protease, as determined by specific substrates, inhibitors and consensus sequence motifs. In analogy to other allergens and/or proteases, we deduce that Phl p 1 might enhance the permeability of the epithelium, influence T helper cells to bias Th2, and increase the IgE production of plasma cells. Thus, the group I allergens seem to be the crucial components in a pollen extract which can mediate sensitization and enhance the triggering of symptoms leading to the persistence of a grass pollen allergy.

Introduction

The allergen structure plays a crucial role in the sensitization and in the provocation process. The answer to the question why a certain antigen becomes an allergen must lie in its structural characteristics. We first investigated the IgE-reactive epitopes in group I allergens of grasses (here Phl p 1 and Hol l 1). Such structural elements can cause allergic attacks upon exposure, and they are the goals for diagnostic and specific immunotherapy. Second, we tried to determine structural features that might cause the sensitization process. This attempt should help to understand the mechanism of the allergen-specific immune reaction and probably to prevent allergy.

Determination of IgE-Reactive Epitopes

The group I allergens of timothy and velvet grass pollen consisted of 240 amino acids and revealed a sequence identity of 90% [1]. rHol l 1 and its fragments obtained by subcloning were expressed as fusion proteins in *Escherichia coli* and were used to perform an epitope mapping [2]. Fifty patient sera were screened by Western blotting. The results indicated that at least four independent IgE-binding regions existed on rHol l 1. To localize continuous epitopes, we synthesized overlapping decapeptides covering the complete Phl p 1 sequence and analyzed them for IgE reactivity. One

epitope at the N-terminus was recognized by a few sera. Phl p 1 bears several posttranslational modifications [3–5]. The influence of disulfide formations on the IgE binding was examined on nPhl p 1 by an ELISA. While denaturation by 6 M guanidine hydrochloride did not cause any change, the IgE reactivity of reduced and carboxymethylated Phl p 1 was generally reduced by 25%. This emphasizes the impact of disulfide bridges on the structure of Phl p 1.

The carbohydrate structure of Phl p 1 which bears an α 1,3-bound fucose at the innermost N-acetylglucosamine was shown to be the essential configuration of an IgE-binding epitope [4]. IgE reactivity to glycans of similar structure was demonstrated for several allergens. To determine whether carbohydrate structures themselves can cause mediator release, we isolated N-linked glycopeptides from bromelain, which shows a similar carbohydrate structure to Phl p 1, and coupled them to BSA. The conjugates elicited histamine release from basophils of a patient sensitized to carbohydrate determinants. Since Phl p 1 partly forms dimers, we assume that such a reaction can be caused by carbohydrate chains alone, even in the absence of IgE against peptide epitopes.

In summary, these results indicate that the protein conformation and posttranslational modifications are involved in the generation of IgE-reactive epitopes. Because of the multitude and diversity of the epitopes, no single eliminations seem to promise to engineer safe immunotherapeutics.

Determination of the Protein Function

When we expressed Phl p 1 in the eukaryotic *Pichia pastoris* system, we observed a severe degradation of the recombinant protein. The fragmentation was not caused by yeast enzymes but by rPhl p 1 itself. By APIZYM test (BioMérieux, Lyon, France), rPhl p 1 could be identified as a protease, which is specific for basic amino acid residues. Identity of Phl p 1 as a cysteine proteinase was confirmed by experiments using specific substrates and inhibitors [6]. Initially, the enzymatic activity was not observable with nPhl p 1. But preincubation with an appropriate cysteine-protease-activating buffer resulted in a detectable reactivity. This classification was confirmed by sequence alignments. Whereas the complete C1 cysteine proteases showed a homology of only about 10%, the three consensus regions forming the catalytic site revealed a considerable degree of structural similarity as depicted in figure 1.

Except for the asparagine residue in the third domain, Phl p 1 fulfills the typical criteria for the classification as α C1 protease: the presence of the active cysteine-histidine

		position
	*	
Papain	V K N Q G - S C G S C W A F S A V V T I E G I I	16-38
Der p 1	I R m Q G -g C G S C W A F S G V a A T E s a y	25-47
Phl p 1	I f k s G r g C G S C F e i k c T k p e a c s g	62-85
	*	
Papain	C G N K V D --- H A V A A V G Y -	153-167
Der p 1	n G yq p n y -- H A V n i V G Y s	160-175
Phl p 1	e G t K V t f -- H v e k g s n p n	143-158
	* *	
Papain	P N Y I L I K N S W G T G W G E N G Y I R I K R	168-191
Der p 1	vd Y w I V R N S W d T n W G D N G Y gyfaa	180-203
Phl p 1	dk W I a L K e S W G A i W r i d t p e v L K g	184-207

Fig. 1. Comparison of C1 cysteine proteinases (papain and Der p 1, a major allergen of the house dust mite) in the three consensus motifs forming the catalytic site (important amino acid residues are indicated by asterisks). Bold printed, capital letters mark identical, capital letters mark similar amino acid residues to the papain sequence.

pair in the corresponding sequence motifs [7], a tryptophan-rich C-terminal region [7], the similar size of the proteins and their common cellular location.

These results clearly indicate Phl p 1 to be an unactivated protease. Nothing is currently known about the activation of nPhl p 1 in vivo.

Implications of Phl p 1 for the Pathomechanism of Type I Allergy

As a proteolytic enzyme, Phl p 1 might serve to mediate the sensitization process on different levels. Here, we discuss assumptions, drawn from observations of other enzymes and/or allergens, especially the Der p 1 of the house dust mite.

Activated Phl p 1 might increase the permeability of the nasal and lung mucosa and thus reach the immune system. This was shown for several proteolytic enzymes [8]. The enzyme can influence T helper cells to cause a bias to Th2. Recently, Schulz et al. [9] demonstrated a direct effect of Der p 1 on the IL-2 receptor of T helper cells. Cleavage of this receptor caused a lower proliferation of T cells and a decreased interferon- γ secretion.

Finally, the active enzyme can directly influence plasma cells to increase the IgE production. This process is induced

by enzymatical cleavage of CD23 from B cells [10, 11]. By this, an important inhibitory signal for IgE synthesis is suppressed [12]. Apart from that, sCD23 can interact with CD21 on B cells and provoke an induction of IgE synthesis [13]. CD23 liberation can be inhibited by reagents, e.g. α -antiprotease [12]. Since several cysteine protease in-

hibitors exist in the saliva and skin [14], we assume that allergic patients might lack such inhibitors and are therefore destined to be sensitized. Once an individual has been sensitized, a wide variety of IgE-reactive epitopes can repetitively elicit allergic reactions.

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