

Allergenic Activity of a Major Grass Pollen Allergen Is Elevated in the Presence of Nasal Secretion

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Phl p5 is a major allergen of timothy grass and causes rhinitis and bronchial asthma in nearly all patients allergic to grass pollen. The biochemical processing of this molecule by the nasal mucosa at its first encounter and possible changes of its biologic activity are unknown. Two isoforms of the allergen were expressed in *Escherichia coli* and subsequently purified. Conversion of these preparations to various forms with molecular size between 10 and 20 kD in the presence of nasal secretion was observed. Surprisingly, in skin prick test assays with allergic patients the mixture of converted peptides caused significantly higher allergic response when compared with the parent protein. Allergenic activity of the recombinant N-terminal Phl p5a and the C-terminal Phl p5b as measured by skin prick test and histamine release assays was significantly higher than that of the respective parent molecules. Using pancreatic rather than nasal secretion, Phl p5b was completely degraded and its allergenicity was almost completely reduced. Proteolytic degradation converts Phl p5 to defined fragments with increased allergenicity. Complete degradation of Phl p5 on the mucosa could be a preventive strategy to destroy its potency for the induction of an allergic response. Bufe A, Gehlhar K, Schramm G, Schlaak M, Becker W-M. Allergenic activity of a major grass pollen allergen is elevated in the presence of nasal secretion.

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Because of increasing prevalence, allergic diseases constitute a growing problem for public health in Northern Europe and North America (1). Nearly 30% of bronchial asthma, allergic rhinitis, and atopic dermatitis are induced by grass pollen allergens. Thus, these molecules represent a major task for diagnostic and therapeutic research. Once inhaled into the human respiratory system pollen releases the allergens by contact with the mucosa (2). Depending on their biochemical characteristics such as molecular weight, isoelectric point, solubility, or enzymatic activity the allergenic molecules can be stable enough to facilitate penetration through the epithelial layer, reaching those levels where they are recognized by the non-specific or specific defense system (3). In a sensitized patient the production of specific IgE antibodies directed towards the allergens is a prerequisite for the immunologic response leading to allergic rhinitis and bronchial asthma. These IgE antibodies are bound to the effector cells in the submucosa via the high affinity IgE receptor (4). Once binding of the allergen by specific IgE antibodies crosslinks at least two IgE receptors, the signal for the release of mediators is transduced into the cell. In this interaction between antigen, IgE, and IgE receptor, the significance of the structural character of allergenic molecules is still not fully understood. No characteristic pri-

mary, secondary, or tertiary structures of allergens associated with specific IgE antibody response have been identified so far (5). Until now analysis of three-dimensional structures of allergens has not revealed any specific folding features of allergens (6, 7). The interaction of allergens with IgE seems to depend only on the presence of B-cell or IgE-binding epitopes. These epitopes may be linear, as identified for instance on the major cat allergen *Fel d 1* (8). More often they depend on conformational stability of the molecule (9, 10) and the avidity of IgE antibodies towards single linear epitopes is significantly reduced. Under these conditions it would be expected that degradation of allergens decreases the allergenic activity. This has been shown with a few allergens such as major birch pollen allergen *Bet v1*, which was cleaved by half and completely lost its IgE reactivity (11) and with major mite allergen *Der p2*, which did not bind IgE after breakage of disulfide bonds (12). In contrast, the biologically highly active C-terminal component of major timothy grass pollen allergen Phl p5b retained its allergenic activity (13). Thus, degradation of proteins could be an efficient nonspecific defense mechanism for some, but not all, allergens. How allergens are degraded and primarily processed in the mucosa and whether this processing could influence the allergenic activity of these molecules has never been studied. In this article we report that the allergenic activity of major timothy grass pollen allergen Phl p5 is elevated rather than decreased in the presence of nasal secretions. The question is investigated whether the additional activation of these proteins is induced by the conversion of the parent allergenic molecules into lower molecular weight forms with increased allergenicity. In this context B-cell epitope analysis of monoclonal antibodies recognizing Phl p5 allergens served as a prerequisite to identify biologically active protein

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fragments. The influence of nasal and pancreatic secretion on the allergenicity of Phl p5 is compared.

METHODS

Construction of Recombinant Peptides of Phl p5a

The recombinant peptides were either cloned by the PCR technique or subcloned after restriction analysis always using the Phl p5a plasmid pPHLP5A1913 (14) as templates.

Nt-Pep and Ct-Pep (Phl p5a). Construction of both subclones is described elsewhere (14). The inserts of these pBluescript plasmids were subcloned into the appropriate pQE vector (Qiagen, Chatsworth, CA) via *Bam*H I and *Hind* III restriction.

Pep 1,2,3,4,5,6 and 2-4 (Phl p5a). Peptide clones were amplified by PCR using the following primers and PCR conditions:

P1sens (Pep1: AA 32 to 81): ATATGGATCCGGTAAGGCGAC-GACCGA

P1anti: ATATAAGCTTCTCCGCGAAGGCCTTGT

PCR conditions: 95°/0.5 min, 54°/0.5 min, 72°/1.5 min, 35 cycles

P2sens (Pep2: AA 65 to 126): ATATGGATCCACAGGACGT-TCGGCGCAAC

P2anti: ATATAAGCTTCGACGTAGGCGTCGTA

PCR conditions: 95°/0.5 min, 54°/0.5 min, 72°/1.5 min, 35 cycles

P3sens (Pep3: AA 98 to 156): ATATGGATCCGCGCTCACCTC-CAAGCT

P3anti: ATATAAGCTTGGGATGACCTTGACCTCCTC

PCR conditions: 95°/0.5 min, 56°/0.5 min, 72°/1.5 min, 35 cycles

P4sens (Pep4: AA 132 to 189): ATATGGATCCGAGGCGCTC-CGCATCAT

P4anti: ATATAAGCTTAGACGGTGAACCTTGTCGTTG

PCR conditions: 95°/0.5 min, 54°/0.5 min, 72°/1.5 min, 35 cycles

P5sens (Pep5: AA 182 to 286): ATATGGATCCCCCGCCAAC-GACAAGTTCACC

P5a complete-anti: ATATAAGCTTTACACAAATGGCAAT-GCGTGC

PCR conditions: 95°/0.5 min, 56°/0.5 min, 72°/1.5 min, 35 cycles

P6sens (Pep6: AA 244 to 286): ATATGGATCCAAGGGCAT-CACCGCCATGTCC

P5a complete-anti: see above.

Primers P2sens and P4anti (Pep2-4: AA 65 to 189), PCR conditions: 95°/0.5 min, 54°/0.5 min, 72°/1.5 min, 30 cycles

Subcloning and Sequencing

All restriction fragments and PCR products were purified from 1.5% agarose gel, restricted with the appropriate enzymes, and ligated into the expression vector pQE (Qiagen) or pMal-c (New England Biolabs, Beverly, MA). The correct reading frame was controlled by sequencing in both directions according to the dideoxy method of Sanger and colleagues (15) with the T7 Sequencing Kit (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Expression of Recombinant Peptides

Expression of recombinant peptides for epitope analysis was induced with 2 mM IPTG in 10 ml cultures of *Escherichia coli* K12 XL1-Blue containing the recombinant pQE plasmids. At zero hour and after 3 and 5 h of induction, lysates were analyzed on Western blot with monoclonal antibodies.

Purification of Recombinant Phl p5a and b

The recombinant proteins Phl p5a and Phl p5b were cloned by polymerase chain reaction. Expression and purification was performed as described elsewhere (16, 17).

Cloning of C-terminal Peptide of Phl p5b

The C-terminal peptide was constructed by the PCR technique using pPHLP51912 (17) as template. The length was designed according to the 13-kD portion of Phl p5b identified by crystallization as described earlier (13).

P-Ct-sens (Pep.Ct-Phl p5b: AA 130 to 265): ATATGGATCCAA-GATCCCCGCGGCGAG

P-Ct-anti: ATATAAGCTTTCAGACTTTATAGCCACC

PCR conditions: 95°/1 min, 56°/1 min, 72°/2 min, 30 cycles.

The PCR product was purified from 1.5% agarose gel, restricted with the appropriate enzymes, and ligated into the expression vector pMal-c (New England Biolabs).

Purification of N-terminal Peptide of Phl p5a and C-terminal Peptide of Phl p5b

Purification of Phl p5a N-terminus was performed on an Ni²⁺-nitrilotriacetate-resin column according to the instruction of the manufacturer (Qiagen), followed by gel filtration on a Superdex™ 7S HR 10/30 column (Pharmacia Biotech Inc., Uppsala, Sweden). Phl p5b C-terminus was purified as described earlier (16) for the parent Phl p5b via amylose-resin column (Pharmacia). Maltose-binding protein and the recombinant protein were cleaved by factor Xa (New England Biolabs) using appropriate buffer conditions. Recombinant peptide was separated from MBP by ion-exchange chromatography using a DEAE-column on FPLC (Pharmacia).

SDS-PAGE Immunoblotting

Proteins were separated by SDS-PAGE (12%) according to Laemmli (18). After SDS-PAGE, proteins were either silver-stained in the gel, following a procedure mentioned elsewhere (19), or subsequently transferred onto a nitrocellulose membrane by semidry blotting for 30 min at 0.8 mA/cm on a Fastblot (Biometra, Göttingen, Germany). Membranes were cut into strips and used for immunologic detection with monoclonal antibodies or patients' sera in a procedure as previously described (20).

Monoclonal Antibodies and Sera

Monoclonal antibody (mAb) Bo1 (21) recognizes Phl p5a and Phl p5b from crude pollen extract. mAbs BG6 and Bo9 (22) identify only Phl p5a and Phl p5b, respectively. Both are markers for internal Group V allergen differentiation in timothy grass pollen. Control serum samples were acquired from healthy persons who had no clinical history of allergy, IgE antibodies in the normal range, and negative RAST results. We collected patients' sera in the Out-patients Allergy Department at the Medical Clinic of Borstel, Germany. Atopic phenotype was confirmed by clinical history and diagnosis on the basis of a highly positive skin prick test response to grass pollen allergens and specific IgE antibodies greater than 17.5 kU/L as measured in the CAP System (Pharmacia).

Nasal and Pancreatic Secretion

Nasal secretion was collected from allergic patients and from two healthy control subjects by forced excretion of mucus. The secretion was diluted with NaCl 0.9%, thoroughly mixed, and centrifuged. The supernatant was used for further analysis.

Pancreatin was purchased from Kali-Chemie Pharma (Hannover, Germany) as granulate, thoroughly broken with pestle and mortar, and suspended in NaCl 0.9% to a concentration of 100 U/ml of protease.

Skin Prick Tests (SPT) with Recombinant Peptides

SPT was done following the guidelines for clinical diagnosis of allergic diseases (23). Selected fragments were dissolved to a concentration of 100 µg/ml and applied in a standard SPT using physiologic sodium chloride, 1.7 mg/ml histamine-dihydrochloride and 5,000 BU timothy grass pollen extract (Allergopharma, Reinbek, Germany) as controls. Wheal areas and flaring were measured and compared by calculation of surface in mm².

Histamine Release Assay

Blood samples were taken and prepared as described earlier (13): 1 × 10⁵ basophils per ml were stimulated with different dilutions of selected recombinant peptides. Histamine concentrations were measured by RIA (Pharmacia) according to the instructions of the manufacturer. Maximal histamine release was determined in supernatants of disrupted basophils to be about 0.4 to 1 µg per 10⁵ cells.

RESULTS

Fragmentation of Phl p5a by Nasal Secretion

Recombinant timothy grass pollen allergen Phl p5a (16) was expressed in the maltose-binding protein vector pMal-c as fusion partner and purified via amylose-resin column followed by cleavage with factor Xa and ion-exchange chromatography. The purified protein presented as a single band in SDS-PAGE at 32 kD (Figure 1, lane 3). Upon incubation with nasal secretion the parent protein disappeared from the gel and was converted to a number of fragments with molecular size between 12 and 30 kD (Figure 1, lane 4), appearing as additional bands besides the original nasal secretion proteins (Figure 1, lane 2). To demonstrate the presence of allergenic peptides among the converted fragments, the recombinant protein was incubated with nasal secretion in a dose-dependent manner for 30 min, and Western blot was performed. Using a patient's serum as probe it turned out that at least four of the converted fragments between 13 and 20 kD were recognized by IgE antibodies (Figure 2). It also revealed that these peptides retained a relative stability towards proteolytic degradation shown by very slowly disappearing converted fragments in the higher nasal secretion dosages. Comparing the effects of nasal secretion from allergic patients with those from healthy control subjects, no difference between the degradation pattern was observed (data not shown). These results indicate the presence of proteases in the nasal secretion, which can primarily degrade a major allergen from timothy grass pollen. It is also shown that this processing does not necessarily lead to complete degradation of an allergenic component.

Biologic Influence of Allergen Degradation

In order to evaluate the biologic influence of this degradation process, the recombinant allergen was used in skin prick test of allergic patients in the presence or absence of nasal secretion. This *in vivo* assay is regarded as a relevant indicator of allergenic activity and specificity and depends on the mediator release from human skin mast cells. The wheal areas induced by the recombinant allergen, which was either incubated with nasal secretion (column 5) or without nasal secretion (column 4), as shown in Figure 3. NaCl 0.9%, histamine and nasal se-

cretion alone served as controls. Surprisingly, the activity of the recombinant allergen increased significantly in the presence of nasal secretion by a mean of 50%. Thus, conversion of the Phl p5 allergen to low molecular weight fragments is accompanied by an increase of allergenic activity. This would mean that the converted peptides as opposed to the parent molecules induce an elevated allergic response. To provide evidence for this hypothesis, at least some of the peptides had to be identified. For this purpose monoclonal antibodies specific for Phl p5 were used to characterize the converted peptides.

Epitope Analysis of Monoclonal Antibodies

Three monoclonal antibodies served as a marker for Phl p5 allergen. They distinguish between the two isoforms a and b. mAb Bo1 recognizes both molecules (21), whereas mAb BG6 binds to Phl p5a and Bo9 only to Phl p5b (22). Because little is known about the epitopes of these mAbs on the allergens, an epitope analysis was performed first. Nine overlapping recombinant peptides were cloned and expressed in the 6xhis-tag vector pQE. Reactivities of the mAbs were determined in immunoblots. The results in Figure 4 demonstrate that mAb Bo1 needed a relatively large C-terminal portion of Phl p5 for effective binding. As opposed to that, mAb BG6 recognized a more sequential region on the N-terminus of Phl p5a, reaching from AA 98 to AA 131. The fact that Pep2 and Pep4 were not recognized by mAb BG6 indicates that the three AA 129 to 131 (Thr, Leu, Ser) play a crucial role in the interaction between the allergen and the mAb. Earlier results in the context of highly homologous peptides from *Holcus lanatus* (Schramm and colleagues [9]) together with data presented here show that Bo9 bound to Phl p5b in the region of AA 163 to AA 215 and partly included the Bo1 binding epitope. Thus, mAb Bo1 recognized C-terminal peptides of all Phl p5 isoforms, mAb BG6 bound to N-terminal parts of Phl p5a, and mAb Bo9 bound to C-terminal fragments of Phl p5b.

Differential Fragmentation of Isoallergens

In order to characterize the peptides converted in the presence of nasal secretion, a partial digestion of recombinant Phl p5a was performed, and the fragments were analyzed with the mAb by immunoblotting. It can be seen in Figure 5 that at least three peptides were recognized by mAb BG6. Considering the molecular weight of these fragments, it is obvious that they

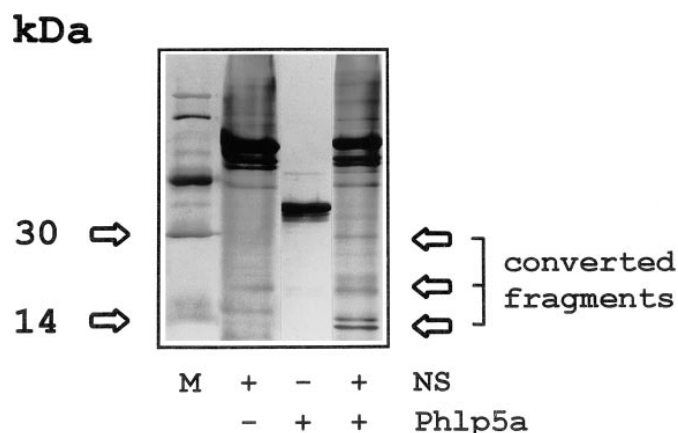


Figure 1. Processing of recombinant Phl p5 by nasal secretion. Gel electrophoretic separation (silver stain) of purified Phl p5a in the absence (lane 3) and in the presence (lane 4) of nasal secretion taken from an allergic patient. Arrows on the right depict novel protein fragments as compared with the control of nasal secretion alone (lane 2). Protein marker in lane 1.

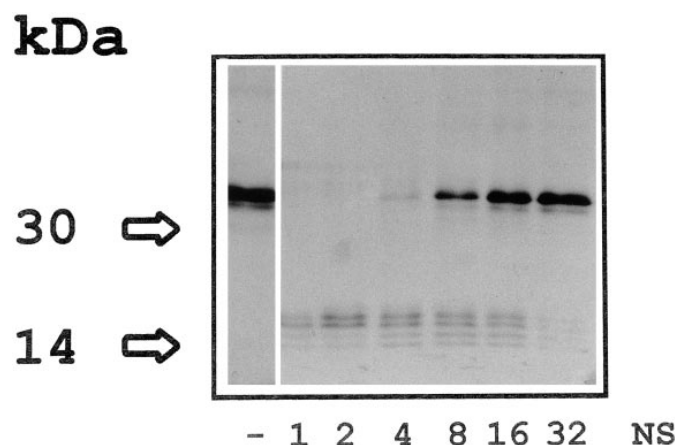


Figure 2. Dose-dependent processing of recombinant Phl p5 by nasal secretion. Western blot of purified Phl p5a in the presence of serially diluted nasal secretion using specific IgE antibodies from a patient's serum as probe.

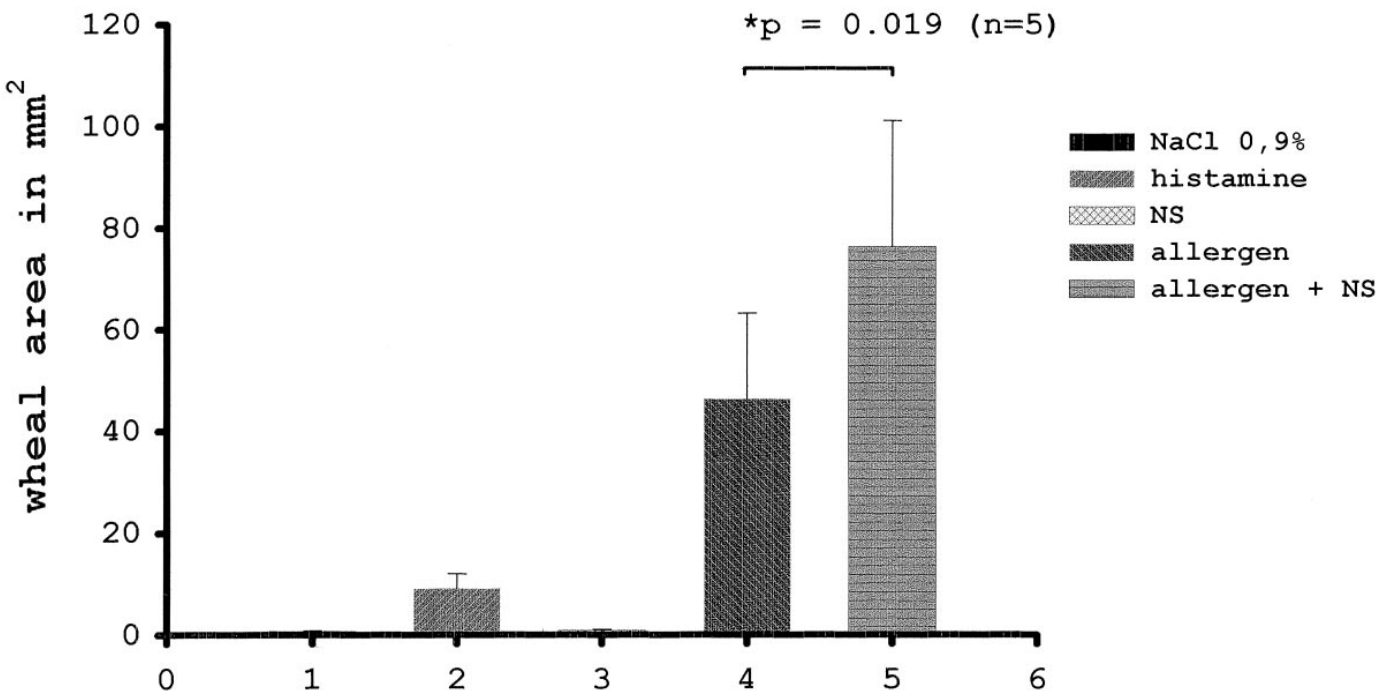


Figure 3. Allergenic activity associated with recombinant Phl p5 in the absence or presence of nasal secretion. NaCl 0.9% (1), histamine (2), nasal secretion alone (3), 100 μ g/ml purified rPhl p5a (4), and the same concentration of the allergen with nasal secretion (5) were assayed for specific allergic response in skin prick test with five allergic patients.

represent preferentially N-terminal peptides. Although weakly, one fragment is bound by mAb Bo1 and is thus a more C-terminal peptide. Using recombinant Phl p5b for partial digestion (Figure 5), only C-terminal peptides could be identified because a probe for N-terminal peptides is missing.

Allergenic Activity of Fragments

Because N-terminal fragments of Phl p5a and C-terminal fragments of Phl p5b are now identified, it was of great importance to question whether these peptides still exhibit allergenic activity. In a histamine release assay the ability of the

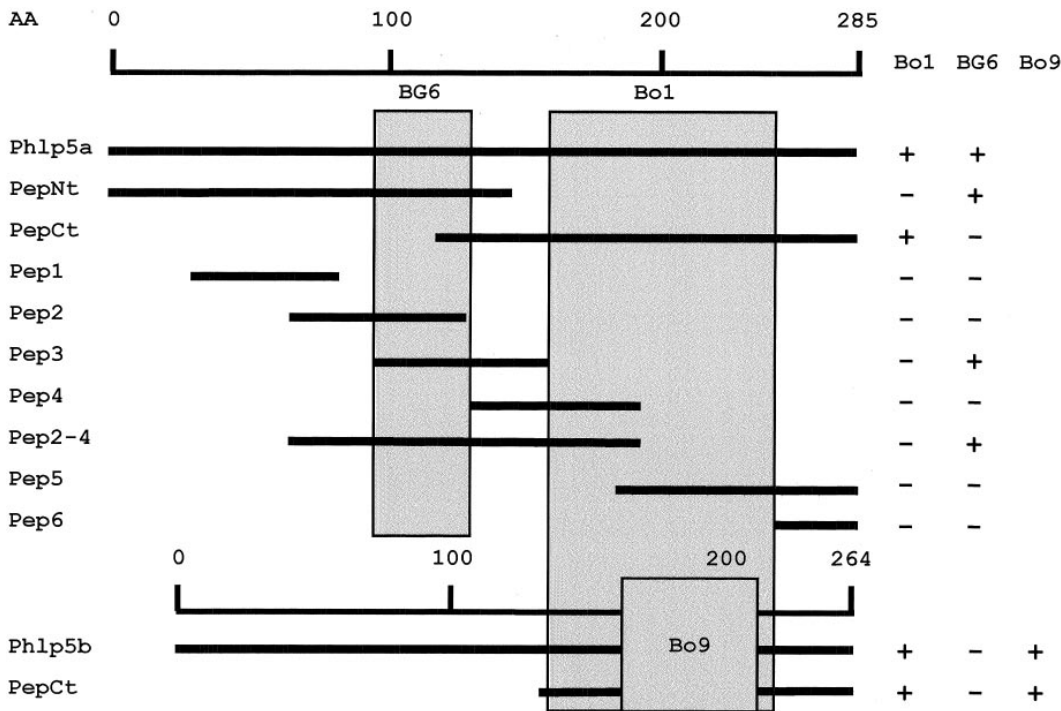


Figure 4. Epitopes of Group V mAbs Bo1, BG6, and Bo9 identified on Phl p5a and b. Diagram of overlapping recombinant peptides derived from Phl p5a and b showing the immunoreactivities of the mAbs with the respective fragments (+ = positive reaction, - = negative reaction). Shaded boxes indicate the sequence regions at least required for binding.

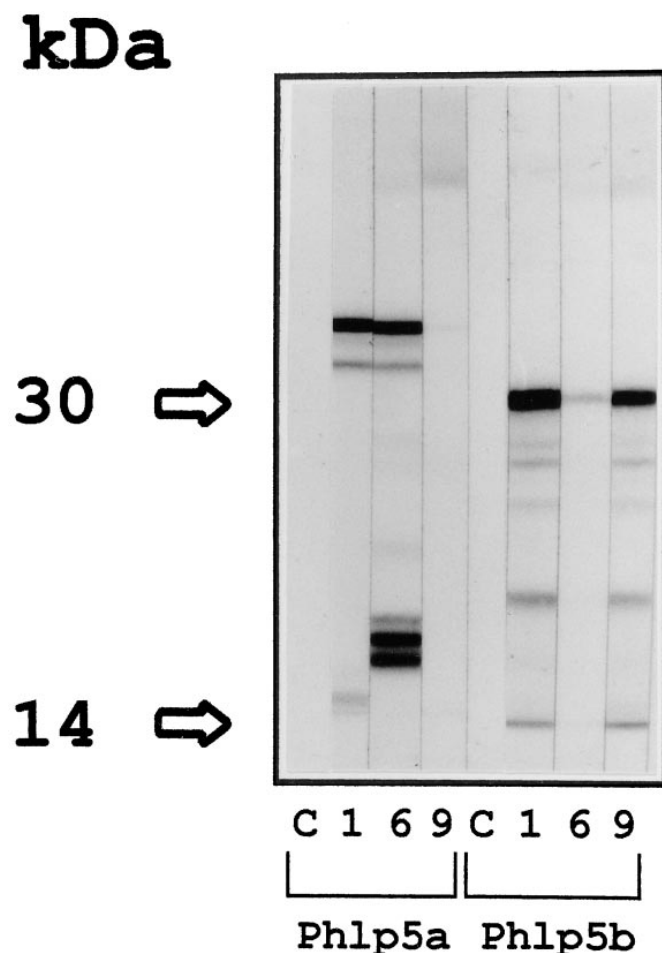


Figure 5. Immunoreactivities of Group V specific mAbs with peptides converted by nasal secretion. Western blot of purified recombinant Phl p5a and b after incomplete digestion with nasal secretion. Single stripes were probed with different mAbs: C = control with secondary antibody; 1 = mAb Bo1; 6 = mAb BG6; 9 = mAb Bo9. For details see METHODS.

purified recombinant N-terminal Phl p5a and the C-terminal Phl p5b fragments to induce mediator release from human basophils of an allergic patient was tested and compared with the parent allergens. The histamine release in relation to the concentration of the four molecules is shown in Figure 6. The N-terminus of Phl p5a was biologically 20 times more active and the C-terminus of Phl p5b eight times more active than the respective parent protein as shown by different molar concentrations causing 50% of maximal histamine release. Thus, N- or C-terminal fragments of the major allergen Phl p5 can have an elevated allergenic activity. This observation is confirmed by similar results from the skin prick test shown in Figure 7. The application of the C-terminal Phl p5b is accompanied by a significant increase of wheal area in five patients compared with its parent molecule. Taken together these results imply that nasal secretion amplifies the allergic response of Phl p5 by conversion of the protein to relatively stable fragments with elevated allergenic activity.

Influence of Pancreatin on Allergenic Activity of Phl p5b

Because it is obvious now that proteolytic digestion of Phl p5 in the nasal mucosa does not necessarily protect a patient from an allergic response, it will be of great interest to find a

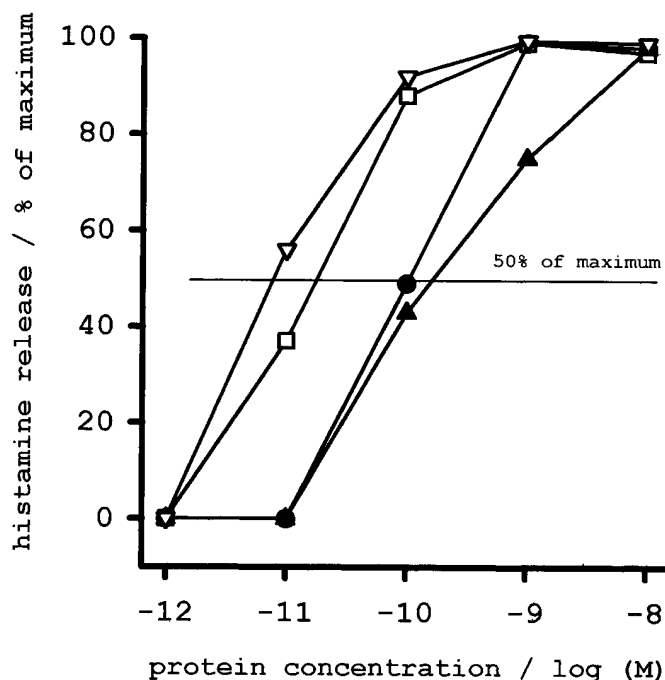


Figure 6. Comparison of allergenic activity associated with mature Phl p5 isoforms and two converted peptides in histamine release. Different concentrations of parent recombinant Phl p5a (closed triangles), of recombinant Phl p5a N-terminal portion (open triangles), of parent Phl p5b (closed circles), and of recombinant Phl p5b C-terminal portion (open squares) were assayed for induction of histamine release from human basophils. For details see METHODS.

substance that will degrade the allergen in such a way that no allergic activity is retained. It is well known that physiologic secretion of pancreas contains proteases that degrade nearly all proteins that have passed through the stomach. Recombinant allergen Phl p5b was incubated in the presence or absence of pancreatin, a standardized solution of pancreatic secretion normally used for patients with indigestion. This was compared with the degradation effect of nasal secretion. An immunoblot probed with mAb Bo1 demonstrating that Phl p5b was completely degraded in the presence of pancreatin, whereas nasal secretion only led to a partial digestion of the allergenic molecule as seen earlier, is shown in Figure 8. Furthermore, when applying the allergen incubated with pancreatin in the skin prick test (Figure 9), there was a significant reduction of wheal areas in comparison with the allergen alone. Pancreatin and NaCl 0.9% served as a negative control, and lack of wheals was indicative of the specificity of the reaction. These data confirm that partial degradation is the mechanism that led to increased biologic activity of Phl p5 allergens. Furthermore, it demonstrates loss of allergenic activity accompanied by complete degradation.

DISCUSSION

No published data exist so far concerning the primary processing of allergens in the nasal mucosa. It has been shown that the major mite allergen *Der* p1 acts like a serine protease (24), and it could augment its permeability in the bronchial epithelium by its own enzymatic activity (25). Furthermore biochemical interaction of allergens with respiratory secretions has been described in terms of solubility of allergenic molecules in the mucosa or binding of allergens with carbohydrase

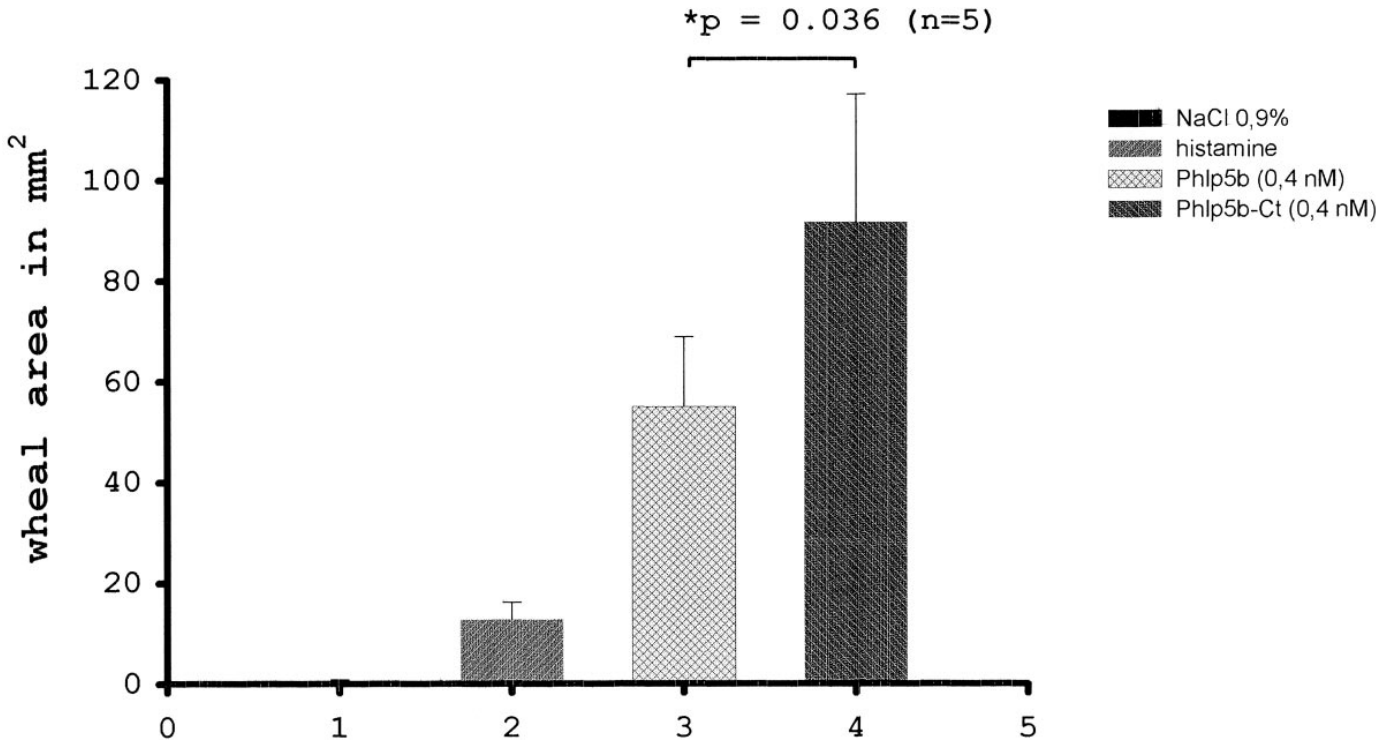


Figure 7. Allergenic activity associated with a mature Phl p5 isoform and a converted peptide in skin prick test. NaCl 0.9% (1), histamine (2), 0.4 nM purified rPhl p5b (3), and 0.4 nM purified Phl p5 C-terminus (4) were assayed for specific allergic response in skin prick test with five allergic patients.

activity to specific carbohydrate moieties on respiratory mucins (26). The influence of proteolytic activity in the mucosa on the stability of allergens and their biologic activity has never been investigated.

In this report we describe the conversion of a major grass pollen allergen to relatively stable low molecular forms in the presence of nasal secretion. The mixture of converted fragments induced an elevated allergic response as compared with the native allergen. So far only the complete allergenic pro-

teins have been thought to represent the actual molecules to induce sensitization and immunologic response in allergic disease. It has been proposed to use immunodominant peptides of grass pollen allergens with no allergenic activity to inhibit binding of the active proteins to the effector cells (10). Furthermore, major birch pollen allergen Bet v1 was demonstrated to lose its allergenicity when converted into a nonanaphylactic T-cell epitope containing fragments (11). But none of these fragments has ever been found in the physiologic environment of the respiratory mucosa. In our case, however, in the presence of nasal secretion, a number of IgE-binding peptides were identified. The proteolytic process did not seem to be a patient-specific phenomenon since it was observed as well when using nasal secretion of healthy control subjects.

Epitope analysis of the grass Group V specific monoclonal antibodies enabled us to better characterize the converted fragments. The fact that Group V specific mAb Bo1 recognized a conformational epitope on the C-terminal portion of Phl p5 facilitated the identification of structurally compact peptides derived from the parent allergen. A further advantage was the possibility to classify the peptides as N-terminal or C-terminal fragments. Although the exact length of the converted fragments was not determined, in any case, however, the localization of binding sites and the subsequent identification of the protein regions the fragments belong to justified the usage of adapted recombinant peptides in a model to compare biologic activities of the different molecules.

The observation of a marked increase in allergenic activity associated with the short N-terminal and C-terminal peptides compared with the parent proteins suggests that the generation of these products in the presence of nasal secretion may arise from a physiologic process. The principle of increasing biologic activity by cleavage of molecules is a general phe-

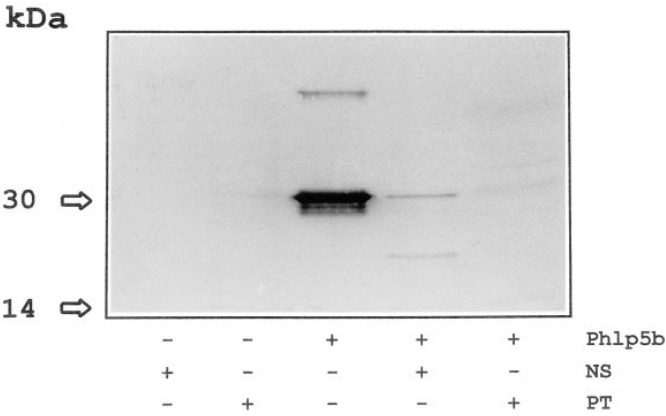


Figure 8. Comparison of allergen processing between nasal and pancreatic secretion. Western blot of purified Phl p5b in the presence of nasal (NS/lane 4) and in the presence of pancreatic secretion (PT/lane 5) compared with nasal secretion (lane 1), pancreatic secretion (lane 2), and recombinant Phl p5b (lane 3) alone. Probe was group V mAb Bo1.

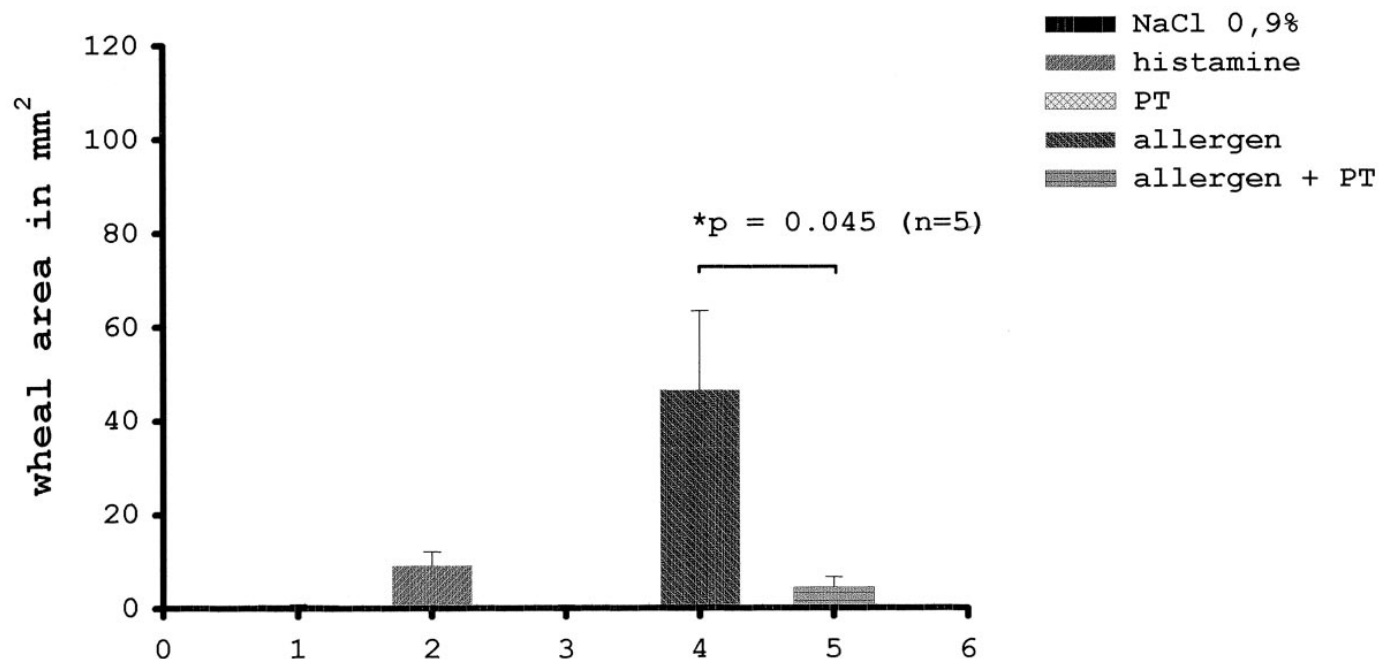


Figure 9. Allergenic activity associated with recombinant Phl p5 in the absence or presence of pancreatic secretion. NaCl 0.9% (1), histamine (2), pancreatic secretion (PT) alone (3), 100 µg/ml purified rPhl p5b (4), and the same concentration of the allergen with pancreatic secretion (5) were assayed for specific allergic response in skin prick test with five allergic patients.

nomenon seen in a number of different biologic systems; in our case, limited proteolysis led to a different bioavailability of the allergen. Similar processes were described for the interaction of bacterial antigens with the host when free lipid A as a degradation product of lipopolysaccharides becomes the actual active molecule (27). The elevation of the allergic response by the mixture of converted peptides can thus be attributed to increased biologic activity of the molecules themselves and not to any adjuvant substances in the nasal secretion. It is noteworthy that the conformational stability of these peptides may contribute to this phenomenon, as was shown for the C-terminal portion of grass pollen allergen Phl p5b isolated by crystallization (13). Another reason for increased biologic activity of the converted peptides could be primary sensitization to the fragments rather than to the intact Phl p5, resulting in a cross-reactivity of fragment-specific IgE with the complete molecules. From the results presented in this report it conveys that the two Phl p5 isoforms are converted to different fragments by nasal secretion. This observation confirms their different structure (16). But it also stresses the significance of isoforms in the allergic immune response, especially since their processing in the nasal mucosa would lead to an increased and even more complex number of bioavailable active allergenic fragments. Furthermore, this phenomenon might explain why certain fragments of specific isoforms are immunodominant and others are not (9). Whether an individual variability in primary processing of allergens in the mucosa is associated with the fact that allergic patients are sensitized to different allergen groups remains an open but very important question.

The composition of active enzymes, especially proteases in the nasal mucosa is not fully understood, and little is known about their activity (28, 29). The most important proteases of the respiratory mucosa are contributed by the mucosal mast cells (30) with the predominant proteases such as tryptase, chymase, and carboxypeptidase. Although the specific protease content of nasal secretion is not defined, it conveys from

our report that gastrointestinal proteases such as those in pancreatic secretions show a more effective proteolytic activity. This is in accordance with the fact that Group V grass pollen allergens induce predominantly inhalant allergies. The significant loss of allergenic activity using recombinant Phl p5b in skin prick test with pancreatic secretion compared with the parent protein alone indicates that complete destabilization of allergens on the mucosa would mean suppression of an allergic response. The same principle is applied when feeding hydrolyzed proteins to children with food allergies (31). Because avoidance of pollen allergens is impossible, a novel strategy to destroy the allergens before they induce an immunologic response would be the therapeutic use of well-tolerated but still active proteases on the respiratory mucosa during the specific pollen season. Before such a concept can be realized, more knowledge on structural and biochemical data of all the other pollen allergens involved in the induction of allergic response must be collected, and the processing of pollen proteins in the nasal mucosa must be understood in more detail.

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