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Comparison of Four Grass Pollen Species Concerning Their Allergens of Grass Group V by 2D Immunoblotting and Microsequencing

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Summary: The identication and characterization of allergenic components is a vital step towards improving diagnosis and therapy. Members of the grass family (Poaceae) reveal a high cross-reactivity among each other caused by the close phylogenetical relationship. In order to investigate the variability between allergenic components, we studied the allergen grass group V, one of the major allergens. Pollen extracts of 4 different tribes (timothy grass *(Phleum pratense)* – Agrostidae, perennial rye grass *(Lolium perenne)* – Festuceae, meadow velvet *(Holcus lanatus)* – Aveneae, and rye *(Secale cereale)* – Triticeae) of the Festucoideae subfamily were separated by 2D PAGE and investigated by immunoblotting using patients' poolserum and monoclonal antibodies (raised against group V allergens of timothy grass pollen). The antibodies identify different allergens in the four grass species. The components vary from 30–50 kDa and pI 4.8–7.0. The eight NH_2-terminal amino acids were determined and indicated high similarities between the different components. These results cast doubt on the suitability of classifying allergens into groups based only on their molecular mass, isoelectric point and N-terminal sequence analysis. It suggests to classify allergens according to their IgE-reactive epitopes.

Key terms: Grass group V, immunoblotting, microsequencing, pollen allergens, two-dimensional polyacrylamide gel electrophoresis (2D PAGE).

Grass pollen are the most common allergens causing immediate type reactions that are mediated by IgE-antibodies. Usually the allergenic symptoms are not provoked by only one grass species but by exposure to other grass pollen as well. The reason for their cross-reactivity to allergens of different grass pollen species is the close phylogenetical relationship[1,2]. For immunotherapy, e.g. hyposensitization therapy, the treatment with well characterized extracts is considered that should only contain relevant IgE-reactive components[3,4]. It is, therefore, necessary to investigate whether or not the allergenic components of different grasses are identical.

In Northern and Central Europe about 90% of patients suffering from pollinosis reveal IgE-reactivity against the major allergen of timothy grass pollen *(Phleum pratense)* termed Phl p V[5]. By cross radio immuno electrophoresis (CRIE) Matthiesen and Løwenstein[6] demonstrated that similar immunological reactivities are also detectable in many other grass pollen. The variability of cross-reactivity of IgE antibodies was investigated by means of RAST-inhibition tests in several groups[7,8]. Although the degree of cross-reactivity was determined between different grasses, the structure of the allergenic molecules and the IgE-reactive epitopes remained obscure. Klysner

Abbreviations:
IEF: isoelectric focusing, IgE: immunoglobulin E, PAGE: polyacrylamide gel electrophoresis, P′: hydroxyproline, pI: isoelectric point, SDS: sodium dodecyl sulfate, 2D: two-dimensional.