Currently, the IUIS allergen database contains 12 allergen and isoallergen sequences from C. thummi thummi. The Uniprot database has demerged entry P02225, listed in the database for Chi t 7, into 7 entries, 5 from C. thummi thummi and 2 from C. thummi piger that are identical to 2 of the sequences from C. thummi thummi. Consequently, the 16 unique amino acid sequences of the mature C. thummi allergens were aligned using ClustalX2, a neighbor-joining tree was generated from the alignment and a percent sequence identity matrix was built to evaluate appropriate nomenclature.

Results: Pairwise sequence alignments showed that sequences belonging to allergens Chi t 5, 6, 7 and 8 possess sequence identities to Chi t 3 between of 51 and 63%. Chi t 1, 2, 4, and 9 diverge to a greater extent from Chi t 3 (<50% identical) and from each other. Phylogenetic tree analysis suggests the clustering of Chi t 1, 3, 7, and 8, while Chi t 1, 2, 4, 5, and 9 form separate clades.

Conclusions: Based on these analyses, the IUIS Allergen Nomenclature Sub-Committee renames Chi t 5, 6, 7, and 8 isoallergens of Chi t 3, even though their sequence identities to Chi t 3 are below the 67% threshold previously defined for isoallergens. The remaining hemoglobins, previously designated Chi t 1, 2, 4 and 9 will retain their previous names.

16 Pollen Allergens Differ From Nonallergic Pollen Proteins By Their Lower Extent of Evolutionary Conservation
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Background: Pollen contains hundreds of different proteins. However, only a small fraction of them have been identified to be allergenic. We aimed to test the hypothesis that most pollen proteins are non-allergenic due to their high extent of sequence conservation among non-related species.

Methods: Data on the composition of pollen proteomes of birch (Betula pendula), plantain (Plantarum judaica) and timothy grass (Pleum pratense) were obtained from the literature. Sequences were downloaded from UniProt and manually classified into allergens and non-allergens. Complete proteome sequences of 3 dicotyledonous species (Arabidopsis thaliana, Populus trichocarpa and Vitus vinifera), 2 monocotyledons (Oryza sativa subsp. japonica and Zea mays) and one moss (Physcomitrella patens) were downloaded from ENSEMBL Plants. Sequences of pollen proteins were compared to these proteomes by using BLAST and the hits yielding the highest sequence identity recorded taking into account only sequence alignments at least 40 residues in length. The distributions of maximum sequence identities of allergens and non-allergens from each species were compared using the Mann-Whitney test.

Results: Allergens from birch and pollinating pollen were significantly (P < 0.001) less similar to proteins from monocots than non-allergenic pollen proteins. Median sequence identities to the nearest rice and maize homologues were 49 and 52% for birch allergens, 86 and 85% for birch non-allergens, 37 and 37% for pollitory allergens, and 87 and 89% for pollitory non-allergens. Similarly, timothy grass pollen allergens were significantly (P < 0.0001) less similar to dicot proteins than non-allergenic pollen proteins. Median sequence identities to the nearest homologues were 43 to 44% for allergens and 81 to 83% for non-allergens. A comparison of all 3 pollen proteomes to sequences from the moss P. patens yielded similarly significant differences.

Conclusions: Pollen allergens belong to evolutionary less conserved protein families than non-allergenic pollen proteins. The continual exposure of the human immune system to nearly identical and hence highly cross-reactive conserved proteins from multiple pollen and plant food species most likely leads to the induction of immunological tolerance rather than allergic sensitization. This study was supported by grants P-22559-B11 (to CR) and SFB-F01802 (to HB) from the Austrian Science Fund.
Methods: Extracts from North American short ragweed (Ambrosia artemisiifolia) pollen were investigated by mass spectrometry (MS), 2D-PAGE and immunoblotting. Furthermore, Amb a 1 isoallergens were purified and IgE reactivity determined by immunoblotting and IgE inhibition.

Results: 2D-PAGE and MS of ragweed extract proved the presence of all 5 known Amb a 1 isoallergens, of which Amb a 1.01 represents the dominant form. Additionally all other ragweed allergens known by sequence (Amb a 3, Amb a 4, Amb a 5, Amb a 6, Amb a 8, Amb a 9, Amb a 10) were identified. The highest IgE reactivity by immunoblotting was observed for Amb a 1.01 followed by Amb a 1.03; other Amb a 1 isoallergens as well as other detected ragweed allergens showed only weak IgE reactivity. All isoallergens with the exception of Amb a 1.04, which is only of low abundance in ragweed extract, were purified. Similar to the immunoblot analysis with crude extract, the purified isoallergens Amb a 1.02 and Amb a 1.05 showed weak IgE binding, whereas Amb a 1.01 and Amb a 1.03 had high IgE reactivity. First IgE inhibition experiments suggest that Amb a 1.01 contains all relevant IgE epitopes.

Conclusions: Amb a 1.01 is the most abundant Amb a 1 isoallergen, and presumably the most important ragweed allergen. However, a larger panel of ragweed-allergic subjects has to be analyzed with regard to IgE and T cell reactivities, to be able to choose a candidate for a recombinant vaccine for specific immunotherapy of ragweed allergy.

19 Proteomic Analysis of Major and Minor Allergens From Isolated Pollen Cytoplasmic Granules
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Background: Grass pollen is one of the most important vectors of aeroallergens. Under atmospheric conditions, pollen grains can release pollen cytoplasmic granules (PCGs). The allergens associated with these intrinsic sub-fractions induce, in laboratory animals as well as in asthmatic patients allergic and inflammatory responses. The aims of this study were to characterize and identify the intrinsic allergens of PCGs, to compare them with those of pollen grains.

Methods: PCGs were isolated from Phleum pratense pollen by osmotic shock. The water-soluble proteins were extracted from pollen grains and their PCGs. Nine out of 26 grass sensitized patient sera were selected on the basis of previous ELISA and immunoblotting results showing IgE specific binding to numerous grass pollen allergens. IgE-binding proteins were analyzed by 1- and 2D-immunoblotting using grass pollen-sensitized patient sera. Once located, allergens were characterized by mass spectrometry.

Results: 2D gels of pollen and PCGs extract revealed about 100 and 40 proteins respectively, with a large spectrum of Mr (10–94 kDa) and pI (<4.5–10.0). More proteins as well as more allergens in pollen than in PCGs were detected by immunoblotting. Several of the allergens listed in the IUIS nomenclature - Phl p 1, 4, 5, 6, 11 and 12 - were found in pollen and PCGs extracts while Phl p 11 was found only in PCGs and Phl p 2 as well as Phl p 13 only in pollen extract. Some other allergens, not listed in the IUIS nomenclature, were also characterized in both pollen and PCGs extracts.

Conclusions: Since the major grass pollen allergens were found in PCGs and because of their small size, these sub-micronic particles should be considered as very potent sensitizing and challenging respirable vectors of allergens. We demonstrate here that PCGs are at least as much dangerous as pollen grains.

20 Cross-Reactivity Between Olive Pollen and 3 Species of Grasses in Madrid, Spain
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Background: The most common allergenic pollens in patients with pollinosis in Central Spain are grasses and olive pollen, with a prevalence of positive skin prick test results of 94 and 61%, respectively. Shared proteins have been described in olive and grasses, such as profilins, polcalcin and trypsin inhibitors, but there are few in vitro studies analyzing the potential cross reactivity between both species. The aim of the present study was to analyze the protein composition of both allergens extracts, and the allergenic cross-reactivity between O. europea and different grass pollen species.

Methods: Seventy-two (72) patients (mean age 10.4 years) were included in the study. All of them suffered rhinoconjunctivitis and/or asthma and were sensitized to olive and/or grass pollen. Specific IgE of the individual patients against O. europea, and to the grass species: Dactylis glomerata, Phleum pratense and Trisetum panicum were determined by ELISA. Inhibition assays were performed to verify allergenic cross-reactivity between grass species and olive. Mass spectrometry analysis was performed to characterize the extracts and establish if there are common proteins in both, grass and olive pollens, that could act as cross reactive proteins.

Results: Three different sensitization patterns were observed: 1) sensitization to olive and grass pollen, 2) sensitization to olive and not to grasses and 3) sensitization to grasses and not to olive. Different pools of sera were mixed according to this classification and used for the different assays. Correlation coefficients found for the 3 grass species were significant (P < 0.0001; Spearman), but not for olive pollen (P = 0.14; Spearman). Proteomic analysis revealed the presence of more than 40 common proteins in grasses and olive pollens, but inhibition assays demonstrated no allergenic cross-reactivity between both families.

Conclusions: There is no in vitro crossreactivity between O. europea and Grass pollen extracts, in spite of the allergens and the large number of common proteins shared by these pollens. We can conclude that sensitization to olive and grasses is species specific.

21 Molecular Properties and Immunological Reactivity of Arabidopsis EXPB1, a Nonallergic Homologue of Grass Group 1 Allergens
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Background: Grass group 1 allergens are glyco-proteins of about 30kDa that are highly soluble and profusely released by grass pollen upon hydration. They bind to IgE antibodies that initiate the allergic response causing hay fever, seasonal asthma, and related immune responses in humans. Bermuda grass (Cynodon dactylon; subfamily Chloridoideae) is an important source of seasonal aeroallergens in warm tropical and sub-tropical areas worldwide. Improved approaches to diagnosis and therapy of allergic diseases require a thorough understanding of the structure and epitopes on the allergen molecule that are crucial for the antigen-antibody interaction. In order to understand structural basis of IgE reactivity of group 1 allergen Cyn d1, we have pursued a comparative genomic approach to search for hypoallergenic or non-allergenic homologues.