immunotherapy using pollen extracts derived from one or several different 
Pooideae species. While for several species the most important allergens 
(group 1 and group 5) have been identified, other allergens have either not 
been identified or sequence data are still missing. We have used mass spec-
trometry (MS) together with genetic and immunological methods to identify 
allergens in various grass pollen extracts.

Methods: Pollen extracts of 6 different grass species (Phleum pratense, 
Holcus lanatus, Lolium perenne, Dactylus glomerata, Festuca pratensis, 
Poa pratensis) and a mixture thereof were analyzed. For identification of 
allergens by MS, extracts were subjected to enzymatic digestion. Result-
ing peptides were separated by liquid chromatography and analyzed by 
tandem mass spectrometry. Protein identification was performed by 
searching both the NCBIPlant release and an individually designed data-
base. The presence of individual allergens was confirmed with allergen-
specific monoclonal antibodies. Unknown sequences were determined fol-
lowing cDNA synthesis from pollen RNA and allergen sequence amplifi-
cation by PCR.

Results: Fes p 1 and Fes p 5 were identified by the PCR approach. MS 
analysis of pollen extracts from the 6 individual species resulted in detection 
of all known allergens including the newly identified Fes p 1 and Fes p 5. 
Based on the homology of allergens from different grass species, previously 
unknown sequences of representatives of groups 2, 3, 4, 7, 11, 12 and 13 
were detected by MS in investigated extracts with high sequence coverage. 
Group 6 allergens could not be identified in some of the analyzed extracts. 
These findings are supported by immunological analyses and thus demon-
strate the specificity of the applied method. Members of all allergen groups 
were identified in an extract mix prepared from pollen of all 6 grass species 
studied.

Conclusions: The most important grass allergens (group 1 and group 5) were 
detected in all extracts. In addition all other known allergens of the assayed 
species and homologues thereof could also be identified, thus demonstrat-
ing the quality of the tested extracts.

13 Gene Expression Pattern of Arabidopsis EXPB1, a Nonallergenic 
Homologue of Grass Group 1 Pollen Allergens
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Background: Grass pollen allergy is one of the most common allergies 
worldwide. Group 1 allergens constitute the major allergenic component of 
grass pollen with more than 85% of grass pollen allergic patients showing 
IgE reactivity. These are highly immunologically cross-reactive glycoproteins 
especially expressed in pollen of all grasses. Alignments of the amino-acid 
sequences of grass group I allergens derived from diverse grass species reveal 
up to 95% homology. It is therefore likely that these molecules share a similar 
biological function.

Methods: RT-PCR analysis, In situ hybridisation, Promoter-GFP construct 
design, plant transformation and analysis of transgenic plants.

Results: Sequence comparison has identified a homologue (B-expansin 
clone At2g20750 or EXPB1) in Arabidopsis of the Cyn d 1 gene. The 
EXPB1 protein is 42% similar to the Cyn d 1 protein. This gene represents 
a member of a small multigene family in Arabidopsis. RT-PCR analysis 
showed expression only in floral not vegetative tissues. In situ hybridisation 
using 150 bp region of the 3 ’UTR of the Arabidopsis gene as probe showed 
specific expression in mature Arabidopsis pollen. We further cloned the 
promoter region for the Arabidopsis EXPB1 and prepared and GFP fusion 
constructs. These constructs were then introduced in to Arabidopsis plants 
by floral dip method. GFP-promoter fusions showed high level of expres-
sion in tri-cellular pollen.

Conclusions: Our study provides evidence that EXPB1, a non-allergic 
homologue of grass group 1 pollen allergens, gene is expressed in mature 
pollen.

14 Comprehensive Expression of Recombinant House Dust Mite 
Allergens for Component-Resolved Diagnosis
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shima, Japan; 4Takanohashi Central Hospital, Hiroshima, Japan.

Background: Allergen-specific immunotherapy (SIT) is the only promising 
treatment of allergy. However, current SIT has limitations such as a need for 
long-term medication and a risk of systemic anaphylaxis. Those issues are 
raised mainly because current SIT procedure is carried out using crude 
allergen extract, which may also induce a harmful neo-sensitization. Use of 
defined recombinant allergens would be a preferable alternative for the next 
generation SIT vaccine as well as for the development of component-resolved 
diagnosis (CRD), which enables to prescribe a patient-tailored vaccine.

Objective of this study is to construct a production system of recombinant 
dust mite (Dermatophagoides farinae) allergens, and to test their 
usefulness for molecular diagnosis.

Methods: Thus far, the WHO/IUIS allergen nomenclature subcommittee has 
approved 24 Dermatophagoides allergens. Among them, we sought to 
express 20 groups of D. farinae allergens (Der f 1, 2, 3, 4, 5, 6, 7, 8, 9, 
10, 11, 13, 14, 15, 16, 17, 18, 20, 21, and 22) using the Escherichia coli 
cold shock expression system. We also tried to express additional new antigens 
[Mag133 (a highly-conserved UK114/YER057c/YjgF family member), 
DFA22 (a new group 2 family member), and DFA67 (porexidoxin)] that 
we originally identified as major allergens with high IgE-binding frequencies. 
IgE-binding ability of those recombinant allergens was assessed by western 
blot analysis. We also tested whether these allergens were applicable for the 
development of CRD.

Results: We confirmed successful expression of above D. farinae allergen 
molecules as soluble recombinant proteins. Western blot analysis revealed 
that these recombinant allergens retained IgE-binding capacity. We also 
found that house dust mite-allergic patients showed differential IgE-binding 
signatures against them, suggesting that our recombinant allergens are useful 
to determine sensitized allergen molecules in individual patients.

Conclusions: Here we carried out the comprehensive expression of 
recombinant D. farinae major allergens. The recombinant allergen repertoire 
offers an essential platform for the future molecular diagnostics of dust mite 
allergy.

15 A Bioinformatic Approach to Allergen Nomenclature Applied to 
Allergens From the Non-Biting Midge Chironomus thummi thummi
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sity of Nebraska - Lincoln, Lincoln, NE.

Background: Representatives of the family Chironomidae (non-biting 
midges; order Diptera) are found worldwide. Freeze-dried chironomid larvae, 
predominantly of the species Chironomus thummi thummi are frequently used 
as fish food and are an allergen source for fish keepers and persons employed 
in the manufacture of fish food. At present, 9 allergens of C. thummi thummi 
have been assigned an official designation by the WHO/IUIS allergen no-
omenclature sub-committee: Chi t 1 to 9. All of them are hemoglobins with
16 Pollen Allergens Differ From Nonallergenic 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), Pellitory (Parientaria judaica) and timothy grass (Phleum 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 Vitis 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 Pellitory 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 Pellitory allergens, and 87 and 89% for Pellitory 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 evolutionarily 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.

17 Sensitization to Betula verrucosa and RBET V1 in Spain

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Background: Sensitization to Betula verrucosa pollen is common in North Central Europe. In Spain, it is also common in patients from Galicia, a region located in the Northwest of Spain. Birch trees are abundant in this region and several birch species have been described. The main objectives were to determine the differences in the IgE reactivity to B. verrucosa and rBet v 1 between patients from Sweden and Spain, as well as the contribution of the major allergen Bet v 1 to the overall reactivity of B. verrucosa. Allergenicity was also compared using a serum pool derived from sera of North American (USA) patients.

Methods: IgE reactivity to B. verrucosa and rBet v 1 was measured in sera from 44 Spanish and 21 Swedish patients. rBet v 1 was produced as N-terminal His-tagged fusion protein and purified as originally described. All of them were sensitized to the pollen of this tree species and suffered from allergic rhinitis during the pollenation period. rBet v 1 contribution was determined by inhibition ELISA using 3 pools of sera: from Spanish, North American and Swedish patients. IgE binding pattern was evaluated by Western blots using the same pools of sera. Specific IgE binding was expressed in arbitrary units.

Results: Specific IgE to B. verrucosa was detected in 50 from the 65 sera analyzed (mean = 3.77 ± 3.71 Units). Specific IgE levels to rBet v 1 were 3.13 ± 3.32 Units. Immunoblot assays confirmed specific IgE binding to rBet v 1, and also to other allergens present in the extract. The Spanish pool presented reactivity to more allergens than the Swedish pool. ELISA Inhibition assays, performed with a native extract and rBet v 1, revealed a significant contribution (>80% inhibition) of rBet v 1 to the allergenicity of the extract, with no differences according to the origin of the sera.

Conclusions: Bet v 1 has a great importance in birch allergy in Galicia and Sweden. Nevertheless, there are differences in the IgE recognition pattern according to the rest of birch allergens. Major allergen from birch, Bet v 1, significantly contributes to the allergenicity of B. verrucosa in Galicia.

18 Proteomic and Immunological Characterization of Ragweed Allergens

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Background: The prevalence of sensitization to ragweed has risen in North America and across Europe. Although the precise lyase Amb a 1, the major allergen of ragweed, was identified as long ago as the 1960s, little is known about the allergenicity of the 5 Amb a 1 isoallergens and other allergens present in ragweed pollen. Ragweed extracts and purified Amb a 1 isoallergens have now been characterized for their allergenic potential to determine whether a single Amb a 1 isoallergen, several isoallergens or a combination with other allergens should be included in a recombinant SIT vaccine.