

2004

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AAAAI

## Scientific Contribution

Dear Specialist

We welcome you to San Francisco and the 60th Meeting of the American Academy of Allergy, Asthma & Immunology.

An impressive amount of original, scientific documentation will be presented and we have selected some of the abstracts which we hope will be of interest to you.

This booklet contains 8 abstracts within the field of allergy vaccination and diagnostics. The abstracts demonstrate the collaboration between allergy specialists and ALK-Abelló and illustrate our long-standing tradition of research cooperation with the international scientific community.

Please join us at ALK-Abelló stand no. 1816 for further interesting discussions.

We wish you an enjoyable and fruitful congress.

Yours sincerely  
**ALK-Abelló A/S**



Henrik Jacobi  
*Executive Vice President*  
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# Scientific Contribution

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# The blocking activity of birch pollen specific immunotherapy induced IgG<sub>4</sub> is not qualitatively superior to that of other IgG subclasses

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## Rationale

Allergen specific IgG antibodies induced by specific immunotherapy (SIT) interfere with the allergen IgE interaction and act as blocking antibodies *in vitro*. It has been hypothesised that IgG<sub>4</sub>, as opposed to other IgG subclasses, is particularly important in this function, which may play a role for the clinical efficacy of SIT. In this study, we compared the inhibitory capacity of IgG<sub>4</sub> alone versus non-IgG<sub>4</sub> IgG from SIT treated birch pollen allergic patients.

## Methods

IgE depleted serum samples from 14 SIT treated birch pollen allergic patients were separated into purified IgG<sub>4</sub> and IgG<sub>4</sub> depleted IgG. The allergen binding activities of IgG and the IgG mediated inhibition of allergen binding to autologous IgE were detected using <sup>125</sup>I labelled rBet v 1.2801, a recombinant variant of the major allergen of *Betula verrucosa* pollen. Binding avidities were determined by saturation binding analysis.

## Results

When the sera were depleted of IgG<sub>4</sub>, the ratio between inhibition and binding activities was found to be the same as in unseparated serum, indicating a simple relationship between inhibition and binding activities. In contrast, a significant, but less than twofold higher relative blocking activity was found in the purified IgG<sub>4</sub> fraction. There was no significant difference between the binding avidities (1/K<sub>d</sub>) measured in IgG<sub>4</sub> depleted IgG and purified IgG<sub>4</sub>.

## Conclusion

These results suggest that SIT induced specific IgG<sub>4</sub> contributes to the IgG mediated blocking of allergen binding to IgE in a simple quantitative manner and not by a particular intrinsic blocking activity.

# Alutard® SQ grass demonstrates clinical efficacy in subjects with seasonal allergic rhinoconjunctivitis in a large-scale, double-blind, placebo-controlled study of specific allergy vaccination (The AVANZ Study)

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## **Rationale**

Evaluation of the clinical efficacy and safety of Specific Allergy Vaccination (SAV) with Alutard® SQ grass in the doses 100,000 SQ-U and 10,000 SQ-U.

## **Methods**

Subjects aged 18 to 60 years with grass pollen induced allergic rhinoconjunctivitis not adequately controlled by symptomatic treatment were included. In all, 203 subjects were randomised to 100,000 SQ-U, 104 to 10,000 SQ-U and 103 to placebo. Updosing was performed over 8 weeks. Maintenance treatment was given every 6 weeks +/- 2. Subjects completed a daily diary card on symptoms and use of allergy medication. Allergic symptoms were assessed weekly by Visual Analogue Scale (VAS) and Quality of Life (QoL) was measured. Subjects were given free symptomatic medication during the study.

## **Results**

In the 100,000 SQ-U group, reductions were obtained in symptom and medication scores and VAS compared to placebo ( $p < 0.001$ ). The 10,000 SQ-U group obtained reduction in symptom score ( $p < 0.013$ ). Reduction in symptom/medication score over placebo was 41/48% in the 100,000 SQ-U group compared to 24/21% in the 10,000 SQ-U group (peak season). Statistically QoL was significantly better in subjects who received active treatment compared to placebo. Allergic reactions to injections were reported in all groups and most frequently in the 100,000 SQ-U group. Most systemic reactions were non-specific or mild (WHO position paper). Non-life threatening systemic reactions all responded to symptomatic allergy treatment and adrenaline was used on only one occasion. No anaphylactic reactions were reported.

## **Conclusion**

SAV with Alutard® SQ grass 100,000 SQ-U is clinically effective, superior to 10,000 SQ-U and well tolerated.

# Mapping of Der p 2 antibody binding epitopes by site directed mutagenesis

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## Methods

rDer p 2 was subjected to site directed mutagenesis at six selected surface positions (K6, K15, H30, E62, H74, K82) distributed over the entire molecular surface. rDer p 2 mutants containing one (N=6), two (N=2), three (N=1), four (N=1) and six (N=1) mutated amino acids were expressed in *Pichia pastoris* and analysed by IgE inhibition and monoclonal antibody (mAb) binding experiments.

## Results

The IgE inhibition experiments demonstrated that mutations in position K6, H30 and E62 interfered marginally with IgE binding whereas mutations at K15, H74 and K82 significantly reduced IgE binding. A cumulative effect was observed for mutations at H74 and K82, and the double mutant reduced IgE to a level comparable to that of the six-position mutant. Three out of four mAbs raised against nDer p 2 were affected by the mutations. The single point mutations at K15 and H74 abolished the interaction between two of the mAbs and rDer p 2 mutants. Concomitant binding analysis of the mAbs indicated that at least 2 distinct epitopes could be identified on nDer p 2.

## Conclusion

Site directed mutagenesis of rDer p 2 identified surface exposed amino acid residues, which participate in both IgE and mAb binding epitopes. Other surface exposed amino acid residues of Der p 2 seem to be located in areas not involved in the binding of the investigated antibodies.

# Quality control of non-standardised allergenic extracts: major allergen and IgE binding of birch, English plantain and olive pollen

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## **Rationale**

The objective of these studies is to apply quality control techniques used to determine the potency of standardised allergenic extracts to assess the quality of non-standardised weight/volume, PNU, and alum adsorbed extracts. An outcome of this objective is the determination of appropriate methods to manufacture consistent, well-characterised extracts.

## **Methods**

Non-standardised birch, English plantain and olive pollen extracts were tested. Multiple consecutive lots manufactured over 2-3 years were measured for major allergens Bet v 1, Pla l 1 and Ole e 1 using direct binding ELISAs obtained from ALK-Abelló, Madrid and validated in our laboratory. IgE binding ELISAs were performed using sera pools from at least 5 patients allergic to the allergen. Electrophoresis and immunoblotting were also performed.

## **Results**

Most extracts had good consistency by either IgE binding or major allergen, although some lots show a significant deviation from the average. English plantain and birch had a few lots that were much lower in major allergen than the average. Aqueous extracted olive pollen extracts showed consistency as did glycerin extracted pollens, however, a significant reduction in extractability of the major allergen with glycerin was observed. Some pollen source lots showed significant differences in Ole e 1. IgE binding ability was well correlated with Bet v 1 in B extracts. Major allergens were also measured in alum adsorbed extracts.

## **Conclusion**

These studies demonstrate the benefit of testing potency in non-standardised extracts and will provide the means to produce consistent products in the interim before FDA standardised products become available.

# Prevalence of sensitisation to mugwort pollen allergens and association with food allergy to peach

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## Rationale

The aim of this work was to assess *in vivo* the pattern of sensitisation to an array of *Artemisia vulgaris* pollen allergens in a Mediterranean population, and to study the possible association of sensitisation to Art v 3 and other relevant LTP allergens: Par j 1 and Pru p 3.

## Methods

24 mugwort pollen allergic patients were selected on the basis of positive SPT, specific IgE and positive nasal provocation tests. SPT was performed with pure natural pollen allergens Art v 1, Art v 3, Art v 60-kDa (no systematic name yet) and Par j 1, and with a peach extract with known Pru p 3 concentration. *In vitro* assays included measurement of specific IgE to pure allergens and ELISA inhibition among LTP allergens.

## Results

The three mugwort allergens elicited a positive SPT in 70-80% of patients. A significant correlation, both in wheal areas and IgE levels, was observed between the mugwort extract and each of the mugwort allergens. Fourteen patients had a positive SPT to peach extract, and a highly significant correlation between the skin responses to peach extract and Art v 3 was found. Art v 3 significantly inhibited the binding of IgE from three patients' sera to Pru p 3. Pru p 3 and Par j 1 could not inhibit the IgE binding to the other allergens.

## Conclusion

It has been shown that Art v 1, Art v 3 and Art v 60-kDa are major mugwort pollen allergens. Besides, Art v 3 seems to be the primary sensitising agent in some peach allergic patients.

# Specific IgE binding of rHev b 12 is restricted to fruit allergic patients

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## Rationale

Lipid transfer proteins (LTP) have been identified as relevant allergens but their role in latex allergy is unclear. This study aimed to produce a recombinant latex LTP (rHev b 12) to clarify its IgE binding properties.

## Methods

A recombinant generated Maltose Binding Protein (MBP) rHev b 12 fusion protein was used to study the IgE binding by the CAP method. 48 sera of atopic patients from Germany (N=34) and Spain (N=14) with fruit allergy were examined. 13 of these patients had an additional latex allergy, 12 were sensitised to latex.

## Results

rHev b 12 specific IgE was observed in four Spanish peach allergic patients (CAP values: 0.88-2.27 kU/L), whereby one had an additional latex sensitisation. Two sera from cherry allergic German patients with latex sensitisation displayed also Hev b 12 specific IgE antibodies (0.68 and 0.96 kU/L). All other sera and the MBP controls revealed negative results (CAP values: <0.35 kU/L).

## Conclusion

Latex LTP (rHev b 12) specific IgE binding in peach and cherry allergic patients seems to be a result of partially common IgE binding epitopes with the LTPs of peach (Pru p 3, 65%) and cherry (Pru av 3, 61%). Although rHev b 12 seems to have minor relevance as a latex allergen in Central Europe, its significance as a cross-reactive allergen in Mediterranean countries like Spain has to be kept in mind.

# Immunological characteristics of heterodimeric recombinant Fel d 1

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## **Rationale**

Exposure and sensitisation to domestic cat (*Felis domesticus*) is a significant risk factor for developing allergic rhinitis and asthma. Earlier studies have shown that majority of the cat allergic patients develop allergic responses against major cat allergen Fel d 1. Several attempts have been made to produce a recombinant (r) Fel d 1 as heterodimer. We have recently shown that baculovirus expression system is able to produce rFel d 1 that has compatible characteristics to nFel d 1.

## **Methods**

In the present study, an immunological characterisation of rFel d 1 in comparison to the purified nFel d 1 and cat allergen extract (IMP Fel d, ALK-Abelló) was performed by use of IgE inhibition, lymphocyte proliferation and basophile histamine release assay(s).

## **Results**

In inhibition assay against cat allergen extract, natural and rFel d 1 were able to inhibit 87% of the binding of cat allergen specific serum IgE antibodies. The basophile histamine release responses with rFel d 1 and nFel d 1 showed to be in line with the responses given by the cat allergen extract. Comparable T-cell responses towards natural and rFel d 1 were obtained in lymphocyte proliferation assay and when the response of Fel d 1 specific T-cell lines was investigated even though nFel d 1 seemed to be recognised more frequently.

## **Conclusion**

These results show that heterodimeric rFel d 1 elicits immunological responses that are comparable to nFel d 1. The present study demonstrates that recombinant allergen(s) with well-defined biochemical and immunological characteristics is feasible for development of therapeutic vaccines.

# Biochemical characterisation of recombinant Phl p 6 and comparison with natural Phl p 6 and recombinant Phl p 5

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## Rationale

House dust mites and grass pollen are the most important allergen sources worldwide. Here, we report the characterisation of recombinant Phl p 6, a major grass pollen allergen from *Pbleum pratense*, using standard biochemical methods.

## Methods

Recombinant Phl p 6 was expressed in *Pichia pastoris* and recombinant Phl p 5.0107 was expressed in *E. coli*. Both were purified to homogeneity and analysed by SDS-PAGE. rPhl p 6 and nPhl p 6 were analysed by immunoblotting and Rocket Immuno Electrophoresis (RIE). Melting temperatures of rPhl p 6, nPhl p 6 and rPhl p 5 were determined by Circular Dichroism (CD).

## Results

Recombinant Phl p 6 migrated in reduced silver stained SDS-PAGE and immunoblots as nPhl p 6. When analysed by RIE rPhl p 6 and nPhl p 6 formed precipitates of similar morphology. Furthermore, rPhl p 6, nPhl p 6 and rPhl p 5 gave rise to similar spectra (local max at 192 nm and local min at 208 and 222 nm) when analysed by CD. CD temperature denaturation analyses showed similar melting temperatures,  $58.5 \pm 0.5^\circ\text{C}$  (mean  $\pm 95\%$  CL) (rPhl p 6) and  $59.0 \pm 0.3^\circ\text{C}$  (nPhl p 6) and  $65.3 \pm 1.4^\circ\text{C}$  (rPhl p 5), respectively.

## Conclusion

An expression and purification procedure has been established enabling the production of recombinant Phl p 6 having biochemical characteristics and immunochemical activity corresponding to that of the natural allergen from grass pollen.

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