

Scientific contribution



Dear colleague

Largest study of allergy vaccination to date

Last summer more than 400 Britons with hay fever participated in the largest clinical study of allergy vaccination to date.

The study documents that treatment with Alutard® SQ significantly reduces allergy symptoms and the use of symptom-relieving medication.

In addition, we know from the PAT study that allergy vaccination with Alutard® SQ reduces the risk of allergic hay fever developing into asthma.

Both studies strongly support the WHO recommendation of allergy vaccination as a cornerstone in the global approach to allergy treatment. Hence, we have chosen to sum up the effects of allergy vaccination in the words:

**Allergy vaccination:
Curing allergy – preventing asthma**

At our symposium, the details of the remarkable British study will be presented by the main investigator, Anthony J. Frew, and we will discuss the benefits of allergy vaccination further.

Review on sublingual immunotherapy

The other most recent development within allergy treatment is sublingual immunotherapy. During the last decade, the clinical efficacy of this treatment has been increasingly substantiated, not least thanks to the efforts of ALK-Abelló.

At our symposium, Christof Ebner will look into the future of sublingual immunotherapy with focus on immunological mechanisms.

Finally, the Henning Løwenstein Research Award 2003 will be presented to an appointed young scientist who has shown excellence within the field of allergy.

This booklet contains 28 abstracts within the field of allergy vaccination and diagnostics. These abstracts demonstrate the collaboration between allergy specialists and ALK-Abelló and illustrate our long-standing tradition of research in cooperation with the international community.

If you wish to comment on or further discuss the enclosed abstracts, please visit us at the ALK-Abelló stand no. 10.

We look forward to meeting you at EAACI 2003.

Yours sincerely
ALK-Abelló A/S

The ALK-Abelló company sponsored symposium

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Allergy vaccination: Curing allergy – preventing asthma

The UK initiative – documenting allergy vaccination

AJ Frew

Southampton General Hospital, Southampton, United Kingdom

From 26 medical centres in the UK, 410 subjects with seasonal allergic rhinoconjunctivitis with confirmed sensitivity to *Phleum pratense* were enrolled into a placebo-controlled, randomised study on the effect and safety of specific immunotherapy. 203 subjects were randomised to receive the recommended 100,000 SQ-U maintenance dose, 104 subjects to a group receiving 10,000 SQ-U and 103 subjects to a placebo group.

Allergen vaccine was administered as 15/16 injections of gradually increasing doses of allergen, over a period of 7-8 weeks. The maintenance dose was administered every 6±2 weeks. The primary efficacy end points were symptom and medication scores. The secondary efficacy end points were subject evaluation of treatment, rhinoconjunctivitis Quality of Life (QoL) questionnaire and visual analogue scale (VAS) for allergic symptoms.

Safety data were collected and analysed separately as local or systemic reactions versus all other adverse events. Adverse events were recorded in accordance with current ICH guidelines. Local reactions were recorded, and systemic reactions were classified according to World Health Organisation (WHO) guidelines (WHO 1998).

During the whole grass pollen season, symptom, medication and combined scores were significantly lowest in the 100,000 SQ-U group (compared to placebo $p<0.001$). The symptom scores in the 10,000 SQ-U group were lower than in the placebo group ($p=0.013$) whereas the difference in medication use and combined symptom and medication use was not statistically significantly different between 10,000 SQ-U and placebo.

In the 100,000 SQ-U group, the median of reduction in symptom and medication was largest during the peak season; 41% and 48% reduction, respectively. For the 10,000 SQ-U group, the reductions were 24% and 21%, respectively. In the 10,000 SQ-U group, the reduction was largest in the whole season; 26% and 32% reduction, respectively. In the 100,000 SQ-U group during the whole season, the reductions were 34% and 54%, respectively.

During the grass pollen season, the 100,000 SQ-U dose was associated with a better quality of life compared to both the 10,000 SQ-U group and placebo ($p<0.001$). The 100,000 SQ-U dose was also associated with the lowest VAS for allergic symptoms score compared to placebo ($p=0.001$). Alutard grass pollen 100,000 SQ was well tolerated and no life-threatening systemic reactions were reported. Local and systemic reactions were reported in all treatment groups,

including placebo, although most reactions were seen in the 100,000 SQ-U group. When required, reactions responded well to symptomatic treatment.

SIT with Alutard SQ grass pollen 100,000 SQ-U was clinically effective and superior to 10,000 SQ-U and placebo. SIT was well tolerated in patients with rhinoconjunctivitis not responding adequately to symptomatic drugs.

Curing allergy and preventing asthma

GB Pajno

Department of Paediatrics, University of Messina, Messina, Italy

Asthma is not a single, well defined disease entity, but consists of various different subtypes. Once asthma has developed, there is a considerable variation in the course of the disease. It is possible for children who develop asthma early in life to experience a remission of their symptoms as they grow older.

However, this may not indicate a complete regression of the inflammatory processes but only an improvement in lung function as a result of lung growth, and children who experience remission may still be at risk of an asthma relapse when they reach adolescence or adulthood. Several prospective studies in the past years have helped to clarify the development of allergen sensitisation in early childhood and its relationship to asthma development. The cornerstone of the treatment of childhood asthma is inhaled steroids (ICS). It has been proposed that early intervention with ICS therapy can be effective in preventing disease progression and the risk for irreversible changes in the airways; there is no doubt that intervention with ICS offers the best opportunity to improve asthma control.

Today SIT “does not take a position as being the last choice of treatment but represents a supplement to drug treatment used in the early phase of the disease”.

Based on clear evidence, SIT has long-lasting clinical efficacy and reduces the probability of developing new sensitivities in children who are allergic only to house dust mites; moreover it can prevent the development of asthma in children with allergic rhinoconjunctivitis caused by birch, timothy, or both.

Nowadays it is essential to select the patients who benefit from this kind of treatment, and it is essential to begin SIT when asthma is not severe and when the patients have only one or few sensitisations.

Despite some unresolved problems concerning SIT, it has been clearly demonstrated that this treatment can be successful, provided that: a) it is initiated as early as possible; b) patients are carefully selected; c) SIT is correctly associated with pharmacological therapies.

Immunological mechanisms and future perspectives of sublingual immunotherapy

C Ebner

University Hospital of Vienna, Vienna, Austria

1. The immunological concept of “oral tolerance”
2. Immunological changes in the course of SLIT and interpretation of these data
3. Comparison with the mechanisms known to be operative in SIT
4. Speculation about the future of SLIT
 - SLIT with more than two extracts for polysensitised patients?
 - Combination of SLIT and SIT?
 - SLIT as treatment of the oral allergy syndrome in birch pollinosis?

Best practices of allergy vaccination and sublingual immunotherapy

L Jacobsen

ALK-Abelló A/S, Research, Hørsholm, Denmark

Subcutaneous allergen specific immunotherapy – Specific Allergy Vaccination (SAV) – has been used for many years. After characterised and standardised allergen extracts were introduced in the 1980s, a lot of carefully performed clinical and immunological research has given a much better understanding of the clinical outcome and immunological mechanisms of the effect. Subcutaneous immunotherapy is regarded as the “golden standard” for immunotherapy although recent years have given much more information about alternative administration routes of allergen extracts. The knowledge about the immunological mechanisms of effect is based on long-term, high dose, subcutaneous administration of allergens and the preventive capacity of the treatment is based on the subcutaneous allergy vaccination concept.

The documented efficacy of allergy vaccination can be described in four levels:

Early effect

- immediate reduction in symptoms/need for medication

Continued effect

- further reduction in symptoms/need for medication during treatment
- reduction in hyperresponsiveness/late phase response

Long-term effect

- persistently reduced symptoms/need for medication after termination of treatment
- persistently reduced hyperresponsiveness/late phase response

Preventive effect

- prevention of new sensitivities and exacerbation of disease (rhinitis into asthma).

The clinical effect of sublingual immunotherapy (SLIT) has been documented with regard to the early effect. A few long-term treatment studies are available, showing that SLIT results in reduced symptoms and need for medication as long as treatment is continued. Recently, one study has indicated a potential long-term benefit in children with asthma. More follow-up studies are needed in order to establish precisely the long-term potential of SLIT and also investigations of potential preventive capacity should be initiated.

WHO has recently recommended in the ARIA report that the indications for SLIT used in high doses basically follow the indications from subcutaneous immunotherapy. It is obvious to consider SLIT as an alternative to subcutaneous immunotherapy yet only a few comparisons have been made. In general these studies show a clinical effect of both SLIT and SAV but the immunological mechanisms related to the effect seem to be different. No study on SLIT has reproduced the immunological effector mechanisms that have been carefully described in the literature in relation to SAV.

SLIT should be regarded as yet another tool together with allergen avoidance, medical treatment and SAV for the allergist in choosing the optimal treatment for the individual allergic patients. In the future comparisons with conventional medical treatment should be initiated as this would add important information to the potential of specific immunotherapy and facilitate the doctor in making the right choice for his patients.

Early immunological changes induced by sublingual and subcutaneous specific immunotherapy in children with allergic respiratory disease

C Antúñez², R Santos¹, JL Corzo¹, JA Cornejo-García², C Mayorga², MJ Torres², A Jurado¹, M Blanca³

¹Servicio Alergia Infantil, Hosp. Materno Infantil, Málaga, Spain, ²Laboratorio de Investigación Hosp. Carlos Haya, Málaga, Spain, ³Servicio de Alergia, Hosp. Carlos Haya, Málaga, Spain

Background

Immunotherapy has been shown to be a safe and effective treatment for both allergic rhinitis and asthma and is recommended by the World Health Organisation. Though injection immunotherapy (SCI) is a practical and effective means of reducing sensitivity to allergens, sublingual immunotherapy (SLI) has attracted considerable attention in recent years, but its efficacy and the basic mechanisms involved are not well known. We evaluated the early immunological changes induced by both SCI and SLI in children with allergic respiratory disease sensitised to *Dermatophagoides pteronyssinus*.

Methods

Two groups of children were evaluated in a pilot study: Group 1 (N=15) treated with SCI and Group 2 (N=13) treated with SLI (both from ALK-Abelló S.A.). The immunological evaluation was performed before and one month after starting immunotherapy. Total and specific IgE antibodies were determined by immunoassay, and the lymphocyte subset, cellular activation markers and intracellular cytokines by flow cytometry. Statistical comparisons were made between the basal and 1-month determinations in both groups.

Results

No changes in either total or specific IgE antibodies were found in either group. In Group 1 there was a significant increase in the CD4 subpopulation and monocytes (CD14) with a decrease of B-lymphocytes, whereas no changes in the lymphocyte subpopulation were observed in Group 2. In both groups there was a significant increase in activated CD4 cells and a decrease in activated CD8 cells. Although there was a decrease in both groups in the production of the cytokines studied (IFN- γ , TNF- α , IL-2, IL-4, IL-10 and IL-13) this was only significant for IL-2 and TNF- α in the CD4 subpopulation. Significant differences detected between the two types of immunotherapy were: SCI induced a decrease of IL-13 in CD4 and CD8 lymphocytes whereas SLI only induced a reduction of IL-4 in CD8 lymphocytes.

Conclusions

Both types of immunotherapy induce immunological changes, even immediately after administration of the treatment, with an increase in the activation lymphocytes. These changes at least at the times studied seem to be more important in SCI, although they can also be detected after SLI.

Allergy – Living & Learning: Immunotherapy in respiratory allergic diseases

T Chivato¹, *L Jacobsen*², *J de Monchy*³, *R Dahl*⁴, *E Valovirta*⁵, *P Andersen*²
¹*Hospital Aire, Department of Allergology, Madrid, Spain,* ²*ALK-Abelló A/S, Research, Hørsholm, Denmark,* ³*Academisch Ziekenhuis Groningen, Groningen, The Netherlands,* ⁴*University Hospital of Aarhus, Department of Respiratory Diseases and Allergology, Aarhus, Denmark,* ⁵*Turku, Allergy Centre, Turku, Finland*

Introduction

Allergy – Living & Learning (A-L&L) is a European initiative with the purpose of increasing the level of awareness, knowledge and understanding of people living with allergies in order to improve the general status of care available to allergy sufferers. The objectives were to identify the management of the respiratory allergic diseases and investigate the consequences of specific diagnosis and treatment.

Material and methods

The knowledge of allergic patients was examined in 10 countries. The A-L&L study consisted of three phases; a qualitative study, a pre-testing phase and a quantitative study (25 questions were included in the final version of the questionnaire covering several subjects: age, sex, symptoms, treatments, severity, quality of life, allergy tests, expenses, level of knowledge, etc.).

7,000 patients with asthma and hay fever were identified by random telephone screening from 10 European countries (Austria, Denmark, Finland, Germany, Italy, Netherlands, Norway, Spain, Sweden and the United Kingdom). The target population was aged between 16 and 60 years.

Results

About 20% of allergic patients do not receive treatment of any kind. About 80% of allergic patients use some form of medication: Alternative therapy (7%), depot medications (8%), allergy vaccines (16%), inhaler (28%), pills/tablets (50%), nasal spray/drops (46%) and eye drops (33%). About one-third of allergic patients are dissatisfied with their treatment.

Conclusions

An important number of allergic patients in 10 European countries are undertreated. The use of immunotherapy in respiratory allergic diseases (hay fever and bronchial asthma) is surprisingly low.

Sublingual immunotherapy with a latex extract (SLIT-LATEX): Tolerance and evolution of skin reactivity

A Cisteró-Bahima¹, J Sastre², E Enrique¹, S Quirce², MM San Miguel¹, M Fernández², R Alonso¹, B Gandarias³, S Martín³

¹Institut Univ. Dexeus, Barcelona, Spain, ²Fundación Jiménez Díaz, Madrid, Spain,

³ALK-Abelló S.A., Madrid, Spain

Background

The variety of products containing latex is considerable, making avoidance of exposure impossible. Specific immunotherapy (IT) with latex able to safely reach high concentrations in a short period of time may be the most promising therapeutic tool for the treatment of this increasing problem of sensitisation to latex.

Methods

A multicentre study was carried out to evaluate tolerance and evolution of skin symptoms after sublingual treatment with *Hevea brasiliensis* (500 µg/mL of total protein) SLIT-LATEX. The build-up phase followed a rush schedule and was administered at the hospital. A maximum dose of 500 µg was reached in four days. The maintenance phase consisted of 100 µg/day doses three times a week. Skin reactivity was evaluated by SPT, rubbing test and latex-glove use test, before the beginning of IT (T₀), after build-up treatment (T₁) and 10 weeks after the beginning of IT (T₂).

Results

1,044 doses were administered (366 build-up and 678 maintenance) to 26 patients (5 men/21 women), mean age 35±7.5 years. All of them had cutaneous symptomatology and in most cases (88.5%) also respiratory symptoms. 11.5% of patients reported previous history of anaphylaxis.

All patients reached the maintenance dose of 500 µg. 20.1% of the doses occasioned immediate local discomfort and 3.3% delayed gastrointestinal distress. None required treatment. There was a 3.6% systemic reactions (SR) per dose, being 2.1% immediate SR and a 1.5% delayed. 55.3% of SR received treatment, consisting of antihistamines (26.3%), β₂-agonists alone (5.3%) or associated to corticosteroids (18.4%). Adrenaline was administered precautionary in one patient who had immediate dyspnea twice.

At T₁, skin reactivity decreased in use test (p=0.003) and also in rubbing test, although not significantly (p=0.07). At T₂, both use and rubbing test decreased significantly (p=0.0004 and p=0.004, respectively). On the contrary, no change was detected by the parallel line assay for SPT at T₁ or T₂ as compared to T₀.

Conclusions

In conclusion, sublingual administration of high doses of SLIT-LATEX on a rush schedule under clinical observation is an efficient treatment able to decrease the skin reactivity to latex in just 4 days.

This is a particularly suitable schedule for patients who find it difficult to interrupt their work shift.

Double-blind, placebo-controlled study of immunotherapy with *Parietaria judaica* in mass units: Clinical efficacy

M Ferrer¹, A Peláez¹, D Hernández², R Alamar², A Cisteró-Bahima³, E Enrique³, S Martín⁴, B Gandarias⁴

¹Hospital Clínico, València, Spain, ²Hospital La Fe, València, Spain, ³Institut Univ. Dexeus, Barcelona, Spain, ⁴ALK-Abelló S.A., Madrid, Spain

Background

Parietaria judaica pollen has a high prevalence in the Mediterranean area, and its capacity to produce rhinitis and/or asthma as well as its long pollination period is largely documented.

Method

We carried out a multicentre study to evaluate clinical efficacy and tolerance of a *P. judaica* extract 25 BU/mL (1,5 µg/mL Par j 1), Pangramin® Depot UM.

Results

57 patients monosensitised to *P. judaica* with rhinitis and/or asthma were recruited. Immunotherapy (IT) was started in 52 patients, and 42 followed the treatment for 20 months (22 patients with active treatment, active group (AG) and 20 patients with placebo, placebo group (PG)). There were 13 drop-outs (5 AG and 8 PG) and 2 withdrawals (1 AG and 1 PG). The reasons were in none of the cases related to IT.

Patients completed diary cards for registration of symptoms and medication intake during 8 weeks. Cards were issued at three different times (T₉₉ during the pollination season before the beginning of IT (PS-1999), T₀₀ one year after PS-2000 and T₀₁ PS-2001). Pollen counts corresponding to all periods were available.

A statistically significant decrease was observed in the allergic symptomatology in AG (p<0.01 T₀₀ and p<0.05 T₀₁) and not in PG. There was also a significant difference between both groups (p=0.01). Differences in the medication intake were also found (p=0.03). Similar results were observed in AG related to symptoms + medication (p<0.01 T₀₀ and p<0.05 T₀₁), and statistically different to PG (p=0.0007).

If we study “healthy days”, meaning days without symptoms and without any intake of anti-allergic medication, there was a significant increase only in AG (p<0.001 T₀₀ and p<0.01 T₀₁). There was also a significant difference of “healthy days” between both IT groups (p<0.01).

Conclusion

In conclusion, the extract of *P. judaica* 25 BU/mL, Pangramin® Depot UM, is able to reduce symptoms and medication intake and significantly increase the number of healthy days in AG, which was not observed in PG.

3-D modelling and IgE-epitope mapping of the major peach allergen Pru p 3

G García-Casado¹, LF Pacios², A Diaz-Perales¹, R Sánchez-Monge¹, M Lombardero³, FJ García Sellés⁴, F Polo³, D Barber³, G Salcedo¹
^{1,2}Departamento de Biotecnología, ¹E.T.S. Ingenieros Agrónomos and ²E.T.S. Ingenieros de Montes, Universidad Politécnica, Madrid, Spain, ³Departamento I+D, ALK-Abelló S.A., Madrid, Spain, ⁴Servicio de Alergia, Hospital Virgen de la Arrixaca, Murcia, Spain

Background

Lipid-transfer proteins (LTPs) are relevant plant panallergens that are present both in foods and pollens. Pru p 3, a major peach allergen in the Mediterranean area, is among the best characterised members of the LTP protein family. Its value as a diagnostic tool for *Rosacea* fruit allergy has been demonstrated both *in vivo* and *in vitro*.

Methods

A pool, as well as individual sera, from patients with peach allergy and positive skin prick test to Pru p 3 was used. 3-D modelling was achieved by using five experimentally available structures of Pru p 3 homologues (>50% sequence identity) from rice and corn as templates. Theoretical prediction of potential IgE binding regions was performed by selecting specific residues on the molecular surface displaying prominent electrostatic potential features. Synthetic peptides (10 mers; 5 amino acids overlapping) covering the full Pru p 3 sequence were used to detect IgE epitopes by ¹²⁵I-anti-IgE immunodetection.

Results

Pru p 3 showed a 3-D structure comprising four α -helix and a non-structured C-terminal coil (residues 76-91). Amino acid regions around positions 23-36, 39-44 and 80-91 were predicted as potential epitopes according to their relevant surface and electrostatic properties (putative conformational epitopes). IgE immunodetection of synthetic peptides allowed to identify regions 11-20, 31-40 and 71-80 as major sequential epitopes of Pru p 3.

Conclusions

Main conformational and sequential amino acid regions of Pru p 3 potentially involved in IgE-binding were located. These data can help to search for hypoallergenic forms of Pru p 3 recombinants.

Rhinitis due to sensitisation to *Casuarina* pollen. Identification of allergens

JJ García-González¹, M Lombardero², M Barceló¹, S Fernández¹, MA Negro¹, MJ Carmona¹, JM Vega-Chicote¹, A Miranda¹, M Muñoz¹, E Reina¹, D Barber²
¹Hospital Regional Universitario Carlos Haya, Málaga, Spain, ²R&D Department, ALK-Abelló S.A., Madrid, Spain

Introduction

The genus *Casuarina*, commonly known as Australian pine, belongs to the family *Casuarinaceae*. Most of the 60 known species are native in Australia, although some are found in Southeast Asia and in the southwestern region of the Pacific. In Spain, the most common species are *C. cunninghamiana*, *C. stricta* and *C. equisetifolia*. The pollen grain is of medium size and is triporate, isopolar and radially symmetric. In our area, it pollinates in the months of October and November. In 1997 (JJ García-González *et al.* Allergy 1997;52:11-7), we conducted an aerobiologic and preliminary clinical study, and new clinical data as well as the identification of *Casuarina* allergens are now included.

Material and methods

The clinical study was carried out in 500 consecutive subjects between 14 and 58 years of age, who were sent to our service after being suspected of having atopic rhinitis. We performed skin prick tests with common aeroallergens (ALK-Abelló S.A.) and *C. cunninghamiana* and *C. equisetifolia* pollen extracts. The nasal provocation tests were performed using a Rhinospir 164 device with an extract of *C. cunninghamiana*. *Casuarina*-specific serum IgE determinations were done by the RAST technique. IgE immunoblotting with patients' serum was performed after SDS-PAGE of *Casuarina* pollen extract (15% polyacrylamide gel and non-reducing conditions).

Results

Fourteen subjects (2.8%) were found to have positive skin tests to both species and no differences were detected. Seven patients had positive rhinomanometry and *Casuarina*-specific serum IgE (>0.35 kU/L). IgE immunoblotting with individual sera revealed the presence of different IgE-binding bands with an apparent molecular weight of approximately 10-12 kDa (detected by 4 out of 7 patients), 14 kDa (3/7), 17 kDa (3/7), 20 kDa (2/7) and 50 kDa (5/7). Preincubation of patient serum with *Lolium perenne* pollen extract before the *Casuarina* IgE immunodetection led to the disappearance of the 14 kDa and 17 kDa bands in the blotting.

Conclusions

We have clearly established the presence of IgE sensitisation to *Casuarina* pollen in 7 patients (positive skin and nasal provocation tests and serum specific IgE). No relevant differences have been found between the two *Casuarina* species. The *Casuarina* allergen profile was determined and 2 protein bands (10-12 kDa and 50 kDa) may be considered as major allergens. The 14 and 17 kDa bands cross-react with *L. perenne* and probably correspond to profilin.

Tolerance of sublingual immunotherapy of *Lolium perenne* administered at high doses

F Gozalo Reques¹, I Ojeda², JL Estrada¹, P Ojeda², MJ Alvarez¹, S Martín³, P Rico³

¹Hospital de León, León, Spain, ²Clínica de Asma y Alergia, Madrid, Spain,

³ALK-Abelló S.A., Madrid, Spain

Background

We designed a multicentre prospective study to assess tolerance of high sublingual doses of a biological standardised *Lolium perenne* extract.

Methods

Sublingual swallow immunotherapy (SLIT) was co-seasonally administered (January to June) in 70 patients with rhinitis associated with mild asthma due to grasses. 3 different starting doses were studied: 0.04, 0.2 and 1 µg of Lol p 5. In all cases the achieved maintenance dose was 8 µg of Lol p 5. The extract was administered from monodose vials not containing phenol that were discarded after a single administration. Build-up phase was taken daily (Monday to Friday) and always under allergist supervision. Maintenance was administered 3 times a week (Monday, Wednesday and Friday).

Results

70 patients with a mean age of 28.4 ± 9.8 years were included, 50% were males. 48.6% also suffered from asthma and 10% presented Oral Allergic Syndrome. 60% of the patients were polysensitised mostly to *Cupressaceae* or *Plantago*.

Throughout the 6 months of treatment, a total of 6,059 doses were administered; 1,816 during build-up phase and 4,243 at maintenance. All patients but one (98.6%) reached the established maximum dose. For that patient only, the dose had to be reduced to 2 µg of Lol p 5.

In 473 doses symptoms appeared after SLIT administration (7.8%): 6.1% local reactions (LR), 1.4% systemic reactions (SR) and 0.26% both LR + SR. 72% of the SR occurred during the build-up. No anaphylaxis occurred. No treatment was required for 98% of the LR and for 72% of the SR. Regarding LR, the only administered treatment was antihistamines. For SR, antihistamines were used in 9 cases (8.6% of the SR), inhaled beta agonist in 12.5% and inhaled corticosteroids in 2.9%. No epinephrine was needed.

No statistically significant differences were observed in tolerance between the different starting doses. Higher frequencies of reactions were related to higher concentrations studied, reaching more than 6% of SR with doses higher than 2 µg of Lol p 5. The nature of the side effects was mainly orolabial itching followed by gastrointestinal manifestations, naso-ocular and pharyngeal symptoms.

Conclusions

These results show that SLIT at high doses of *L. perenne* is safe and well tolerated. It seems that a significant increase in the starting dose is not associated with an increase in side effects. However, due to the increase of reactions observed with doses higher than 2 µg of Lol p 5, we do not recommend to exceed this quantity of allergen.

Characterisation of antibody (murine) binding epitopes on various house dust mite group 2 allergens

H Henmar¹, H Ipsen¹, J Skovsgaard¹, C Bolwig¹, F Polo², D Barber², O Duffort²
¹ALK-Abelló A/S, Research, Hørsholm, Denmark, ²ALK-Abelló S.A., Madrid, Spain

On a world-wide basis, the allergens derived from house dust mites are probably the most ubiquitous and there is a strong evidence that exposure to mite allergens is a highly significant risk factor for developing allergy. There are at least 11 different allergens in the mite extract. The most important are group 1 and group 2 allergens. Group 1 and 2 allergens are present in all house dust mite species studied. Der p 2 has an extensively homology with Der f 2.

Structurally, group 2 allergens from house dust mite (HDM) are very similar and it has been shown that patients IgE cross-react within the various species, indicating common epitope pattern(s).

Murine monoclonal antibodies (mAb) were raised towards natural Der p 2 in Balb/c mice. 22 clones were selected and tested in the Biacore system for reactions towards the various Der p, Der f, Eur m recombinant and/or natural homologous HDM group 2 allergens. The mAb were evaluated by the kinetics of the interaction between antibody and allergen and by epitope mapping in concomitant binding.

Twelve mAb were able to bind nDer p 2 and nDer f 2 in the Biacore system. The 12 mAb could be grouped according to their off rate constant; slow = a and intermediate = b. Some of the b antibodies in complex with rDer p 2 wt dissociate faster than the complexes formed with nDer p 2. Three of the 12 monoclonal antibodies additionally recognise epitopes on Eur m 2 allergen, indicating that the antibodies investigated react with at least 2 different epitopes on nDer p 2. Concomitant binding analysis performed with the 4 highest affinity antibodies indicates that at least 2 distinct epitopes exist on nDer p 2. However, combining kinetic and concomitant data indicates that the four highest affinity antibodies define at least 3 distinct epitopes.

In conclusion, 12 mAb raised towards nDer p 2 cross-react extensively with nDer f 2 but to a much less degree with Eur m 2, and at least 3 distinct epitopes must be assumed in order to explain the kinetic analysis.

Comparison of dog allergens in commercial and self-prepared extracts with special attention to different breeds

A Heutelbeck, T Ahrens, E Hallier, TG Schulz
Occupational Health, Georg August University, Göttingen, Germany

Background

Dogs are relevant sources of allergens in the environment. This is also reflected in the number of patients with known allergy to dogs described to be approximately 16%. In some cases the commercial diagnostic tests show only slightly positive or even negative results despite clearly dog-related symptoms. Relevant results can be produced only with extracts of hair of their own dogs.

Methods

We compared the protein pattern of extracts from different commercial and self-made dog allergen extracts by SDS-gel electrophoresis. We used the raw material of four commercial dog allergen extracts (Allergopharma, ALK-Abelló, Bencard, HAL) and hairs of the breeds of dogs which are quantitatively relevant in Germany. Protein extracts were separated using SDS-PAGE (15% gel) and stained with Coomassie and Silver. Molecular weights (MW) were estimated by comparison to commercially available MW standard mixtures.

Results

In all commercial extracts, distinct protein fractions were observed at 18, 25 and 69 kDa and a weaker one at 43 kDa, as described in other investigations. Most of the commercial extracts showed additionally strong bands with MW >14 kDa. The majority of the bands in the self-prepared extracts of different breeds displayed a MW lower than 30 kDa. Especially in the extracts of hairs of breeds such as Yorkshire terrier, cocker spaniel and poodle, we found protein bands in the MW range lower than 14 kDa, which are missing in the commercial extracts.

Conclusion

All four commercial extracts investigated showed only minor differences in protein pattern. Can f 1 appeared in all commercial and self-prepared extracts, but in some extracts only very weakly. We newly described protein bands with a MW <14 kDa with a lack in commercial extracts. Further investigations are in preparation to elucidate the relevance of these particular proteins for sensitised patients. If the results with commercial dog allergen extracts are inconsistent, tests with hairs of own dogs should be performed.

Epitope grafting: The building of a conformational Bet v 1 epitope on Mal d 1

J Holm, M Ferreras, L Hansen, J Haugel-Nielsen, H Ipsen, K Lund, MD Spangfort, JN Larsen
ALK-Abelló A/S, Research, Hørsholm, Denmark

The binding energy in antibody-antigen complexes is a result of a combination of attractive forces and entropy increase, caused by the almost complete expulsion of water molecules from the interface. In other words, antibody binding to protein antigens relies on a perfect fit in the topographies of the interacting molecular surfaces. We have previously described the crystal structure of the complex between Bet v 1 and the Fab fragment of the murine monoclonal antibody, BV16. Here we show that the BV16 epitope can be built on a homologous molecule, Mal d 1, by site directed mutagenesis.

The BV16 antibody was raised by immunising mice with purified Bet v 1 and does not cross-react with the homologous major allergen from apple, Mal d 1. This can be explained by 5 amino acids differing between Bet v 1 and Mal d 1 in the epitope. These 5 amino acid residues were substituted in order to build the BV16 epitope on Mal d 1. The substitutions had a dramatic effect on the binding of birch allergic patients' IgE, which increased from 0% to 40% as compared to Bet v 1. Histamine release from human basophils increased from 0% to 100% relative to Bet v 1, although at a 4 times higher concentration. Interestingly, Biacore experiments showed that the K_d-values for the two complexes were identical ($(2.4 \pm 1.4) \times 10^{-10}$ M and $(2.7 \pm 0.4) \times 10^{-10}$ M) suggesting that the exact topography of the BV16 epitope has been "grafted" to a homologous molecular "scaffold".

In conclusion, engineering of allergenic epitopes by structural and molecular biology may represent a useful strategy for the development of safer and more efficient vaccines for allergy vaccination.

Meta-analysis of allergen specific immunotherapy in children based on available peer-reviewed publications

L Jacobsen¹, E Alvarez-Cuesta², E Valovirta³

¹ALK-Abelló A/S, Research, Hørsholm, Denmark, ²Dept. of Aller. Clin. Immunol., Madrid University Hospital, Madrid, Spain, ³Turku Allergy Centre, Ped., Turku, Finland

As part of an overall review of immunotherapy studies, we performed a meta-analysis on efficacy outcome parameters from published studies in which we were able to identify absolute data on the number of patients that improved as a result of treatment. Studies were selected for analysis if they were randomised and controlled and the allergen extract used could be identified. A total number of 10 studies fulfilled these criteria. Calculations of odds-ratio for improvement were performed by use of Mantel-Haenscel statistics calculating the relative "risk" of improvement.

Eight studies had included symptoms of asthma as end point for efficacy measure. The common odds-ratio for improvement of asthma symptoms in these studies were 11.9 (7.7-17.9). Five of these asthma immunotherapy studies had included allergen bronchial provocation test as an absolute measure of improved bronchial tolerance to allergen. The odds-ratio for successful reduction in bronchial sensitivity to allergen was estimated to 9.1 (4.5-18.3).

Only few studies focusing on rhinoconjunctivitis as end point for efficacy fulfilled the above criteria. Two studies report the number of patients that improve as a result of treatment. In these studies the odds-ratio for improvement was 12.4 (4.3-35.8). From two studies we were able to calculate the odds-ratio for reduced conjunctival sensitivity measured by conjunctival provocation test to 3.6 (1.1-11.6).

Unfortunately, only a limited number of studies report detailed information about the relative number of children that improve as a result of immunotherapy. Based on the data available, we find a highly significant odds-ratio for improvement and conclude that allergen immunotherapy in children suffering from allergic rhinoconjunctivitis and/or asthma is highly effective.

Five-year follow-up on the PAT study. A 3-year course of specific immunotherapy (SIT) results in long-term prevention of asthma in children

L Jacobsen¹, C Möller², S Dreborg³, HA Ferdousi³, S Halcken⁴, A Høst⁵, LA Norberg⁶, A Koivikko⁶, E Valovirta⁶, B Niggemann⁷, U Wahn⁷
¹ALK-Abello A/S, Research, Hørsholm, Denmark, ²Umeå University, Ped., Umeå, Sweden, ³Linköping University, Ped., Linköping, Sweden, ⁴Sønderborg Hospital, Ped., Sønderborg, Denmark, ⁵Odense University, Ped., Odense, Denmark, ⁶Turku Allergy Centre, Ped., Turku, Finland, ⁷Charité University, Ped., Berlin, Germany

Rhinitis frequently precedes the onset of asthma. Efficacy of SIT for pollen allergy has been confirmed in several studies and long-term clinical effect up to 8 years after termination of 2-4 years' immunotherapy has been reported. Recently, it was shown that a 3-year course of SIT in children with hay fever caused by allergy to grass and/or birch pollen significantly reduced the risk for development of asthma during this treatment period (odds ratio 2.52 (1.3-5.1) in favour of SIT). In order to investigate the long-term prevention of SIT on the development of asthma in children with seasonal allergic rhinoconjunctivitis, we performed a 5-year follow-up (two years after termination of treatment) of these patients.

From 6 paediatric allergy centres, 208 children, 6-14 years of age (mean 10.7 years), with grass and/or birch pollen allergy but without any other clinically important allergy were recruited. All had moderate to severe hay fever symptoms but at inclusion none reported asthma with need of daily treatment. After the initial season, 205 children were stratified and randomised to receive either SIT for 3 years or to an open control group. The contents of major allergen per immunotherapy maintenance injection given every 6 weeks (\pm 2 weeks) corresponded to 20 μ g Phl p V (grass) and 12 μ g Bet v I (birch) (Alutard SQ 100,000 SQ units/ml). Both groups received symptomatic treatment limited to oral loratadine, topical levocabastine or sodium cromoglycate and, if not responding to these drugs, nasal budesonide. The development of asthma was monitored through clinical evaluation and post-seasonal visual analogue scale.

Improvement in hay fever and conjunctival provocation test results achieved during treatment persisted during the 2-year follow-up. During the 0-season before randomisation, 20% of the children had mild asthma symptoms during the pollen season(s). Among patients without asthma before immunotherapy, the SIT treated group had significantly less asthma (SIT: 15/75, control: 38/67) after 5 years as evaluated by clinical symptoms (odds-ratio 2.68 (1.3-5.7)) in favour of SIT for prevention of asthma (n=142). Significantly fewer patients with asthma reported an increase in asthma scores ($p < 0.01$).

Conclusion

SIT for 3 years with standardised allergen extracts in an optimal dose has shown a long-term and preventive effect on later development of asthma in children with only seasonal rhinoconjunctivitis.

Discordance analysis of dog dander specific IgE assay from two *in vitro* systems

N Johansen, U Brink-Andersen

ALK-Abelló A/S, In Vitro Diagnostic Business Unit, Stenløse, Denmark

Background

As part of the initial in-house validations, method comparison studies were conducted comparing Bayer ADVIA Centaur to Pharmacia CAP FEIA and Magic Lite SQ systems for dog dander (e5) specific IgE analysis. 115 patient samples collected from ALK-Abelló's sample bank were tested by all three methods. The clinical diagnoses of this population were unknown. A good correlation to the Pharmacia CAP method above class 2 was shown. However, 17% of the samples were found to be discordant, mostly in classes 1 and 2 in the CAP system and negative in the ADVIA Centaur assay.

The purpose of the study was to investigate the discordant sample population reactivity and the reason for the difference between ADVIA Centaur and CAP FEIA dog dander sIgE assays.

Methods

ADVIA Centaur (AC) and Pharmacia CAP FEIA (CAP) methods were used for sample analysis of sIgE concentration. CRIE and CAP inhibition sIgE assays were used for the characterisation of the allergen assay reagents and the sample allergen reactivity. CAP inhibition analyses were performed using sample inhibition with dog, cat and dust mite extracts – also major allergen protein (Fel d 1) was used. A pre-incubation of sample with the Pharmacia allergen CAP sponge (dog, cat or dust mite) was used to identify the different sample allergen reactivities.

Result

For the discordant samples, the sIgE assays showed higher concentrations for cat and dust mite specific IgE than for dog in both AC and CAP systems. The CRIE on concordant samples shows strong reactivity to dog major allergen. The CRIE on discordant samples shows strong reactivity to cat major allergen. The extract CAP inhibition assays showed an equal or stronger inhibition by cat extract, cat purified major allergen or dust mite allergen extract for discordant samples. Pre-incubation of the sample with the allergen CAP before assay in the CAP system also showed stronger inhibition by cat or dust mite CAP for discordant samples. For concordant samples the inhibition study showed stronger inhibition from dog extract in the dog IgE assay.

Conclusion

A fairly good concordance was found between the ADVIA Centaur and CAP FEIA dog dander sIgE assays. In the discordance analyses all immuno-chemical analyses indicate a cross reactivity and/or cross contamination in the CAP system, as also reported in the literature. The ADVIA Centaur dog dander sIgE assay is less sensitive to cross reactivity and cross contamination.

Diversification of the IgE binding in immunoblotting of commercial and self-prepared cow allergen extracts

C Junghans, A Heutelbeck, TG Schulz, E Hallier

Occupational Health, Georg August University, Göttingen, Germany

Background:

In some patients commercial test kits of cow allergen do not confirm the obviously cow related symptoms. The aim of this study was to investigate four different commercial cow allergen extracts from Allergopharma, ALK-Abelló, Bencard and HAL compared with self-prepared cow allergen extracts of different cattle breeds by immunoblotting. We used the sera of 38 German farmers with asthma and rhinoconjunctivitis caused by cattle. The cow related symptoms could be confirmed by specific IgE antibodies in the sera of 68% of the patients.

Method

The raw material of the commercial cow allergen extracts as well as hairs of the farmers' own cattle were extracted by a 24-hour incubation in a 0.1 M NH_4HCO_3 solution.

Results

The pattern of the immunoreactions with cow allergens differed in the sera of the various farmers. After immunoblotting we found distinct bands in all symptomatic farmers, despite the fact that twelve farmers had a negative RAST result. Bands with molecular weights in the range between <14 and about 67 kDa were observed; the reactivity with the major allergen bos d 2 at 20 kDa was detected in all farmers though not in every case as the strongest band. An association of the sensitisation pattern and the races of the cattle was not detectable. In contrast to our self-prepared extracts, some proteins of the same molecular weight did not show a distinct band in some of the commercial extracts.

Conclusion

The most striking result of our investigation lay in the altered capacities of proteins in some of the commercial extracts to bind with IgE antibodies. We speculate that some proteins had lost their ability to react with IgE antibodies. This may be caused by methods of production. Another reason might be the too low concentration of allergens in the commercial extracts. The lack of the allergen IgE binding capacity of some commercial cow allergen extracts may be a possible explanation for the differences between clinical symptoms and results obtained with commercial test in some patients.

Purification and characterisation of Cup s 1, major allergen of *Cupressus sempervirens* pollen

RI Monsalve¹, D Barber², RC Panzani³, M Villalba⁴, R Rodriguez⁴

¹ALK-Abelló S.A. & Complutense University, R&D, Departamento de Bioquímica y Biología Molecular I, Madrid, Spain, ²ALK-Abelló S.A., R&D, Madrid, Spain, ³Instituto Francés, Allergenos, Marseille, France, ⁴Universidad Complutense, Bioquímica y Biología Molecular I., Madrid, Spain

Background

Allergy to common cypress (*Cupressus sempervirens*) is a widespread pollinosis in Mediterranean areas. In spite of this its major allergens have not been isolated and characterised as has been the case with allergens of closely related species as *Cupressus arizonica* or *Juniperus spp.* This is probably due to the peculiar composition of *C. sempervirens* pollen.

Method

Aqueous extract of pollen of *C. sempervirens* has been concentrated and diafiltrated to eliminate contaminating components. Several chromatographic methods and conditions have been used to find the best method for the isolation of the major allergen (Cup s 1); ion exchange and size exclusion chromatography have been carried out in order to achieve this purification.

Results

Size exclusion chromatography allows the purification of Cup s 1 and makes characterisation possible (N-terminal, amino acid and mass spectrometry analysis). IgE from 83% of patients allergic to cypress recognise the band corresponding to this allergen. Fractionation methods employed for isolating homologous allergens (e.g. Cup a 1 from *C. arizonica* pollen) did not allow purification of Cup s 1, confirming the complications originated by the special characteristics and components of the pollen of *C. sempervirens*.

Conclusions

Pollen extraction and exhaustive diafiltration of the aqueous extract followed by size exclusion chromatography allows the purification of the major allergen of *C. sempervirens*, Cup s 1. The availability of this purified allergen will allow the development of methods for a much more convenient purification and quantification of Cup s 1, necessary in both diagnosis and immunotherapy of cypress allergy.

Double-blind, placebo-controlled study of immunotherapy with *Parietaria judaica* in mass units: Tolerance

A Muñoz¹, A Basomba¹, A Cisteró², E Enrique², A Peláez³, E Burches³, B Gandarias⁴, P Rico⁴

¹Hospital La Fe, València, Spain, ²Institut Univ. Dexeus, Barcelona, Spain,

³Hospital Clínico, València, Spain, ⁴ALK-Abelló S.A., Madrid, Spain

Background

Parietaria judaica pollen has a high prevalence in the Mediterranean area, and its capacity to produce rhinitis and or asthma as well as its long pollination period is largely documented.

Methods

In this study, clinical efficacy and tolerance of *P. judaica* 25 BU/mL, 1.5 µg/mL Par j 1 (Pangramin® Depot UM) were evaluated.

Results

57 rhinitis patients with/without asthma and monosensitised to *P. judaica* were recruited. During 20 months, 28 patients received active treatment (AG) and 29 placebo (PG). The average age was 34.7±10.4 years, and the average time since the onset of the disease was 7.9±7.8 years. 54.4% of patients were women.

There were 13 drop-outs (5 AG and 8 PG) and 2 withdrawals (1 AG and 1 PG). The reasons were in none of the cases related to immunotherapy (IT). All patients reached the previously established maintenance dose of 20 BU (1,2 µg Par j 1). 1,527 doses were administered, 803 in AG and 724 in PG.

There were 25 adverse reactions, representing 1.6% of doses (2.6% AG and 0.6% PG). 5 out of 25 were local reactions (LR) and 20 were systemic reactions (SR). Subcutaneous nodules were observed at 8 occasions (1 AG and 7 PG). These remitted without treatment. No LR needed treatment.

Related to SR, 16 were in AG and 4 in PG. The nature of SR was: 10 symptoms in upper respiratory tract (7 AG and 3 PG), 4 in lower respiratory tract (all of them in AG), 4 were cutaneous symptoms (all of them in AG) and 2 unspecific manifestations (1 AG and 1 PG). All SR were delayed and mild. No case of anaphylactic shock was observed. 55% of SR did not need treatment. Antihistamines were administered at 8 occasions and β₂ agonist just once.

Conclusion

On the basis of our experience, tolerance of Pangramin® Depot UM, *P. judaica* 25 BU/mL, can be qualified as very good.

Double-blind, placebo-controlled study of immunotherapy with *Parietaria judaica* in mass units: Immediate and delayed cutaneous response

A Muñoz¹, A Basomba¹, A Cisteró², E Enrique², A Peláez³, M Ferrer³, B Gandarias⁴, S Martín⁴

¹Hospital La Fe, València, Spain, ²Institut Univ. Dexeus, Barcelona, Spain,

³Hospital Clínico, València, Spain, ⁴ALK-Abelló S.A., Madrid, Spain

Background

Parietaria judaica pollen has a high prevalence in the Mediterranean area, and its capacity to produce rhinitis and or asthma as well as its long pollination period is largely documented.

Methods

We carried out a multicentre study to evaluate clinical efficacy and tolerance of a *P. judaica* extract 25 BU/mL (1,5 µg/mL Par j 1), Pangramin® Depot UM.

The evolution of immediate and delayed cutaneous response was evaluated after specific immunotherapy (IT). Immediate cutaneous response was assessed by skin prick test (SPT) with 4 concentrations (500, 100, 20 and 4 BU/ml), each by duplicate at six different times: T₉₉=Pollen Season (PS) 1999, before the beginning of IT, T₀=immediately before the beginning of IT, T₃=three months after the beginning of IT, when the maintenance dose is reached, T₀₀=PS 2000, T₁₂=one year after the beginning of IT, T₀₁=PS 2001.

The area of wheals was measured by planimetry and changes by Parallel Line Assay (PLA). Delayed cutaneous response was assessed by intracutaneous test (ICT), injecting 0.02 ml of 0.39BU/ml. This test was done in T₉₉, T₀₀ and T₀₁. The mid-diameter of the wheal was measured at 6, 24 and 48 hours.

Results

52 rhinitis patients with/without asthma monosensitised to *P. judaica* received active treatment (n=26, AG) or placebo (n=26, PG). At baseline, both groups were homogeneous for SPT and ICT. There was a statistically significant decrease in SPT in AG (p<0.01 in T₃, T₀₀, T₁₂ and T₀₁) not observed in PG. There was also a significant difference between both IT groups (p<0.01 in all assessments after IT: T₃, T₀₀, T₁₂ and T₀₁).

Similar results were observed with ICT: a significant decrease was seen in the AG at 6h (p<0.05 in T₀₀ and p<0.001 in T₀₁) and at 24h in T₀₁ (p<0.05). On the contrary, response did not decrease in the PG. A statistically significant difference was as well observed between groups at 6h (p=0.0076 on T₀₀ and p=0.0071 on T₀₁) at 24h (p=0.0092 on T₀₀ and p=0.035 on T₀₁) and at 48h on T₀₁ p=0.033. During the study the kinetic of ICT in AG compared with PG was statistically different in the three assessments (p=0.0003 at 6h, p=0.012 at 4h and p=0.013 at 48h).

Conclusion

We can summarise that Pangramin® Depot UM, *P. judaica* 25 BU/mL is able to reduce significantly the immediate and delayed cutaneous response.

Biological standardisation of a *Blomia tropicalis* extract

JA Nadal¹, T Carrillo², F Schamann³, D Solé⁴, C Gonzalez de la Cuesta⁵,
M Lombardero¹, S Martín¹

¹ALK-Abelló S.A., Madrid, Spain, ²Hospital Dr. Negrín, Las Palmas de Gran Canaria, Spain, ³Hospital Insular, Las Palmas de Gran Canaria, Spain, ⁴Escola Paulista de Medicina da UNIFESP, Sao Paulo, Brazil, ⁵Hospital Santa Maria Madre, Orense, Spain

Background

An international multicentre trial has been developed for the biological standardisation and determination of sensitivity and specificity of a *Blomia tropicalis* extract.

Methods

A total of 143 patients from four different clinics in tropical and non-tropical areas (2 in the Canary Islands, 1 in Brazil and 1 in Northwest Spain) were selected according to clinical history and previous diagnostic tests.

Studied population comprised 58 *Blomia*-sensitised patients, 26 non-atopic patients and 18 atopic patients not sensitised to *Blomia*, all of them from tropically located clinics. A third control group of 41 patients sensitised to mites but residing in a non-tropical area (Orense, Northwest Spain) was included to assess cross-reactivity. Home dust samples collected from four patients in Orense revealed an elevated mite pressure but an absence of *B. tropicalis*.

All patients and control subjects were tested by Skin Prick Test (SPT) with four concentrations of the *B. tropicalis* extract (3, 0.6, 0.12 and 0.024 mg/ml) and with a *Dermatophagoides pteronyssinus* 100 BU/ml (40µg/ml Der p 1 and 20µg/ml Der p 2) ALK-Abelló extract. Blood samples were collected for specific IgE determination.

Results

Biological standardisation was performed in 53 *B. tropicalis* sensitised patients. Cutaneous response to histamine at 10 mg/ml was equivalent to 0.36 mg/ml of *B. tropicalis* extract.

SPT results show 100% sensitivity with the two highest concentrations tested, decreasing to 86.8% and 58.5% with 0.12 and 0.024 mg/ml, respectively. Specificity varied for each of the three control populations and extract concentration, ranging from 10.3 to 100%.

Specific IgE analysis reached 79.3% sensitivity and a specificity of 41.5%, for patients sensitised to mites and 100% for atopic patients not sensitised to mites.

Conclusions

SPT with the *B. tropicalis* extract of 10 HEPs presents a sensitivity of 93.4%. Consistently with other *in vivo* and *in vitro* studies, an important cross-reactivity with *D. pteronyssinus* was found. This cross-reactivity limits specificity to 20.5% at 10 HEPs for mite-sensitised atopic patients. At the same extract concentration, we obtained 100% specificity for atopic patients not sensitised to mites.

A double-blind, placebo-controlled birch allergy vaccination study: Inhibition of CD23-mediated serum-IgE facilitated allergen presentation

RJJ van Neerven¹, M Arvidson², H Ipsen¹, S Rak², SH Sparholt¹, PA Würtzen¹
¹ALK-Abelló A/S, Research, Hørsholm, Denmark, ²Sahlgrenska Hospital, Göteborg, Sweden

The clinical efficacy of specific allergy vaccination (SAV), previously called specific immunotherapy, is well documented. The working mechanism of this treatment, which has been used for almost a century, is not completely known at present. Allergen-specific CD4⁺ T-lymphocytes are activated at extremely low allergen concentrations *in vivo* possibly as a direct result of serum IgE facilitated allergen presentation (S-FAP). In a previous study, we have shown that this process can be inhibited by long-term birch SAV sera.

In the present study, we have analysed sera from birch allergic patients in a randomised, double-blind, placebo-controlled clinical trial for their ability to mediate S-FAP. Birch-specific IgE levels had not changed after SAV. Bet v 1-specific IgE levels however were significantly decreased ($p < 0.05$) and Bet v 1-specific IgG4 levels had increased significantly after SAV ($p < 0.001$). None of these effects were observed in the placebo group. Serum levels of Phl p 5-specific IgG4 as a control allergen were not affected. When the sera were tested for their ability to induce S-FAP, a complete abrogation of the ability to mediate S-FAP was noted in the sera from patients receiving active treatment ($p < 0.001$), but not in the control group. This inhibition of S-FAP seemed to be associated with the reduction in the ratio between Bet v 1-specific IgE and IgG4 antibodies in serum, but a clear correlation between antibody concentrations and T-cell activation could not be demonstrated. Finally, it was possible to demonstrate binding of allergen-IgE complexes to B-cells in freshly isolated PBMC and to purified B-cells by flow cytometry experiments, further supporting the notion that this mechanism is important for T-cell activation *in vivo*.

In conclusion, the present study clearly shows that SAV leads to an inhibition of S-FAP, a mechanism that is needed to obtain optimal T-cell activation at the very low allergen concentrations present *in vivo*. This novel mechanism may explain at least in part the increased allergen threshold levels found in allergen provocation tests and the reduction of late phase reactions observed after SAV.

Tolerance of sublingual immunotherapy of grasses administered in disposable monodose vials

I Ojeda¹, F Gozalo Reques², P Ojeda¹, JL Estrada², S Martín³, P Rico³

¹*Clínica de Asma y Alergia, Madrid, Spain,* ²*Hospital de León, León, Spain,*

³*ALK-Abelló S.A., Madrid, Spain*

Background

An open multicentre study was designed to assess the tolerance, accept and compliance rates of swallow-sublingual immunotherapy (SLIT) presented in disposable monodose plastic-containers (DMPC) and following a fixed treatment schedule.

Methods

58 patients underwent SLIT during 3 months. All patients suffered from seasonal rhinitis associated or not with mild asthma. A biological standardised extract mix of five grasses (*Phleum, Lolium, Poa, Festuca* and *Dactylis*) without phenol was used. The initial treatment was manufactured in a set of 7 blisters, each of them with 5 DMPC. The volume of each dose was fixed (0.4 ml) and DMPC was discarded after administration. The administered doses were: 0.001, 0.002, 0.006, 0.01, 0.05, 0.16 and 0.5 µg of group 5. Build-up doses were taken daily from Monday to Friday under allergist supervision. Maintenance dose was established at 0.5 µg of group 5 and administered three times a week.

Results

58 patients with a mean age of 27.6 ± 8.0 years, 33 males/25 females, received SLIT. All of them had symptoms of rhinitis, 93% also conjunctivitis and 50% asthma. Most of the patients were polysensitised and 31% monosensitised to grasses.

2,961 doses were administered, 1,924 during the build-up phase and 1,037 at maintenance. There were no side effects in 94.7% of the administrations. In 158 cases patients referred symptoms after SLIT administration; 93 local side effects (3.1% of the doses) and 65 systemic side effects (2.2%). Neither anaphylaxis nor severe reactions occurred during SLIT. Antihistamines were administered in 1 local reaction, beta agonist in another patient who presented respiratory symptoms and inhaled corticosteroids in 2 cases due to naso-ocular symptoms. In 95.5% of the doses, treatment schedule remained unmodified.

SLIT compliance was excellent or good in 98% of the patients. The accept of the DMPC was classified as easy to use by 96.2% and 100% of the patients and allergists, respectively. Tolerance was considered as very good by 88.5% of the patients and by the specialists in 93.9% of the cases. New treatment schedule was positively rated by 95.9% of the patients.

Conclusions

These results show that SLIT used in DMPC is well tolerated and provides a useful alternative, being strongly accepted by all participating allergists and raising patient compliance close to 100%. A fixed volume in DMPC simplifies the treatment schedule and avoids the use of preservatives.

Sublingual immunotherapy: Tolerance on a large sample of a starting dose higher than usual

M Pozzan¹, V Bordignon²

¹ASL 16, Allergology Service, Padua, Italy, ²Allergologist, Bassano Del Grappa (VI), Italy

Background

Allergen-specific immunotherapy is traditionally begun with very low dosages for tolerance and safety reasons. Sublingual immunotherapy (SLIT) has shown an excellent tolerance in several clinical trials. We sought to check the tolerance of SLIT when started with dosages higher than usual, as to shorten the build-up phase and to increase patient's compliance.

Methods

We selected 872 patients (486M/386F, age range 2.5-65 years, mean 22.81), clinically monosensitised to inhalant allergens (main allergens: grass 298, mites 285, *Corylaceae*/*Betulaceae* 158, and *Parietaria* 37) and suffering from rhinoconjunctivitis and/or asthma (asthma in total 430, rhinitis in total 865). All patients or their parents gave an informed consent to take part in the study. SLIT was started when patients were symptom-free with a dose 125 times higher than the dose normally administered with a biologically standardised SLIT preparation commercially available (ALK-Abelló, Milan, Italy), i.e. 1 drop from vial 3 (200 STU/mL) instead of 1 drop from vial 0 (1.6 STU/mL). The amount of the major allergen administered for the most common allergens ranged from 0.005 mg (Par j 1) to 0.18 mg (Bet v 1). The first administration was performed under medical surveillance, and in case of good tolerance the following ones were self-administered by patients. Patients or their parents were instructed to report immediately any local and/or general side effects possibly related to the allergen administration and had the availability of rescue drugs to control allergy symptoms, if any. In 81 patients the administration of 1 drop from vial 3 was repeated daily (morning) for one week, in 522 patients twice daily (morning and evening) for one week and in 239 patients three times a day (morning, midday, and evening) for one week before going on with the following scheduled administrations.

Results

Only an 8-year-old child sensitised to mites and taking the allergen twice a day suffered from a slight itching of the buccal mucosa lasting three days and self-resolved without need for drugs or interruption of the treatment.

Conclusions

SLIT administration for the most common allergens can be safely started with dosages of major allergens in the range 0.005 to 0.18 mg.

Efficacy and safety of sublingual immunotherapy (SLIT) with grass pollen in children with seasonal allergic rhinoconjunctivitis to grass pollen

C Rolinck-Werninghaus¹, C Liebke¹, W Leupold², CP Bauer³, J Kuehr⁴, H Wolf⁵, J Schnitker⁶, B Niggemann¹

¹Charité-Virchow-Klinikum, Klinik und Poliklinik für Kinderheilkunde und Kinderchirurgie, Abteilung Pädiatrische Pneumologie und Immunologie, Humboldt Universität, Berlin, Germany, ²Universitätsklinikum Carl Gustav Carus, Klinik und Poliklinik für Kinderheilkunde, Dresden, Germany, ³Kinderklinik der Technischen Universität, München, Germany, ⁴Klinikum der Albert-Ludwigs-Universität, Kinderklinik, Freiburg, Germany, ⁵ALK-Scherax Arzneimittel GmbH, Klinische Forschung, Hamburg, Germany, ⁶Institut für Angewandte Statistik Dr. Jörg Schnitker GmbH, Bielefeld, Germany

Background

Subcutaneous immunotherapy is an effective treatment in children with seasonal allergic rhinoconjunctivitis (SAR). Sublingual compared to subcutaneous application of pollen extracts promises advantages for patients' compliance. However, its efficacy and safety in children is still controversial.

Method

97 children with SAR to grass pollen (CAP \geq 2) aged 3-14 years (40% asthma) received SLIT (B.U. Pangramin SLIT[®], ALK-Scherax, Germany, 0.5 mg of 5 major grass pollen allergens, 3x/week) in a prospective, double-blind study in 4 centres in Germany from 01/1999 to 11/2001. Primary end point was a multiple symptom-medication score for changes in seasonal symptoms (eyes 3, nose 4, lung 4 items; score 0-3) and changes in medication (7 rescue drugs, score 0-1) in 2001 compared to 1999, documented daily by the patients (full analysis set: SLIT n=39, placebo n=38). Due to the variation of pollen counts in seasons and centres, scores were adjusted to 1000 pollen/m³ and a 6 weeks' season.

Results

Homogeneity tests revealed that the treatment groups were inhomogeneous with more severe conjunctivitis and higher symptomatic medication in 1998, higher total and grass pollen specific IgE and a higher incidence of atopic dermatitis in the SLIT group. Ocular, nasal or lung symptoms were not significantly reduced by SLIT compared to placebo. However, the medication score improved significantly (P=.003). The multiple end points of symptoms and medication reached statistical significance (P=.049). For the single end points within the placebo group, no significant differences were seen over time. In the SLIT group ocular symptoms improved from 5.1 to 2.9 (P=.026), nasal symptoms from 14.7 to 6.5 (P=.010) and medication score from 4.4 to 1.3 (p<.001). Lung symptoms did not differ. Mild to moderate allergic side effects of treatment were reported in both groups (SLIT: 49%, placebo: 27%, P=.026).

Conclusion

Our study indicates that SLIT with 0.5 μ g major grass pollen allergens 3x/week for 3 years was able to reduce a combined symptom-medication score in children with SAR, based on significant improvements of the medication use, but had no significant effects on ocular and nasal symptoms compared to placebo.

T-cell immune responses during sublingual immunotherapy (SLIT) with grass pollen in children with seasonal allergic rhinoconjunctivitis to grass pollen

C Rolinck-Werninghaus¹, M Kopp², C Liebke¹, J Kuehr², H Wolf³, J Schnitker⁴, B Niggemann¹

¹Charité-Virchow-Klinikum, Klinik und Poliklinik für Kinderheilkunde und Kinderchirurgie, Abteilung Pädiatrische Pneumologie und Immunologie, Humboldt Universität, Berlin, Germany, ²Klinikum der Albert-Ludwigs-Universität, Kinderklinik, Freiburg, Germany, ³ALK-Scherax Arzneimittel GmbH, Klinische Forschung, Hamburg, Germany, ⁴Institut für Angewandte Statistik Dr. Jörg Schnitker GmbH, Bielefeld, Germany

Background

The efficacy of SLIT in children with seasonal allergic rhinoconjunctivitis (SAR) is still in discussion. Studies of its influence on the immune system are rare.

Method

In vitro T-cell responses of 29 children with SAR to grass pollen (CAP \geq 2) receiving SLIT (B.U. Pangramin-SLIT®, ALK-Scherax, Germany, 0.5 mg major grass pollen allergens 3x/week) were analysed for a subgroup of children included in a prospective, randomised, double-blind, multi-centre study. We measured the proliferation of peripheral blood mononuclear cells (PBMC; 3H-Thymidine incorporation) and cytokine secretion to culture supernatants (IL4 + IFN γ , ELISA) after *in vitro* stimulation with grass pollen allergen (20 μ g/ml + 2 μ g/ml) or PHA (3 μ g/ml) before, after 1 and after 2 years of treatment. Furthermore, we investigated intracellular cytokine staining (IL4, IL13, IFN γ , IL10) after non-specific stimulation.

Results

Proliferation of PBMC after allergen- or PHA-stimulation did not differ between the SLIT and the placebo group, at all time points. Furthermore, allergen-induced secretion of IL4 and IFN γ into supernatants remained unchanged by SLIT. After stimulation with PHA, secretion of IFN γ was significantly higher in the SLIT group after 1 year of treatment (2,647 pg/ml vs. 946 pg/ml, P=.001). However, this difference was already seen in pre-treatment experiments (2,022 pg/ml vs. 1,133 pg/ml, P=.057) and was also not significant after 2 years of treatment (631 pg/ml vs. 283 pg/ml, P=.086). Secretion of IL4 to supernatants after coculture with PHA was similar in both groups. In addition, no differences for intracellular staining of IL4, IL13, IFN γ or IL10 were observed. Homogeneity tests had shown that the patient groups were inhomogeneous for total and specific IgE at the beginning of the study (study entry – total IgE: SLIT 282 kU/l, placebo 178 kU/l, P=.026; specific IgE: SLIT 62 kU/l, placebo 24 kU/l, P=.032).

Conclusion

During two years of treatment with SLIT in children, no significant effects on *in vitro* T-cell immune responses were observed. The unstable findings of higher IFN γ secretion after stimulation with PHA in the SLIT group is most likely due to inhomogeneous patient groups despite randomisation.

Double-blind, placebo-controlled dose-response study of clinical efficacy and safety of sublingual immunotherapy (SLIT) with tree pollen extract in children suffering from tree pollen induced hay fever with or without seasonal allergic asthma

E Valovirta¹, Christian Ljørring², L Jacobsen²

¹Turku Allergy Centre, Ped., Turku, Finland, ²ALK-Abello A/S, Research, Hørsholm, Denmark

The objective of the study was to investigate the dose-response relationship in clinical efficacy and safety of sublingual immunotherapy in allergic children suffering from rhinoconjunctivitis caused by allergy to tree pollen.

98 children aged 5-14 years with a clinical history of tree pollen induced allergic rhinoconjunctivitis with/without seasonal asthma for at least two years were included. Allergy to relevant tree pollen were confirmed by positive SPT ≥ 3 mm (Soluprick[®] SQ 10 HEP, ALK-Abelló A/S), positive specific IgE (Magic Lite[®] SQ, \geq class 2) to tree pollen mixture as well as either individual species of the mixture (*Betula verrucosa*, *Corylus avellana* and *Alnus glutinosa*) and positive CPT $\leq 100,000$ SQ-U/ml to tree pollen (Aquagen[®] SQ, ALK-Abelló A/S). The extract used for SLIT was a glycerinated mixture of *B. verrucosa*, *C. avellana* and *A. glutinosa* with a major allergen content corresponding to 12 μ g major allergen.

The children were randomised in 3 groups receiving sublingual immunotherapy up to 18 months. Eighty-four children completed the study. Distributed on 5 days each week the patients received the following:

High dose (n=25): Weekly accumulated dose of 200,000 SQ-U (daily dose: 40,000 SQ)

Low dose (n=32): Weekly accumulated dose of 24,000 SQ-U (daily dose: 4,800 SQ)

Placebo (n=27): Soluprick glycerinated diluent.

The high dose group showed a significant reduction (41%) of combined symptoms/medication score compared to placebo group (p=0.03). The low dose group showed a reduction of 26% (p=0.11). There were no statistical differences between the high dose and the low dose groups. Change from baseline in CPT, MBPT or LPSR did not show any statistically significant differences between treatments and placebo. All patients tolerated the treatment well and there were no difference between the groups with regard to side effects.

Sublingual immunotherapy with tree pollen 200,000 SQ/week significantly reduces allergic symptoms during the tree pollen season in tree pollen allergic patients. SLIT with a tree pollen mix can be considered safe and well tolerated by patients with tree pollen allergy.

ALK-Abelló Companies

Austria

ALK-Abelló GmbH
Bäckermühlweg 59
A-4030 Linz
Tel.: +43 732 385 372
Fax: +43 732 385 37 277
e-mail: office@at.alk-abello.com

China

ALK-Abelló A/S
Rm 1812, 18/F
Metropole Square
2 on Yiu Street, Shatin
Hong Kong
Tel.: +852 2144 5218
Fax: +852 2144 5618
e-mail: hwtsui@alk-abello.com.hk

Denmark

ALK-Abelló Danmark
Borups Allé 177, D4
Postboks 400
DK-2000 Frederiksberg
Tel.: +45 38 16 70 70
Fax: +45 38 16 70 99

Finland

ALK-Abelló, Siviiliike Suomessa
Iluuodontie 17B, 2krs
FI-00980 Helsinki
Tel.: +358 9 341 7350
Fax: +358 9 341 73570

Germany

ALK-Scherax Arzneimittel GmbH
Sülldorfer Landstrasse 128
D-22589 Hamburg
Tel.: +49 40 8 70 70 70
Fax: +49 40 8 70 880 88
e-mail: info@alk-scherax.de

Italy

ALK-Abelló S.p.A.
Via Settembrini 60
(Ang. Via Ramazzotti 12)
I- 20020 Lainate MI
Tel.: +39 0 2 937631
Fax: +39 0 2 93763457
e-mail: alk_abello@allergia.it

The Netherlands

ALK-Abelló bv
Edisonbaan 26
Postbus 1041
NL-3430 BA Nieuwegein
Tel.: +31 30 600 5 790
Fax: +31 30 600 5 792
e-mail: info@nl.alk-abello.com

Norway

ALK Sverige AB,
Filial av Utenlandsk Aksjeselskab
Postboks 218, Økern
Spireavegen 6
N-0510 Oslo
Tel.: +47 2337 9950
Fax: +47 2297 0318

Spain

ALK-Abelló S.A.
Miguel Fleita 19
E-28037 Madrid
Tel.: +34 91 32 76 100
Fax: +34 91 32 76 122
e-mail: smarketing@es.alk-abello.com

Sweden

ALK Sverige AB
Smörhålevägen 3
S-434 42 Kungsbacka
Tel.: +46 30 01 85 45
Fax: +46 30 01 39 10
e-mail: alk.sverige@alk.se

UK

ALK-Abelló (UK) Ltd
2 Tealgate
Hungerford, Berkshire
RG17 0YT
Tel.: +44 1488 68 60 16
Fax: +44 1488 68 54 23
e-mail: angela@alkabello.co.uk

USA

ALK-Abelló, Inc.
Main office
1700 Royston Lane
Round Rock, Texas 78664
Texas
Tel.: +1 512 251 0037
Fax: +1 512 251 8450
e-mail: info@us.alk-abello.com

Manufacturing
35 Channel Drive
Port Washington, 10050 New York
Tel.: +1 516 767 1800
Fax: +1 516 767 4229

Vespa Laboratories, Inc.
1095 Upper Georges Valley Rd
Spring Mills, PA 16875
Tel.: +1 814 422 8165
Fax: +1 814 422 8424
e-mail: vespalab@aol.com

Biopol Laboratory, Inc.
327 East Pacific Avenue
WA 99202 Spokane
Tel.: +1 509 456 7794
Fax: +1 509 455 7965

ALK-Abelló Distributors

Australia

CSL Ltd
45 Poplar Road Parkville
Victoria 3052
Tel.: +61 3 9389 1611
Fax: +61 3 9389 1874
e-mail: ssilk@csl.com.au

Brazil

FDA Allergenic LTDA
Rua de Abolição 413
20755-170 Rio de Janeiro-RJ
Tel.: +55 21 899 9393
Fax: +55 21 289 9290
e-mail: fda.allergenic@attglobal.net

Canada

Western Allergy
151 Brunei Rd., #35
Mississauga, ON L4Z2H6
Tel.: +1 905 290-9952
Fax: +1 905 290-9957

Allergy Canada Ltd.
10 Royal Orchard Blvd., #53102
Thornhill, ON L3T 7RH9
Tel.: +1 905 763-6642
Fax: +1 905 763-7120

Czech Republic

ASCO med spol.s.ro.
Pod Cihelnou 23
16100 Praha 6
Tel.: +420 2 3331 3578
Fax: +420 2 3331 3582
e-mail: ascomed@anet.cz

Greece

Delta Medical S.A.
48, Marathonos Avenue
GR-153-54i
Tel.: +30 210 6615 209
Fax: +30 210 6615 218
e-mail: deltamed@otenet.gr

Hungary

SPIRO-MED Ltd.
Kerepesi ut.26
HU-1148 Budapest
Tel.: +36 1 246 0798
Fax: +36 1 246 3613
e-mail: szego.katalin@fuzio-pharma-hu

Iceland

Thorarensen LYF
Lynghálsi 13
IS-110 Reykjavik
Tel.: +354 530 7100
Fax: +354 530 7101

Indonesia

PT. Lapi Laboratories
Pluit Mall Blok D No. 2
Jl. Raya Pluit Selatan
Jakarta 14450
Tel.: +62 21 6613833
Fax: +62 21 66 30450
e-mail: customerservice@lapi.co.id

Malaysia

Germax SDN. BHD.
Wisma G.A.M., 3rd Floor
Lot 8241, Jalan 225/1A
46100 Petaling Jaya
Tel.: +60 3 7958 3112
Fax: +60 3 7958 3003
e-mail: germax@po.jaring.my

Poland

Viac Handels GesmbH
Alutard Information Office
ul. Godebskiego 5
Warsaw / 05-090 Janki
Tel.: +48 022 715 69 79

Portugal

Bioportugal
Alameda Fernao Lopes 19E
Miraflores
1495-135 Alges (Lisbon)
Tel.: +351 21 41 39 750
Fax: +351 21 41 08 928
e-mail: biop@esoterica.pt

Slovak Republic

ADOS spol.s.ro.
Letná 1
SK-831 03 Bratislava
Tel.: +42 12 444 510 95
Fax: +42 12 442 599 73

South Africa

Laboratory Specialities cc.
197 Fabriek Street
Strydom Park
Randburg 2125 (Johannesburg)
Tel.: +27 11 792 6790
Fax: +27 11 793 1064
E-mail: angien@mweb.co.za

Switzerland

Trimedal AG
Fabrikweg 2
CH-8306 Brüttisellen
Tel.: +41 18 34 00 05
Fax: +41 18 34 00 07
e-mail: info@trimedal.ch

Turkey

Albio Allerji
Mustafa Mazhar Bey sk. 41/4
Selamicesme
Istanbul
Tel.: +90 216 330 2300
Fax: +90 216 418 2007
e-mail: albio@albio.com.tr

Venezuela

Solo Alergias
Avda. Francisco de Miranda
Edificio Centro Plaza
Nivel 3, Local CC-3-77, Paseo La Vereda
Urbanización Los Palos Grandes
Caracas
Tel.: +58 212 284 02 64
Fax: +58 212 284 02 64
e-mail: soloalergias@cantv.net



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ALK-ABELLÓ A/S • BØGE ALLÉ 6-8 • DK-2970 HØRSHOLM • DENMARK • TEL +45 45 74 74 45 • FAX +45 45 74 86 90 • WEB: WWW.ALK-ABELLO.COM