

The 60th Anniversary Meeting of the American
Academy of Allergy, Asthma & Immunology

7-12 March 2003, Denver



Scientific Contribution



Dear Specialist

We welcome you to Denver and the 60th Anniversary 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.

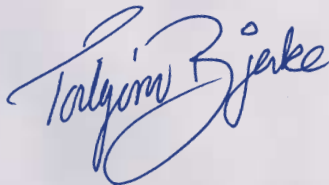
This booklet contains 8 abstracts within the field of allergy vaccination and diagnostics. The abstracts demonstrate the collaboration between allergy specialists and ALK-Abelló and illustrate our long-standing tradition of research in cooperation with the international scientific community.

If you wish to comment on or further discuss the enclosed abstracts, please join us at the ALK-Abelló stand no. 1028.

We wish you an enjoyable and fruitful congress.

Yours sincerely

ALK-Abelló A/S



Torbjørn Bjerke

Executive Vice President

Research & Development

Scientific contribution

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The stability of major allergens in mite and grass allergenic extracts

WT Caples, GA Plunkett
ALK-Abelló, Inc, Round Rock, Texas, USA

Rationale

ALK-Abelló has developed and validated assays to measure major allergen content in house dust mite and grass pollen extracts. Production lots have been analysed for several years. Major allergen content may be used by physicians to guide immunotherapy dosing. This study was initiated to determine the stability of the group 5 major allergen in several grass species pollen extracts and the group 1 and 2 allergens in *D. farinae* and *D. pteronyssinus* mite extracts.

Methods

Monoclonal based ELISA reagents were obtained from ALK-Abelló, Madrid. These assays were validated in our laboratory by determining the performance characteristics and establishing internal controls. Der f 1, Der f 2, Der p 1 and Der p 2 were measured in the corresponding extracts, and group 5 major allergen was measured in pollen extracts from 6 Northern grass species. The major allergen content was measured shortly after manufacture and again after storing at 1-5° C for several months.

Results

Most of the allergen types had at least 3 lots evaluated. 88% of lots with the mean age of 16 months (range 3-33) maintained at least 60% of their major allergen content. 94% maintained at least 50% of their initial value.

Conclusion

This ongoing study shows that group 5 grass allergen and groups 1 and 2 mite allergens have maintained considerable stability during the testing periods.

Basic and clinical immunology (BCI)

Vaccines/other immunotherapeutics

Kinetics and mode of peptide delivery via the respiratory mucosa determine the outcome of Th2 immunity versus tolerance in allergic inflammation of the airways

G Hall¹, L Lund², JR Lamb¹, ER Jarman²

¹*Immunobiology Group, MRC Centre for Inflammation Research, Respiratory Medicine Unit, University of Edinburgh Medical School, Edinburgh, Scotland,*
²*ALK-Abello A/S, Hørsholm, Denmark*

Rationale

Allergen derived peptides may provide a safer alternative to conventional specific immunotherapy. We investigated the efficacy of mucosally administered peptide alone or adsorbed to chitosan in the induction of CD4+ Th2 cell tolerance and protection against pulmonary inflammation induced by sensitisation and challenge with allergen.

Methods

Chitosan is a biocompatible mucopolysaccharide, which enhances the levels of adsorption of compounds delivered via the nasal mucosa. Mice were administered intranasally a peptide containing an immunodominant epitope of the *Dermatophagoides pteronyssinus* (Der p 1) allergen as soluble antigen or adsorbed to chitosan, prior to sensitisation and allergen challenge. Pulmonary inflammation, antigen specific CD4+ T-cell responses and antibody levels in sera were determined.

Results

Mice administered peptide adsorbed to chitosan had significant reductions in airway eosinophilia, which correlated with reduced levels of IL-4 and IL-5 in the bronchoalveolar lavage fluid, a loss of Der p 1 specific T-cell cytokine responses in the periphery and the localised production of IL-10 by antigen specific T-cells in bronchial lymph nodes. Induction of peripheral T-cell tolerance was preceded by transient T-cell activation and IFN- γ production.

Conclusion

This data demonstrates that suppression of airway inflammation by intranasal administration of peptide antigen adsorbed to chitosan is initiated by transient T-cell activation and maintained by the production of IL-10 by antigen specific T-cells in the draining lymph nodes.

Epitope grafting: The shaping of a conformational Bet v 1 epitope on Mal d 1, the major apple allergen

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

Antibody specificity and cross-reactivity are important aspects of allergy diagnosis and specific immunotherapy. 'Perfect fit' is of major importance for the affinity of the antibody-antigen interaction, since the binding energy is composed not only by the enthalpy forces of attraction, but also from a significant entropy contribution by the expulsion of water molecules from the complex-interface.

The crystal structure of Bet v 1, the major birch pollen allergen, in complex with monoclonal murine IgG antibody (BV16) Fab-fragment reveals atomic details of a complete conformational Bet v 1 epitope. Substitution of one single amino acid residue (Glu-45-Ser) in the epitope abolishes binding of the BV16 antibody, and leads to 25-50% reduction in IgE binding comparing individual birch pollen allergic patients. The BV16 antibody does not cross-react with the homologous apple allergen, Mal d 1, having 55% amino acid sequence identity with Bet v 1. Among the 16 amino acid residues in the BV16 epitope, the 5 differing between Bet v 1 and Mal d 1 were substituted in order to shape the BV16 epitope on Mal d 1 resulting in an increase in the binding of patient's IgE 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 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, structural studies enhance the understanding of antibody-antigen interactions with the potential of improving allergy disease management.

Basic and clinical immunology (BCI)

Vaccines/other immunotherapeutics

DNA vaccination reverses established allergen induced Th2 mediated airway inflammation and limits progression towards chronic inflammation and possibly airway remodelling

ER Jarman¹, G Hall², JR Lamb²

¹ALK-Abelló A/S, Hørsholm, Denmark,

²Immunobiology Group, MRC Centre for Inflammation Research, Respiratory Medicine Unit, University of Edinburgh Medical School, Edinburgh, Scotland

Rationale

pDNA vaccines encoding allergens have been shown to prevent the induction of Th2 cytokine mediated airway inflammation in experimental models. Nevertheless, their long-term efficacy in limiting or reversing the progression of established pulmonary inflammation towards chronic inflammation and airway remodelling has not been fully explored.

Methods

Mice exhibiting established airway inflammation, induced by repeated exposure to a major allergen of house dust mite *Dermatophagoides pteronyssinus* (Der p 1) were vaccinated with pDNA constructs expressing an immunodominant region of Der p 1 or an irrelevant *Parietaria* allergen. Pulmonary inflammation was assessed on re-challenge of the airways with Der p 1 and the effect on systemic and localised CD4⁺ Th2 responses was determined.

Results

pDNA vaccinated mice exhibited a reduction in the levels of inflammatory cells recruited into the airways following allergen challenge, reduced goblet cell hyperplasia and mucus production. Reductions in eosinophilia were accompanied by a fall in levels of the Th2 cytokines IL-4 and IL-5 as well as in the profibrotic factor TGF- β 1 in the airways. Reductions in airway eosinophilia were conferred by pDNA vaccines, irrespective of the specificity of the construct. Protection correlated with a reduction in levels of Th2 cytokines produced by allergen reactive CD4⁺ T-cells rather than an increase in INF- γ levels.

Conclusion

pDNA vaccines can prevent progression of long-term established airway inflammatory responses to a major aeroallergen and may protect against the onset of chronic pulmonary inflammation and airway remodelling.

Physicochemical and immunological comparison between natural and recombinant Pru p 3. Potential applications

M Lombardero¹, A Díaz-Perales², ML Sanz³, G García-Casado², R Sánchez-Monge², FJ García-Selles⁴, F Polo¹, P Gamboa⁵, D Barber¹, G Salcedo²

¹ALK-Abelló S.A., Madrid, Spain, ²E.T.S. Ingenieros Agrónomos, Madrid, Spain, ³Clínica Universitaria de Navarra, Pamplona, Spain, ⁴Hospital Virgen de la Arrixaca, Murcia, Spain, ⁵Hospital de Basurto, Bilbao, Spain

Rationale

Pru p 3 is a lipid transfer protein that has been identified as a major peach allergen. Recombinant Pru p 3 has recently been produced in the yeast *Pichia pastoris*.

Objective

We aimed at studying the usefulness of recombinant Pru p 3 as a novel tool for diagnosis of peach allergy.

Methods

The protein folding of natural and recombinant Pru p 3 was compared by means of circular dichroism analysis. IgE-binding capacity of both molecular forms was quantified by ELISA and ELISA inhibition assays, and their biological activity was estimated by basophil activation, histamine release and sulphidoleukotriene production tests. Individual serum or blood from patients with peach allergy was used in the assays.

Results

Natural and recombinant Pru p 3 showed nearly identical circular dichroism spectra, indicating that both protein forms are similarly folded. No difference was detected in the IgE-binding capacity of the two Pru p 3 molecular versions. Furthermore, the natural and recombinant forms displayed equivalent stimulatory activity in all three tests measuring basophil activation and mediator-induced release: Basophil activation and induction of sulphidoleukotriene production were positive in 9 out of 10 patients, and histamine release was induced in at least half of the cases.

Conclusion

Recombinant and natural Pru p 3 have similar physicochemical and allergenic properties, and consequently rPru p 3 can be a useful tool for the diagnosis of peach allergy.

Determination of major allergen protein, Ole e 1, in aqueous and glycerinated allergenic extracts

GA Plunkett, WT Caples, KM Poppe
ALK-Abelló, Inc, Round Rock, Texas, USA

Rationale

Ole e 1 is a major allergen in olive tree (*Olea europaea*) pollen. Similar proteins found in other Oleaceae family species such as lilac, privet and ash, may exhibit cross-reactivity. Measurement of the Ole e 1 micrograms/mL provides a novel quality control parameter and also guidance for immunotherapy dosing. The purpose of this study was to validate the method for measuring Ole e 1, establish internal controls, and measure the Ole e 1 in several tree pollens extracted in aqueous and glycerin solutions.

Methods

Reagents for a 2-site monoclonal ELISA specific to Ole e 1 were obtained from ALK-Abelló, Madrid. Standards were calibrated against affinity and HPLC purified Ole e 1.

Results

The assay range was determined to be between 2 and 75 ng/mL. Repeat testing of 1 extract demonstrated assay precision of about 11% CV. Six olive extracts produced parallel lines to the standard. Specificity was established by testing several non-related species. Ash and privet extracts showed measurable Ole e 1.

Several lots of olive pollen extracted 1:20 w/v in 50% glycerin had an average Ole e 1 content of 38 µg/mL and pollen extracted 1:10 w/v in aqueous diluent had an average of 247 µg/mL.

Conclusion

Olive pollen extracts contain Ole e 1 major allergen as determined by a validated ELISA. Closely related species exhibit cross-reactivity. 50% glycerin extracts contained much less of the allergen than aqueous media.

A persistent and selective activation of Syk or ZAP-70 kinase precedes a Th1 or Th2 on-switch

J Tan¹, A Liu¹, HH Jacobi¹, C Glue², A Millner¹, HO Madsen³, LP Ryder³, A Svejgaard³, JN Larsen¹, PA Würtzen¹, PS Skov², H-J Malling², MD Spangfort¹, LK Poulsen²
¹Biological Allergy Research, ALK-Abelló A/S, Hørsholm, Denmark, ²Laboratory of Medical Allergology, Allergy Unit, National University Hospital, Copenhagen, Denmark, ³Department of Clinical Immunology, National University Hospital, Copenhagen, Denmark

Rationale

The precursor CD4⁺ T-cells differentiate into either Th1 or Th2 cells depending upon antigen stimulation and cytokine environment. It is not very clear whether instructive or/and selective differentiation may contribute to Th cell formation *in vivo*.

Methods

We used the following methods in the present study: Intracellular cytokine staining; immune complex kinase assay and immunoblotting; PNA antisense assay; real time quantitative reverse transcription PCR assay; electrophoretic mobility shift assay.

Results

In addition to TCR, we reported an alternative signalling pathway in which IL-2 or IL-4 together with SDF-1alpha induces a persistent and selective Syk or ZAP-70 kinase phosphorylation, resulting in on-switch of naive CD4⁺ T-cells to Th1 or Th2 cells. An attenuating or increasing Cbl protein activity has been seen in these cells, whereas Cbl-b protein activities are persistent. A selective and persistent NFAT1 or NFAT2 activation has been detected in these cells. Syk or ZAP-70 PNA antisense treatment abolishes Syk or ZAP-70 kinase activity, resulting in selective inhibition of IFN-gamma or IL-4 mRNA and protein expression in the cells induced by IL-2 or IL-4 together with SDF-1alpha.

Conclusion

A persistent and selective activation of Syk or ZAP-70 kinase is essential for Th1 or Th2 cell on-switch. Cbl and Cbl-b protein activities followed by a persistent NFAT1 or NFAT2 activation also play distinct roles in Th1 or Th2 cell on-switch.

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, Hørsholm, Denmark, ²Sahlgrenska Sjukhuset, Göteborg, Sweden

The clinical efficacy of specific immunotherapy, recently named specific allergy vaccination (SAV), 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 facilitated allergen presentation (S-FAP). This process is mediated by allergen capture by specific IgE followed by binding to CD23 which leads to uptake and antigen processing. In a previous study we have shown that this effect is functional at natural serum concentrations and can be inhibited by the addition of 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 were not changed after SAV. Bet v 1-specific IgE levels, however, were significantly decreased ($p < 0.05$), and Bet v 1-specific IgG4 levels were 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.

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 at least in part explain the increased allergen threshold levels found in allergen provocation tests and the reduction of late phase reactions observed after SAV.

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-abelló.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, Sivuliike Suomessa
Iiluodontie 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.
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

Center Laboratories
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: sslk@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

Omega Laboratories Ltd.
11, 177 Hamon
Montréal, Que. H3M 3E4
Tel.: +514 335 0310
Fax: +514 339 1407

Czech Republic

ASCO med spol.s.r.o.
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
Vatnagordum 18
IS-104 Reykjavik
Tel.: +354 568 6044
Fax: +354 530 7001

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

Pharmag S.A.
Ul. Marynarki Polskiej 100
80-557 Gdansk
Tel.: +48 58 342 21 21
Fax: +48 58 342 21 33

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.r.o.
Letná 1
SK-831 02 Bratislava
Tel.: +42 17 442 599 73
Fax: +42 17 444 510 95

South Africa

Laboratory Specialities cc.
197 Fabrik Street
Strydom Park
Randburg 2125 (Johannesburg)
Tel.: +27 11 792 6790
Fax: +27 11 793 1064
E-mail: srmaria @mwweb.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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