ADJUVANT 2012

Adjuvant2012 & AllergenVaccines2012 International Congress

Varadero, Cuba, May 6-11, 2012

AllergenVaccines 2012

Abstracts

Organized and hosted by:

ALAI LATIN AMERICAN ASSOCIATION OF IMMUNOLOGY

Inmunología SOCIEDAD CUBANA

Endorsed by:

WAO WORLD ALLERGY ORGANIZATION
A World Federation of Allergy, Asthma & Clinical Immunology Societies

Consejo Nacional de Sociedades Científicas de la Salud CNSCS

INVITATION

Dear Colleague

On behalf of the Organizing Committee, we are pleased to invite you to join us at **the II International Congress on Allergen Vaccines** and **VI International Congress on Vaccine Adjuvants**, organized by the **Latin American Association of Immunology** (ALAI) and the **Cuban Society of Immunology**. In this occasion, holding both meetings jointly, will enhance the opportunities of cross-fertilization and scientific exchange on several contact points between these fields, on the common ground of immunology and biotechnology. The scientific program is specially designed to foster open and deep discussion of hot topics. Keynote addresses, oral presentations and interactive poster sessions will give a unique opportunity for exchanging ideas and foster cooperation among colleagues from academic and clinical research institutions, industry and regulatory agencies from developed and developing countries.

The venue, located in the beautiful beach of Varadero, provides a paradisiacal environment, which allows for an effective combination of science and relaxation in the friendliest atmosphere.

We are looking forward to having the opportunity of warmly welcoming you at our International Workshops on Adjuvants and Allergen Vaccines in Varadero, Cuba, from 6 to 11, May, 2012.

Oliver Pérez
President Cuban Society for Immunology
President Organizing Committee

Alexis Labrada
Co-President Organizing Committee

Program Summary

Plenary Session

1. Novel Approaches for Allergen Vaccines
2. Adjuvants to Allergy Vaccines
3. Debate: Dose response in allergy and cancer IT
4. WAO-AAAAI Seminar on allergen IT
5. Immunobiology of Allergy

Allergen Vaccines

1. Allergen Structure and Function
2. Adjuvants to Allergy Vaccines
3. Pharmaceutical and pharmacological development of allergen vaccines
4. Regulatory outlook, allergen IT and allergy care
5. Clinical trials of allergen IT

Adjuvants

1. New generation of adjuvants
2. Adjuvants for infectious diseases vaccines
3. Adjuvants to chronic infectious and non-infectious diseases
4. Adjuvants for veterinary vaccines

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PROGRAM AT A GLANCE

Day	Morning	Afternoon	Evening
5th	Arriving to Havana, Cuba (Gaviota package)		Free
6th	**9:00.** Old Havana Tour Transfer to Varadero (Gaviota package)	**14:00.** Registration **17:00.Opening Ceremony Vigia room** Welcoming dinner Bacunayagua Restaurant	Free
7th	**9:00. Plenary Session Vigia room** Novel approaches for allergen vaccines and allergy immunotherapy	**14:00. Vigia room 1.** Allergen structure and function **14:00. Vigia room 2.** New generation of adjuvants **17:30. Vigia room 1.** Optional Course (I)	Free
8th	**9:00. Plenary Session Vigia room** Adjuvants to allergy vaccines	**14:00. PlenaryDebate:** Dose-response in allergy and cancer IT **15:10. Plenary** WAO-AAAAI Seminar on allergen IT **16:30. Plenary** Poster sessionand rum testing	Free
9th	**9:00. Plenary Session Vigia room**Immunobiology of allergy	**14:00. Vigia room 1.** Pharmaceutical and pharmacological development of allergen vaccines **14:00. Vigia room 2.** Adjuvants for infectious diseases vaccines **15:55.Vigia room 2.** Adjuvants to chronic infectious and non-infectious diseases **17:50. Vigia room 1.** Optional Course (II)	Free
10th	**9.00. Vigia room 1.** Regulatory outlook, allergen IT and allergy care **9.00. Vigia room 2.** Adjuvants for veterinary vaccines	**14:00. Vigia room 1.** Clinical trials of allergen immunotherapy **16:00. Vigia room. Plenary** Closing Ceremony **20.00.** Return to Havana of national delegates	Free
11th	**Bilateral meetings**	Return to Havana of foreign delegates	Free

LUNCH

DINNER

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PLENARY OPENING

Sunday afternoon, May 6th

Time	Name	Title
17:00-17:20	Alexis Labrada Co-President Org. Committee National Center of Bioproducts, Cuba	**Welcome**
17:20-18:00	Richard Lockey, Past President WAO, James A Haley Veteran's Hospital, Tampa, Univ. South Florida, USA	**KNA 1.** Asthma, a syndrome of multiple phenotypes and co-morbidities
18:00-18:40	Oliver Pérez, President SCI Finlay Institute, Cuba	**KNA 2.** Shooting to some immunological paradigms using potent adjuvants

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Plenary Session: Novel approaches for allergen vaccines and allergy immunotherapy

Monday morning, May 7th

Chairs: Fernández Caldas E. and Valenta R.

Time	Name	Title
9:00-9:20	Rudolph Valenta, Medical University, Vienna Austria	**C-1** From mechanisms of allergen-specific immunotherapy towards side effect-free allergy vaccines
9:25-9:45	Fatima Ferreira, University of Salzburg, Austria	**C-2** Development of immunogenic hypo-allergens for SIT
9:50-10:10	Enrique Fernandez Caldas, Inmunotek, Spain	**C-3** New strategies to characterize allergoids: an old problem revisited
10:15-10:35	Margarete Focke Tejkl, Medical University, Vienna Austria	**C-4** Molecular and immunological characterization of BM32, a grass pollen allergy vaccine based on non-allergenic peptides fused to hepatitis PreS
10:40-11:00	**Coffee break**	
	Chairs: van Ree R and Larche M	
11:00-11:20	Ronald van Ree, AMC-University of Amsterdam, The Netherlands	**C-5** The FAST project: Recombinant hypoallergenic approaches for the treatment of food allergy
11:25-11:45	Marianne van Hage, Karolinska University, Sweden	**C-6** Pet allergens and new vaccines
11:50-12:10	Mark Larche, Mc. Master University, Hamilton, Canada	**C-7** Peptide immunotherapy
12:15-12:35	Rod Hafner, Circassia, UK	**C-8** Clinical efficacy of cat-Toleromune peptide immunoterapy. Results from a phase IIB clinical trial

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Vigia room 1: Allergen structure and function

Monday morning, May 7th

Chairs: Chapman M and Mari A

Time	Name	Title
14:00-14:20	Adriano Mari, IDI-IRCCS, Allergen Data Laboratories, Italy	**C-9** Biochemical, immunological, and clinical features of allergenic molecules: do they always overlap?
14:25-14:45	Martin Chapman, Indoor Biotecnologies USA	**C-10** Immunoassay based methods for allergen analysis
14:50-15:10	Anna Pomes, Indoor Biotecnologies USA	**C-11** Structural biology of allergens and allergen/antibody complexes
15:15-15-25	Eduardo Egea, Universidad de Norte, Barranquila, Colombia	**SOP-1**. A novel Device for the detection of surface deposited dust mite allergens. A simple sampling, dilution and analysis technique.
15:30-15:45	**Coffee break**	
	Chairs: Himly M and Briza P	
15:45-16:05	Martin Himly, University of Salzburg, Austria	**C-12** Lessons from recombinant allergens and hypoallergenic variants on protein aggregation allergenicity and immunogenicity
16:10-16:30	Peter Briza, University of Salzburg, Austria	**C-13** Mass spectrometry and proteomics in allergen characterization
16:35-16:55	Marcos Alcocer, Nottingham University, UK	**C-14** Food allergens, lessons from the Ber e 1 model system
16:55-17:05	Yvonne Vissers, Nestlé Research Centre, Switzerland	**SOP-2** Effect of boiling on aggregate formation and on the allergenicity of Ara h 1 and Ara h 2/6 from peanut.
	Optional Course Part I	
17:30-18:30	The Allergome Platform, a course for Molecular Allergologists. Adriano Mari (IDI-IRCCS, Allergen Data Laboratories, Italy)	

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Vigia room 2: New Generation of Adjuvants

Monday morning, May 7th

Chairs: Fresno M and Batista A

Time	Name	Title
14:00-14:30	Manuel Fresno, Centro de Biología Molecular "Severo Ochoa" Spain	**KNA 3:** Toll-like receptors based adjuvants: towards specific induction of protective immune responses
14:35-14:55	Els N. Meeusen, Monash University Australia	**C-15** Effects of different adjuvant formulations on cellular migration, maturation and antigen trafficking from the site of vaccination
15:00-15:20	Thorunn Olafsdottir, University of Gothenburg Sweden	**C-16** Combination adjuvants signaling through TLR9
15:25-15:40	**Coffee break**	
15:40-16:00	Gerardo Guillén, CIGB, Cuba	**C-17** VLP as adjuvants
16:05-16:25	María E Lanio, University of Havana, Cuba	**C-18** Liposomes as immunoadjuvants: Contribution of B-1 cells and immunopotentation with encapsulation of Sticholysins, pore-forming proteins of the sea anemone *Stichodactyla helianthus*
16:30-16:50	Fernando Ferreira, U de la R, Montevideo, Uruguay	**C-19** Saponins from *Quillaja brasiliensis* form ISCOM-like particles
16:55-17:15	Daniela Wojta-Stremayr, Medical University of ViennaAustria	**C-20** Anchor characteristics of artificially membrane anchored cytokines applied as 'natural adjuvants' bound to Virus-like particles are key to their optimal biological function
17:20-17:40	Roberto Grau, Universidad Nacional de Rosario Argentina	**C-21** New developments using probiotic spores as alive adyuvants in vaccines
17:45-18:05	Alexander Batista, TOXIMED, Cuba	**C-22** Understanding adjuvant immunotoxicity mechanisms for improve the safety evaluation during vaccine development

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Plenary Session: Adjuvants to Allergy Vaccines

Tuesday morning, May 8th

Chairs: Russo M and Moingeon P

Time	Name	Title
9:00-9:20	Momtchilo Russo, ICB-University Sao Paulo, Brazil	**C-23** Toll-like receptors in allergy
9:25-9:45	Phillip Moingeon, Stallergenes, France	**C-24** Adjuvants and vector systems for the sublingual route
9:50-10:10	Stephan Scheurer, Paul Ehrlich Institute Germany	**C-25** Clinical trials with TLR ligands for treatment of asthma and allergic diseases
10:15-10:35	Peter Creticos, Johns Hopkins University, USA	**C-26** Clinical trials with TLR ligands for treatment of asthma and allergic diseases
10:40-11:00	**Coffee break**	
11:00-11:20	Tom Holdich, Allergy Therapeutics, UK	**C-27** MPL ® as an adjuvant in allergy vaccines
11:25-11:45	Oliver Pérez, Finlay Institute, Cuba	**C-28** Proteo-Cochleate adjuvant platform for allergic vaccines
11:50-12:05	Wendy Ramírez, National Center of Bioproducts , Cuba	**SOP-3** IgG/IgE shift and Th1 response induced by a *Dermatophagoides siboney* vaccine using the AFPL1® combination adjuvant in sensitized mice
12:10-12:25	Beatriz Tamargo, Cuba	**SOP-4** Immunomodulatory antiallergic effect of a nano-liposome formulation containing *Dermatophagoides siboney* allergens and TLR ligands by sublingual route

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Plenary Debate: Dose-response Relationship in Allergy and Cancer Immunotherapy

Tuesday morning, May 8th

Chairs: Chairs: Lockey R and Fernández LE

Time	Name	Title
14:00-14:15	DesiréeLarenas, Hospital Médico Sur, Mexico	**C-29** Dose-response effect in allergen IT (Task Force Paper EAACI)
14:15-14:30	Jorgen Larsen, ALK-Abello, Denmark	**C-30** Clinical proof of concept for SQ-standardised house dust mite allergy immunotherapy tablets in patients with respiratory allergy
14:30-14:45	Luis E Fernández, Center of Molecular Immunology, Cuba	**C-31** Defining the optimal biological dose: The NGcGM3/VSSP cancer vaccine, a case study
14:45-15:05	General Discussion	

Plenary Session: WAO-AAAAI special seminar on subcutaneous immunotherapy

Time	Name	Title
15:10-15:40	Richard Lockey, James A Haley Veteran's Hospital, Tampa, University of South Florida, USA	**C-32** Allergen Immunotherapy, A 2011 Practice Parameter Update
15:40:16:10	Dennis Ledford, James A Haley Veteran's Hospital, Tampa, University of South Florida, USA	**C-33** Allergen Immunotherapy, Prevention, Recognition and Treatment of Systemic Allergic Reactions
16:10-16:30	Discussion	

Plenary Poster Session and Rum Testing Session 16:30 -19:00

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Plenary Session: Immunobiology of Allergy

Wednesday morning, May 9th

Chairs: Crameri R and Rhyner C

Time	Name	Title
9:00-9:20	Reto Crameri, SIAF, Switzerland	**C-34** The diagnosis is the first step of any allergen specific immunotherapy: are we measuring what we should?
9:25-9:45	Claudio Rhyner, SIAF, Switzerland	**C-35** IgE-binding epitopes: facts and speculations.
9:50-10:10	Phillip Moingeon, Stallergenes, France	**C-36** Allergen-specific CD4+ T cell responses in healthy, allergic individuals and during immunotherapy
10:15-10:35	Winfried Pickl, MedicalUniversity, ViennaAustria	**C-37** Immunosomes for modulation of allergen-specific immune responses
10:40-11:00	**Coffee break**	
	Chairs: Platts-Mills T and Caraballo L	
11:00-11:20	Luis Caraballo, III - University of Cartagena, Colombia	**C-38** Parasite infections and allergen sensitization
11:25-11:45	Neuza Alcantara Neves, ICS-FederalUniversity of Bahia Brazil	**C-39** The effect of single and multiple infections in atopy and wheezing in Latin American children.
11:50-12:10	Leonardo Puerta, III - University of Cartagena, Colombia	**C-40** The role of fattic acid binding proteins in allergy
12:15-12:35	Luciana Mirotti, ICB-University of Sao Paulo, Brazil	**C-41** Neural pathways in allergy

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Vigia room 1: Pharmaceutical and pharmacological development of allergen vaccines

Wednesday morning, May 9th

Chairs: Barber D and Dreborg D

Time	Name	Title
14:00-14:20	Domingo Barber, ALK-Abello, Spain	**C-42** Bioavaliability of allergen vaccines
14:25-14:45	Erika Jensen-Jarolim, Medical University, Vienna Austria	**C-43** Safety aspects of allergen subunit vaccines
14:50-15:10	Sten Dreborg, Uppsala University, Sweden	**C-44** Pharmacokinetics and standardization of mPEG modified allergens
15:15-15:35	Ulla Seppälä, ALK-Abello, Denmark	**C-45** Using mass spectrometry for determination of major allergens in allergen standardization
15:40-15:55	**Coffee break**	

Chairs: Kerkvliet E and Neubauer A

15:55-16:15	Erica Kerkvliet, HAL, The Netherlands	**C-46** Potency tests for modified and alum-adsorbed modified products
16:20-16:40	Betty Goedewaagen, CITEQ biologics, The Netherlands	**C-47** Quality and allergen composition of mite allergen source materials
16:45-17:05	Angela Neubauer, Biomay, Austria	**C-48** Development of BM32 - a Recombinant Vaccine for Immunotherapy of Grass Pollen Allergy: from R&D to first clinical trials
17:10-17:30	Hans Huber, Biomay, Austria	**C-49** GMP manufacturing and quality control of recombinant allergy vaccines

Optional course part II

17:50-18:50	The Allergome Platform, a course for Molecular Allergologists. Adriano Mari (IDI-IRCCS, Allergen Data Laboratories, Italy)

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Vigia room 2: Adjuvants for infectious diseases vaccines

Wednesday morning, May 9th

Chairs: Guzman MG and Romeu B

Time	Name	Title
14:00-14:20	María G Guzman, IPK, Cuba	**C-50** Advances of Cuban dengue vaccine candidates. Adjuvant uses
14:25-14:45	Ernesto Marcos, CIGB, Cuba	**C-51** Virus like particles based on the recombinant capsid protein of dengue-2 enhance the immune response against recombinant proteins
14:50-15:10	Jeffrey J Bowden, Flinders UniversityAustralia	**C-52** Recombinant H1N1/2009 pandemic influenza vaccine containing Advax™ polysaccharide adjuvant: Randomized clinical trial of immunogenicity and safety
15:15-15:35	BelkisRomeu, Finlay Institute, Cuba	**C-53** AFCo1 and AFPL® overcome the thymus-independent stimuli of unconjugated polysaccharide antigens
15:40-15:55	**Coffee break**	

Adjuvants to chronic infectious and non-infectious diseases

Chairs: Christensen D and Acosta A

15:55-16:15	Dennis Christensen, Statens Serum Institut Denmark	**C-54** On the role of vaccine delivery for the induction of functional immuneresponses by TB vaccines
16:20-16:40	Armando Acosta, Finlay Institute, Cuba	**C-55** Adjuvants modulate the immune response to proteoliposomes of *Micobaterium smegmatis* in mice
16:45-17:05	Marta Romano, Scientific Institute of Public HealthBelgium	**C-56** DNA vaccines for the prophylaxis of tuberculosis
17:10-17:30	Erika Jensen-Jarolim, Medical University, Viena, Austria	**C-57** Mimotope vaccines in cancer to induce IgG or IgE
17:35-17:55	Luis E Fernández, CIM, Cuba	**C-58** Towards an Adjuvant for Immuno compromised Scenarios
18:00-18:10	Darien García, CIGB, Cuba	**SOP-5** Co-administration of CR3 with core and surface hepatitis B antigens induces a Th2-Th1 shift of the HIV-specific response

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Vigia room 1: Regulatory outlook, allergen immunotherapy and Allergy Care

Thursday morning, May 10th

Chairs: Rabin R and Fernández L

Time	Name	Title
9:00-9:20	Ronald Rabin, FDA, USA	**C-59** Status of allergen products in USA
9:25-9:45	Detlef Bartel, Paul Ehrlich Institute, Germany	**C-60** Product regulation approach and batch release in Germany
9:50-10:10	Victor Cunha, FDA Allergenic, Brazil	**C-61** Current situation of allergenic extracts registration in Brazil
10:15-10:35	Judith Cartaya, CECMED, Cuba	**C-62** Progress in regulation of therapeutic vaccines and biological products in Cuba
10:40-11:00	**Coffee break**	

Chairs: Fernández Caldas E andLabrada A

11:00-11:20	Jorge F Maspero University of Buenos Aires, Argentina	**C-63** Design of novel sublingual and subcutaneous immunotherapies in Latin American countries
11:25-11:45	Alexis Labrada, National Center of Bioproducts, Cuba	**C-64** Introducing allergen immunotherapy into the public healthcare sector: the Cuban experience
11:50-12:10	Miguel Hinojosa, Hospital Ramón y Cajal, Madrid, Spain	**C-65** Management of occupational allergy
12:15-12:35	Ruth Creticos, USA	**RTD-1 Round Table Discussion.** The impact of allergic conditions on children with speech and language disorders

Clinical Trials of Allergen Immunotherapy

Chairs: Creticos P and Larsen J

Time	Name	Title
14:00-14:20	Peter Creticos, Johns Hopkins University, USA	**C-70** Primary endpoints, validation, and clinical efficacy in trials with subcutaneous and sublingual immunotherapy
14:25-14:45	Jorgen Larsen, ALK-Abelló, Denmark	**C-71** Disease modifying effect of a SQ-standardised grass pollen allergy immunotherapy tablet
14:50-15:10	Andreas Nandy, Allergopharma, Germany	**C-72** Recombinant allergens for allergen specific immunotherapy
15:15-15:35	Reymond C, Anergis, Switzerland	**C-73** Hypoallergenic Bet v 1 derived contiguous overlapping peptides are well tolerated in human and lead to sustained immunological responses
15:40-15:55	**Coffee break**	

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Vigia room 2: Adjuvants for veterinary vaccines

Thursday morning, May 10th

Chairs: Ascarateil S and Sifontes S

Time	Name	Title
9:00-9:30	Stephane Ascarateil, Seppic, France	**KNA. 4** Vaccine adjuvants designed for veterinary and human use
9:35-9:55	Domingo W. Fontisiella, *Fishing Company of Villa Clara (Pescadilla),* Cuba	**C-66** Use of efficiency model in the Aquiculture experimentation
10:00-10.20	Domingo W. Fontisiella, *Fishing Company of Villa Clara (Pescadilla),* Cuba	**C-67** Fish diseases and use of vaccines, probiotics and other pharmaceutical products in aquaculture
10:25-10:45	Sergio Sifontes, Chemical Bioactive Center Cuba	**C-68** Immunochemotherapy using nitrovynilfuran compounds entrapped in immunoactive cochleate structures
10:50-11:05	**Coffee break**	
	Chairs: Fernández Caldas E andLabrada A	
11:05-11:25	Carlos E Pérez, CIGB, Camagüey, Cuba	**C-69** Methodology to design and optimize stable oily formulations using Montanide 888 VG
11:30-11:40	Antonio Miranda, Medical Science University, Holguín Cuba	**SOP-6** Adjuvant use in production of tetanous antitoxin in horses
11:45-11:55	Syduet, González, LABIOFAM Cuba	**SOP-7** Obtainment of aqueous and oily vaccines against paramyxovirus type 1 infection in pigeons and assessment of immune response
12:00-12:10	Mauren Villalta-Arrieta, Universidad de Costa Rica, Costa Rica	**SOP-8** Comparison of the adjuvant activity of aluminum hydroxide and calcium phosphate on the antibody response towards the Bothropsasper snake venom

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Vigia room: Plenary Closure

Thursday morning, May 10th

Time	Name	Title
16:00-16:30	Thomas Platts-Mills, University of Virginia, USA	**KNA 5.** IgE antibody responses: understanding the interaction between adjuvants and route of exposure
16:35	Belkis Romeu (Finlay), Alexis Labrada (BioCen), and Oliver Pérez (Finlay), Cuba	**Closing remarks**

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I. Adjuvants

P1.1 Role of B-1 cells in the immune response against an antigen encapsulated into phosphatidylcholine-containing liposomes. *Y Cruz-Leal, Cuba*

P1.2 Liposomes encapsulating Sticholysins with immunoadjuvant properties to stimulate an antigen-specific cellular immune response. *RJ Laborde, Cuba*

P1.3 Aluminum phosphate as adjuvant improves the immunogenicity of monovalent conjugates against serotypes 1, 14 and 19F of *Streptococcus pneumoniae*. *A Pérez, Cuba*

P1.4 Synthesis and evaluation of fresh aluminum phosphate for its possible use as adjuvant. *L Nadal, Cuba*

P1.5 Comparison of freshly prepared aluminum phosphate and commercial Adju-phos adsorption capacity. *Y Serrano, Cuba*

P1.6 The hen's egg test on chorioallantoic membrane (HET-CAM), useful in vitro assay for assessing irritation properties of immunoadjuvants formulations. *A Batista-Duharte, Cuba*

P1.7 Molecular mimicry between mayor proteins from *Neisseria meningitidis* B and self-proteins is insufficient condition for triggering early post-vaccination autoimmunity in mice and rats vaccinated with OVM-derived adjuvants. *A Batista-Duharte, Cuba*

P1.8 Obtain of aqueous and oily vaccines against *Paramyxovirus* Type 1 infection in pigeons and assessment of immune response. *S González, Cuba*

P1.9 A Recombinant Vaccine of H5N1 HA1 Fused with Foldon and Human IgG Fc Induced Complete Cross-Clade Protection against Divergent H5N1 Viruses. *BJ Zheng, China*

P1.10 Adjuvant effect of bacterial outer membrane vesicles with penta-acylated lipopolysaccharide on antigen-specific T cell priming. *DH Lee, Korea*

P1.11 Preliminary results of AFCo1 and AFPL1 co-administered with rPhl p5 by parenteral and mucosal route in mouse, *M Cuello, Cuba*

P1.12 Diverse adjuvant activity of saponins from the South American flora. *F Silveira, Uruguay*

P1.13 Comparison of the adjuvant activity of aluminum hydroxide and calcium phosphate on the antibody response towards the Bothrops asper snake venom. *M Villalta, Costa Rica*

P1.14 Co-administration of CR3 with core and surface hepatitis B antigens induces a Th2-Th1 shift of the HIV-specific response. *D García, Cuba*

P1.15 IMS4112 and VLP of HBV as adjuvants for a quimeric protein of HIV-1. *I Rodriguez-Alonso, Cuba*

P1.16 Influence of the adjuvant in the immune response to proteoliposomes and liposomes from non-pathogenic mycobacteria in mice. *ME Sarmiento, Cuba*

P1.17 Comparison of the adjuvanticity of different oil/water emulsions prepared with Freund´s adjuvant on the antibody response towards snake venoms. *K Rodríguez, Costa Rica*

P1.18 Very small size proteoliposomes derived from *Neisseria meningitidis*: an effective adjuvant for antigen-specific cytotoxic T lymphocyte response stimulation under leukopenic conditions. *L Oliver, Cuba*

P1.19 Results of repeated dose intramuscular administration of the adjuvant combination Montanide ISA51 VG/VSSP in Sprague Dawley rats. *A. Mancebo, Cuba*

P1.20 Interleukin 2 mutein as adjuvant for anticancer therapies. *JA Sánchez, Cuba*

P1.21 From *Neisseria meningitidis* serogroup B vaccine to Potent Adjuvants. M Lastre, Cuba

P1.22 Physicochemical and Biological Characterization of 1E10 Idiotypic Vaccine. *Y. Machado, Cuba*

P1.23 Consistency of the manufacturing process for active pharmaceutical ingredients for gangliosides vaccines (NGcGM3/VSSP and NAcGM3/VSSP) in BioCen. *M Alvarez, Cuba*

P1.24 Influence of phosphate salts and alum content on to the immunogenicity of a *Dermatophagoides siboney* AFPL1-adjuvanted vaccine. *R Samalea, Cuba*

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II. Novel Allergy Vaccines and Pharmacological Preclinical Evaluation

P2.1 Usefulness of a murine model of respiratory challenge test for the preclinical pharmacological evaluation of novel allergen vaccines. *V Bourg, Cuba*

P2.2 Experimental model of asthma to *Blomia tropicalis* recombinant antigens. *NM Alcântara Neves, Brazil*

P2.3 Potential of Brazilian Natural Products as immunomodulators of allergic diseases. *CA Figueiredo, Brazil*

P2.4 Effects of 4-Alil-2-Metoxifenol in murine model of allergic respiratory disease induced by *Blomia tropicalis* mite. *CA Figueiredo, Brazil*

P2.5 Toxicity assay in repeated doses of *Dermatophagoides siboney* allergen extract in mice. *Y González, Cuba*

P2.6 Enzymatic activity in native and polymerized mite allergen extracts. *JI Tudela, Spain*

P2.7 Beneficial Effect of Hydrolyzed Egg in Allergy. *YM Vissers, Switzerland*

P2.8 Histological analysis as a tool for the preclinical pharmacological evaluation of therapeutic allergy vaccines. *JF Infante Bourzac, Cuba*

P2.9 Dose repeat toxicity and local tolerance of a novel nano-liposome adjuvanted allergen vaccine. *A Morejón, Cuba*

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III. Allergen Immunotherapy, Clinical Trials and Clinical Practice

P3.1 Clinical evolution of allergic patients under immunotherapy with Valergen.mite vaccines. *AO Benítez Goitizolo, Cuba*

P3.2 Adherence and tolerability of mite allergen sublingual immunotherapy with different dosing schedules in asthmatic children. *R Castro Almarales, Cuba*

P3.3 Efficacy and Safety of a Novel Ragweed Allergy Immunotherapy Tablet (AIT) During Peak Season in North America. *P Creticos, USA*

P3.4 Adverse Events related to allergen immunotherapy with standardized House Dust Mite vaccines in asthmatic patients. *AO del Arco Pérez, Cuba*

P3.5 Effectiveness and impact on Quality of Life of SublinualInmunotherapy with House Dust Mite vaccines in allergic conjunctivitis. *I Figueroa García, Cuba*

P3.6 Adverse reactions to allergen immunotherapy using house dust mite vaccines in asthmatic patients. *Y Fuentes Páez, Cuba*

P3.7 Clinical response to subcutaneous immunotherapy (SIT) in polysenzitised profiline positive patients is reflected by the modification of allergen-specific IgE, IgG4, IgE/IgG4- Ratio levels measured by Microarray technique (ISAC 112). *F. Gay-Crosier, Switzerland*

P3.8 3-years course of allergen-specific immunotherapy (ASIT) in children with allergic diseases. *A Gevorkyan, Russia*

P3.9 Immunologic and clinical efficacy of sublingual immunotherapy with House Dust Mite allergen vaccines in pediatric patients with persistent moderate allergic rhinitis. *JV González Abreu, Cuba*

P3.10 Allergy Training and Immunotherapy in Latin America: How Survey-results lead to a Regional Overview. *D Larenas-Linnemann, Mexico*

P3.11 Cycloferon enhances the effectiveness of SCIT. *L Lazarenko, Russia*

P3.12 Preliminary results of a national pharmacovigilance study of allergen injection and sublingual immunotherapy with house dust mite allergen extracts. *M Mateo Morejón, Cuba*

P3.13 Efficacy and safety of immunotherapy with Valergens Dp and Bt extracts in asthmatic adults. *M Morales Irarragorri, Cuba*

P3.14 Sublingual Immunotherapy and Quality of Life in asthmatic patients. *R NaranjoRobalino, Cuba*

P3.15 Assessment of efficacy and safety of allergen immunotherapy with standardized mite allergen vaccines in the clinical practice. *TC Pérez Jiménez, Cuba*

P3.16 A Report of Pharmacovigilance of Adverse Reactions to Allergen Immunotherapy by Sublingual Route in asthmatic patients. *VR Pérez Rodríguez, Cuba*

P3.17 Safety of Sublingual Immunotherapy with Standardized Vaccines of Domestic Mites. *M RonquilloDíaz, Cuba*

P3.18 Adverse reactions to subcutaneous allergen immunotherapy in patients with asthma, rhinitis and /or atopic dermatitis with an ultra rushbuildup immunotherapy. *J Sánchez, Colombia*

P3.19 Elevated tear fluid levels of MIP-1 alfa in patients with atopic dermatitis. *M Mrugacz, Poland*

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IV. Allergen Characterization, Allergen-Specific Diagnostics, Cross reactivity and Exposure

P4.1 Allergic sensitization and IgE binding profile to hen's egg allergens in Cuban adults and children. *D Álvarez Díaz, Cuba*
P4.2 Sensitization to storage mites, wheat and yeast allergens in Cuban bakers. *M Álvarez-Castello, Cuba*
P4.3 Cross-reactivity between *Olea europaea* and *Betula verrucosa* extracts. *B Cases, Spain*
P4.4 Allergic sensitization and IgE/IgG4 profile to Soy food and respiratory allergens in Cuban adults and children. *R Cruz Jiménez, Cuba*
P4.5 Sensitization to mite allergens and its role in atopic dermatitis in children. *A Díaz Rodríguez, Cuba*
P4.6 Serodiagnosis of allergy to *Blomia tropicalis* using recombinant antigens instead of whole mite extract increases the assay specificity. *JC Marques Ponte, Brazil*
P4.7 IgY Policlonal antibodies Conjugated to Colloidal Gold Nanoparticles as a novel tool for the detection of of Dust Mite Allergens. *E Egea, Colombia*
P4.8 A novel Device for the detection of surface deposited dust mite allergens. A simple sampling, dilution and analysis technique. *E*
P4.9 House Dust Mite Fauna and its Relationship to Allergen Skin Tests in Six Mexican States. *BI Fernández Duro, Cuba*
P4.10 Sensitization to *Dermatophagoides pteronyssinus*, *Dermatophagoides siboney* and *Blomia tropicalis* in children from three family physician's offices. *M González León, Cuba*
P4.11 Allergic sensitization and IgE/IgG4 profile to wheat food allergens in Cuban adults and children. *M Jiménez, Cuba*
P4.12 In silico dimerization propensity study of house dust mite allergens from families 5 and 21. *S Khemili, Algeria*
P4.13 Allergen sensitization patterns in Mexican patient with rhinitis symptoms: a prospective nation-wide study. *D Larenas Linnemann, México*
P4.14 Sensitization to three domestic mites in allergic children in a suburban area in Cuba. *MC Reyes Zamora, Cuba*
P4.15 Is sensitisation to *Cupressus spp*. (Cypress) and *Platanus spp.* due to allergen exposure or cross-reactivity? *J Subiza, Spain*
P4.16 Is allergy to peanut important in Cuba? *D Torralba Averoff, Cuba*
P4.17 Blo t 12 as a species-specific marker of *Blomia tropicalis*. *J Zakzuk, Colombia*

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V. Epidemiology of asthma and allergic diseases. Allergy and parasitism

P5.1 Food and nutrition insecurity: a marker of vulnerability to asthma symptoms. *NM Alcantara-Neves, Brazil*

P5.2 Prevalence of asthma and its association with body mass index and abdominal obesity in children from a small Brazilian town. *NM Alcantara-Neves, Brazil*

P5.3 Atopy as a risk factor for recurrent Upper Respiratory Tract Infections in children under 5 years. *A Ciria Martín, Cuba*

P5.4 The speciific IgE Anti *Ascaris lumbricoides* seems to influence the serologic IgE response to Indoor allergens in Colombian pediatric asthmatic patients. *E Egea, Colombia*

P5.5 Sensitisation to Der p1, Der p2, Der p10, *Ascaris suum* and shrimp in a population of allergic patients living on the tropical Caribbean Island of Martinique. *S Lafosse-Marin, Martinique, France*

P5.6 Cross-reactivity between *Dermatophagoides pteronyssinus*, *Ascaris suum, Anisakis simplex,* shrimp, and two tropomyosins of different shrimp species in Martinique. *S Lafosse-Marin, Martinique, France*

P5.7 Association between asthma and IgE levels. *M Martínez, Venezuela*

P5.8 Cross-reactivity between anti-mite and anti-helminth IgE antibodies and their effect in the diagnosis of atopy by IMMUNOCAP assay. *JC Marques Ponte, Brazil*

P5.9 Identification of protein fractions obtained from somatic extract of *Toxocara canis* with immunoregulatory effects on peripheral blood mononuclear cells of allergic and non-allergic donors. *NM Alcantara-Neves, Brazil*

P5.10 Association between FeNO and IgE levels in Patients with allergic asthma. *J Torres, Venezuela*

P5.11 Longitudinal study "Natural history of asthma in Havana, Cuba": results from the first year. *SJ. Venero, Cuba*

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VI. Standardization, Pharmaceutical, Quality and Regulatory Aspects of Allergen Vaccines

P6.1 Allergenic potency of *Parietaria judaica* and *Olea europaea* using different serum pools containing, or not, specific IgE to the major allergens. *E Abel-Fernández, Spain*

P6.2 Quality Risk Management application in the manufacturing process of raw allergenic material of house dust mite vaccines. *PC Alfonso, Cuba*

P6.3 Grass and mite mixtures: how does the proteolytic activity of *Dermatophagoides pteronyssinus* affect *Phleum pratense* extracts? *E Fernández-Caldas, Spain*

P6.4 Process consistency of *Dermatophagoides siboney* purified allergen fraction as the active pharmaceutical ingredient for a novel adjuvanted allergen vaccine. *D Martínez, Cuba*

P6.5 Stability study of food allergen extracts in glycerin and aqueous formulation. *A Más Quintero, Cuba*

P6.6 Stability study of bulk allergen extracts of House Dust Mites in freezing state. *M Odelin, Cuba*

P6.7 A simple method for assessing Ultrafiltration efficiency for allergen extracts. *O Oliva Díaz, Cuba*

P6.8 Validation of sterile filtration processes of house dust mite allergen vaccines. *Y Perea, Cuba*

P6.9 Use of gamma-sterilized rubber stoppers for assuring lower moisture content in lyophilized house dust mite allergenic extracts. *H Pérez, Cuba*

P6.10 Microbial bioburden during the culture process of *Dermatophagoides pteronyssinus*. *M Sánchez, Cuba*

P6.11 Risk Analysis applied to Quality Improvement of the manufacturing process of the Active Pharmaceutical Ingredient of allergen vaccines. *N Seuret Hernández, Cuba*

P6.12 Establishing pharmacopeia a validated sterility test for glycerinated allergen extracts. *A Tsoraeva, Cuba*

P6.13 Cuban National Regulatory Authority: Legal Basis for Allergenic Products. *L Fernández, Cuba*

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Program Summary

Plenary Session

1. Novel Approaches for Allergen Vaccines
2. Adjuvants to Allergy Vaccines
3. Debate: Dose response in allergy and cancer IT
4. WAO-AAAAI Seminar on allergen IT
5. Immunobiology of Allergy

Allergen Vaccines

1. Allergen Structure and Function
2. Adjuvants to Allergy Vaccines
3. Pharmaceutical and pharmacological development of allergen vaccines
4. Regulatory outlook, allergen IT and allergy care
5. Clinical trials of allergen IT

Adjuvants

1. New generation of adjuvants
2. Adjuvants for infectious diseases vaccines
3. Adjuvants to chronic infectious and non-infectious diseases
4. Adjuvants for veterinary vaccines

Optional Course

Poster Sessions

- Session I
- Session II
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- Session V
- Session VI

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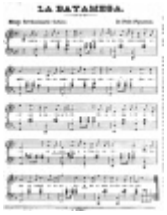

Royal Palm

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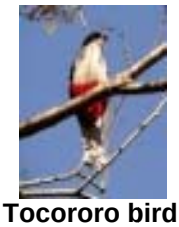

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Adjuvant2012 & AllergenVaccines2012
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Varadero, Cuba, May 6-11, 2012

KNA-1: Asthma, a syndrome of multiple phenotypes and co-morbidities

RF. Lockey

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Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role; in particular, mast cells, eosinophils, neutrophils (especially in sudden onset, fatal exacerbations, occupational asthma, and patients who smoke), T lymphocytes, macrophages, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of coughing (particularly at night or early in the morning), wheezing, breathlessness, and chest tightness. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment". Clinically observed asthma phenotypes, defined as "entire physical, biochemical and physiological makeup in an individual as determined both genetically and environmentally, as opposed to genotype" have been described in a variety of ways, but there are no known consensus descriptions. The National Heart Lung and Blood Institute (NHLBI), The American Academy of Allergy Asthma, Asthma and Immunology (AAAAI), and The American Thoracic Society (ATS) convened a Task Force to develop a consensus to identify phenotypes of asthma. More common phenotypes were identified initially to obtain a general sense of variables that should be considered to identify a unique phenotype. Parameters associated with each phenotype include demographics, clinical characteristics, comorbidities, biomarkers, definition, research questions, and future directions. While some patients may fit one phenotype, others will have characteristics for more than one. Nine phenotypes have been identified and include 1) allergic asthma, 2) non-allergic (intrinsic asthma), 3) infection related asthma, 4) aspirin exacerbated asthma, 5) exercise induced asthma, 6) pre-asthma and wheezing in infants, 7) exacerbation proned asthma, 8) asthma with irreversible airflow limitation and 9) eosinophilic and neutrophilic asthma. Other phenotypes of asthma to be considered for inclusion in the future include occupational, severe, steroid resistant, fatality prone, irreversible and difficult to treat or brittle asthma. While knowledge of phenotypes is important to optimally treat asthma (personalized medicine), diagnosing and treating asthma requires knowledge of conditions that emulate asthma, coexist, and effect treatment. An excellent example of a comorbid or coexisting problem is vocal cord dysfunction. Up to 10% of patients who allegedly have asthma have vocal cord dysfunction and no asthma whereas up to 10% of patients with asthma have concomitant vocal cord dysfunction. While there are a variety of different comorbidities which affect asthma, only the most important will be reviewed in this lecture. These include rhinitis, nasal polyposis, gastroesphageal reflux disease, vocal cord dysfunction syndrome, sleep apnea, bronchiectasis, osteopenia and osteoporosis and psychological problems. In summary, asthma is a syndrome, with many phenotypes and many comorbid and coexisting problems. Evaluation of a patient with asthma must include the characteristics of asthma which help define a phenotype, assessment of its severity, as manifested by the past history of recurrent exacerbations and hospitalizations, and the comorbid and coexisting problems which can play a major role in successful outcomes.

KNA-2: Shooting to some immunological paradigms using potent adjuvants

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Background: In Science, the absolute truth is mostly relative afterwards. Some paradigms have been established regarding antigen immunogenicity. The immunological paradigms selected were: 1) Nano- and micro-particles have been used for parenteral and mucosal immunization, respectively. Nevertheless, the latter was not used for parenteral routes. 2) Thyme-independent polysaccharides (TI-2) neither work in newborns/infants nor induce immune memory response and could induce antibody hyporesponses. Covalent conjugation solves these problems. Could these facts be overcome using adjuvants? 3) Immunotherapy is mainly based in parenteral multidoses. Nevertheless, most allergies are mucosal and limited mucosal vaccination approaches are performed. 4) Vaccines are the most cost-effective strategy to prevent diseases; but vaccine coverage is still insufficient. We are in the gold era of Adjuvants and they are determinant in vaccine formulations. They immunopotentiate the innate immunity, deliver antigens to desired places, and polarize the adaptive immune response to the desired level. **Aims:** Show the effect of microparticles by parenteral routes; to overcome the TI-2 effect using potent adjuvants; to explore the mucosal route in allergy and sexual transmitted infections (STI); and to demonstrate the usefulness of Single Time Vaccination (sintimvas) to increase vaccination coverage. **Results:** We developed the Finlay Adjuvant (FA) Platform based on: Proteoliposome (PL) nanoparticles, afplx® and PL-derived Cochleates microparticles, afcox. A non-PL Co was also obtained. Afco1 works by mucosal and parenteral routes against model and vaccine antigens. Both, AFPL1 and afco1 overcome the TI-2 behavior without covalent conjugation. The mucosal route seems to be a promissory route in allergy and STI vaccination. Sintimvas works at least in mice and should be explored in human. **Conclusion:** Good adjuvantscan overcome these four paradigms.

C-1: From mechanisms of allergen-specific immunotherapy towards side effect-free allergy vaccines

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PendingAbstract

C-2:Development of immunogenic hypo-allergens for sit

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The development of a novel vaccine is a highly complex and demanding process. Once candidates has been designed and produced in the laboratory, they undergo vast series of pre-clinical in vitro and in vivo tests as well as optimization procedures. The most important parameters that should be considered in allergy vaccine design are specificity, safety, and efficacy. Specificity can be improved by substituting allergen extracts for the relevant allergen molecules, thus enabling patient-tailored immunotherapy. Safety issues are mostly related to ige-mediated side effects and can be tackled by genetic engineering of allergens. The efficacy of allergy vaccines strongly depends on its immunogenicity. But what are the determinants of immunogenicity? Generally, immunogenicity depends on the interaction with dendritic cells, which are specialized antigen presenting cells serving as interface between antigens and adaptive immunity. The efficiency of antigen uptake, activation of dcs, and stability to endolyososomal proteolysis are generally considered as important parameters in immunogenicity. In addition, intrinsic properties of the antigen are decisive in many steps of its interaction with dcs and have strong implications for the development of hypoallergenic vaccines. Structural manipulations aiming at the reduction of ige binding activity will certainly result in alterations of the fold and intrinsic properties of the allergen. Thus, the possibility that the newly acquired structural features of engineered hypoallergens will strongly affect their uptake and processing by antigen presenting cells should be taken into consideration in the development of hypoallergens for SIT.

C-3: New strategies to characterize allergoids: an old problem revisited.

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Pending Abstract

C-4: Molecular and immunological characterization of bm32, a grass pollen allergy vaccine based on non-allergenic peptides fused to hepatitis pres

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Pending Abstract

C-5: The FAST project: recombinant hypoallergenic approaches for the treatment of food allergy.

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Over the past decades, major allergens of the most important allergenic foods have been identified, isolated, cloned and expressed as recombinant molecules. This development has sparked off a revolution in allergology towards molecular approaches for diagnosis and therapy. The roles of individual molecules in clinical phenotypes are being elucidated rapidly. One of the first clear examples that individual molecules are associated with clinical phenotypes is allergy to Rosaceae fruits such as apple and peach. We now know that fruit allergy caused by pollen-related PR-10 proteins and profilins is mild and restricted to the oral cavity. On the other hand, fruit allergy caused by lipid transfer proteins is often at the basis of more severe systemic reactions. Molecular diagnostics based on such observations are a promis for the future and will increasingly replace extract-based diagnostics.

Availability of recombinant major food allergens has also revived the interest in immunotherapy for food allergy. First attempts towards the end of the last century to treat food allergy with aqueous extracts were upset by extremely high incidence of side-effects. The advent of recombinant technology now allows evaluation of safer approaches of immunotherapy for food allergy. Recombinant food allergens can be modified into hypo-allergenic molecules that do not cause unacceptable levels of side effects. In 2008, the European Commission decided to fund a consortium to develop hypo-allergenic molecule-based immunotherapy for the treatment of severe fish and fruit allergy. The FAST project has now selected the most promising hypo-allergenic variant of fish parvalbumin and is in the process of selecting one for peach lipid transfer protein. GMP production of mutant fish parvalbumin has successfully been carried out and toxicity studies are in progress. Phase I/iia trials in fish allergic patients are planned to start in the first half of 2013. The coming years will learn whether these approaches meet the expectations to provide a safe and effective treatment for food allergy.

C-6: Pet allergens and new vaccines

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Allergy to furred pets is a common cause of ige-mediated allergic disease. The most important cat (Felis domesticus) allergen is the secretoglobulin Fel d 1. We have previously identified Fel d 5 (iga) and Fel d 6 (igm) as allergens and recently shown that the carbohydrate epitope galactose-α1,3-galactose (α-Gal) is responsible for the ige binding to Fel d 5. Furthermore, that ige to α-Gal causes impaired allergy diagnostics in parasite-infected patients. Screening for ige to Fel d 1 and other allergens without carbohydrates might identify patients with true cat sensitization/allergy in parasite-infested areas. The presence of several dog (Canis familiaris) allergens has been established where Can f 1, Can f 2 and Can f 4 belong to the lipocalin family. We have lately cloned a novel dog allergen, Can f 6, that cross-reacts with lipocalins from cat (Fel d 4) and horse (Equ c 1). We found that 38 out of 100 (38%) dog sensitized subjects had ige against Can f 6. Thus, Can f 6 and homologous allergens may contribute to multi-sensitization and symptoms observed in patients with allergy to furred animals. To obtain new vaccines, using Fel d 1 as a model allergen, we have recently demonstrated in a murine model of cat allergy that covalent coupling of Vitamin D3 to Fel d 1 has beneficial effects on airway inflammation, airway hyperreactivity and Fel d 1-specific immune responses. Thus Fel d 1:VD3 is a promising candidate for use in allergen-specific immunotherapy for cat allergy. Moreover, the concept of direct linking of VD3 to allergens can be extended to allergen-specific immunotherapy with any kind of defined allergen.

C-7: Peptide immunotherapy

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Background: Allergen immunotherapy with whole allergen extracts is limited by ige reactivity which leads to adverse events that may be llife-threatening. Aims: To reduce ige reactivity and improve safety and efficacy using short peptide sequences from allergen proteins, formulated into peptide vaccines, for immunotherapy. Results: We demonstrated that induction of tolerance with peptide vaccines is an active process which reduces allergic sensitivity in the skin, nose, lungs and blood. A single administration of a prototype vaccine, designed to interact with multiple major histocompatibility complex (MHC) alleles, to cat allergic asthmatics resulted in the reduction of cutaneous responses to allergen challenge and reduction of Th1 and Th2 responses to allergen in vitro. IL-10 production by PBMC was increased after peptide therapy. Similar outcomes were seen with bee venom allergen phospholipase A2 (PLA2). Subsequently, we developed a novel in vitro assay that measures the suppressive activity of CD4+ T cells following peptide intervention. The results indicate that peptide immunotherapy induces a population of antigen-specific suppressive or "regulatory" T cells. Most recently we have developed vaccines for cat, ragweed, house dust mite, grass and other allergens that are currently in Phase II clinical trials. Conclusions: Peptide immunotherapy induces immunological tolerance to allergens, while reducing ige reactivity.

C-8: Clinical efficacy of cat-toleromune peptide immunoterapy. Results from a phase IIB clinical trial

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C-9: Biochemical, immunological, and clinical features of allergenic molecules: do they always overlap?

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Diagnosis of IgE-mediated diseases dramatically changed in the last few years. All modern technologies, mostly developed in the last 20 years, helped us to overcome some historical limitations forcing allergists to use extracts instead of molecules, skin testing instead of lab testing. Production of highly purified allergen preparations is now feasible either by advanced biochemical tools or by the recombinant DNA technology. Allergen preparations can be immobilized on rigid glass slides or on micro-beads to be used in IgE detection in patients' sera. We have recently reported several general or focused surveys where the experimental data were extracted by the routine testing procedure. At the same time, the availability of broad panels of allergens on the microarray is allowing us to depict the immunological profiles of single patients, understanding which therapeutic intervention is best suitable for each of them in a personalized fashion. We do consider such testing strategy relevant for therapeutic resource optimization facing at the same time patient's preferences and cost management by healthcare providers.

- Combining allergenic molecule or any other antigen with a microarray technology, supported by IT infrastructures (www.allergome.org) is of great help in daily practice
- The robustness of such support leads to generate wealth of data allowing epidemiological profiling of population worldwide without further investments
- Adoption of the electronic record (Allergome-InterAll) helps in gathering data leading to combined clinical and immunological profiling of single individuals
- Such profiling could drive to the therapy of choice motivating patients, authorities, private and public healthcare providers.

C-10: Immunoassay based methods for allergen analysis

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Immunoassay based methods for measuring specific allergens are an integral part of allergen standardization. They provide measurement of specific allergens in mass units and therapeutic dosing for allergy vaccines. This allows regulatory agencies e.g. The European Directorate for Quality of Medicines to develop international references and certified assays for allergens that can be included in the European Pharmacopoeia. Monoclonal (and polyclonal) antibody based assays are available for the most important allergens from most allergen sources. These assays are sensitive, specific and reproducible. The utility of immunoassays is determined by the quality of the antibodies, their isoform specificity, and purified natural or recombinant allergen standards. Building upon the success of the CREATE project, our group has recently a multiple allergen standard which is a cocktail of eight purified natural allergens, with the protein content of each allergen determined by amino acid analysis. This standard has been used to determine the allergen content of WHO/IUIS and FDA allergen reference materials. In addition, we have developed multiplex array technology for measuring 10 or more allergens simultaneously in a single test. This technology improves standardization because all of the measurements are made under identical conditions. An international multi-center trial carried out in nine laboratories, involving ~32,000 data points, showed that multiplex technology is highly reproducible between laboratories.Moreover, Levey-Jennings plots of low and high QC samples determined over a two year period showed extremely low rejection rates (<2%). Measurement of specific allergens by immunoassay based methods allows evidence-based trials of new vaccines and provides a global strategy for harmonization of allergen measurements.

C-11: Structural biology of allergens and allergen/antibody complexes

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Most B cell epitope mapping studies focus on the identification of linear epitopes by design, and are based on ige antibody recognition of synthetic peptides or recombinant allergen fragments. However, the native conformation of a large number allergens (or fragments) is preserved during sensitization, especially for inhaled allergens, and conformational epitopes are important for ige antibody binding. Even for food allergens, which have traditionally been considered to contain mostly linear epitopes (due to molecular cleavage during food processing or digestion), linear epitopes are not sufficient to explain ige reactivity. Our approach is to identify conformational epitopes on allergens by solving the X-ray crystal structure of allergen-antibody complexes, which allows a detailed analysis of the residues involved in the interaction. We solved the three-dimensional structures of the cockroach allergen Bla g 2 in complex with Fab or Fab' fragments of two monoclonal antibodies that interfere with ige antibody binding, and bind to opposite lobes of this inactive aspartic protease. The Bla g 2-antibody structures provide a model for analyzing antibody recognition of two non-overlapping epitopes and induction of mediator release. The structures of a cross-reacting antibody bound to either Der p 1 or Der f 1 were also solved, and revealed the structural basis of cross-reactivity. Site-directed mutagenesis analysis allowed identification of important amino acids involved in igg and ige antibody binding and led to thegeneration of hypoallergens. It also provided insight into the mechanisms of allergen-antibody interaction, including recognition through carbohydrates. These studies contribute to understand the ige immune response and expand our limited knowledge on conformational epitopes recognized by the B cell repertoire.

SOP-1: A novel device for the detection of surface deposited dust mite allergens. A simple sampling, dilution and analysis technique

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Introduction: Surface- deposited substance and particle sample collection and its latter analysis through dilution and reaction is of interest in the chemical, pharmaceutical and health, amongst others. **Objective:** The present work is related to develop and testing a device for sampling, diluting and analyzing dust mite allergens spread on surfaces, wherein said device comprises a self- cleaning collection system which through a rotating collecting surface and a scraper accumulate the sample. **Methods:** The device comprises a tank- like storage system comprising a weakened area wherein upon having the necessary sample quantity, the rotating surface breaks the weakened area of said storage system, making the sample mix with the solution. Another advantage of the device is it is self- contained. It does not require of any accessories or additional units (such as vacuums in other devices). It is a compact unit for sampling, dilution and analysis. **Results**: This device comprises a transport system allowing for the mixed dust sample containing mite allergens to be analyzed by the detection system. This device operates as an auto- contained unit requiring no direct handling of the material to be analyzed or any dilution agent. This device allows for the verification of the amount of mite allergens being analyzed which was collected. **Conclusions:** We present here a novel invention that could be a new tools in the field of public health for the control and prevention of the biological contamination of dust mite allergens.

C-12: Lessons from recombinant allergens and hypoallergenic variants on protein aggregation allergenicity and immunogenicity

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Allergen-specific immunotherapy (SIT) has been carried out for more than a century and represents a well-accepted treatment for ige-mediated allergic disease. As a general rationale for development of improved SIT vaccines (i) specificity, (ii) safety, and (iii) efficacy considerations have to be made.

I. Recombinant wildtype allergens may be used as active pharmaceutical ingredients for SIT displaying improved **specificity** to allergen extracts. For the most important allergenic sources the corresponding major allergens are available in recombinant form. An array of highly purified and well-characterized molecules have been produced during the past decades.

II. For the improvement of **safety** in SIT the concept of using hypoallergens possessing reduced ige-binding properties has been introduced. Several naturally occurring hypoallergens are currently available. However, it is important to note that the way of recombinant production may exert an influence on the ige-binding properties of the product. Beyond such extrinsic effects also intrinsic properties of allergens may determine antigen uptake/presentation and consequently antibody responses to highly homologous but slightly different isoallergens. Furthermore, genetically engineered hypoallergens with rationally designed structural differences may be constructed.

III. **Efficacy** of SIT can be improved by enhancing immunogenicity of the vaccine. As experiments involving animals shall be reduced *in vitro* immunogenicity assays are needed. Limited susceptibility to lysosomal proteolysis has been linked to immunogenicity. Thus, we have designed an *in vitro* degradome assay correlating well with *in vivo* immunization experiments.

In summary, specificity, safety, and efficacy represent three important criteria for optimization to improve SIT. Prerequisite for cost-effective product development is the availability of techniques for the selection of suitable candidate molecules which meet the needs for future SIT. This will be covered by an ever-growing set of physicochemical methods involving the technological advancement of highly sophisticated instrumentation.

C-13: Mass spectrometry and proteomics in allergen characterization

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In recent years, mass spectrometry became an invaluable method for allergen identification and characterization. Unfortunately, the methodology is complex and tends to be confusing to non-specialists. In this presentation, I will explain the principles of mass spectrometry, how proteins are sequenced by MS and how data base search algorithms work. My focus will be on the mass analyzers commonly used in proteomic research: quadrupole, time-of-flight and orbitrap analyzers, and their combinations in tandem MS. I will also cover practical aspects and present examples and results from my laboratory, like the identification of allergens bound to alum, allergen composition in allergoids and analysis of allergen isoforms in complex pollen extracts.

C-14: Food allergens, lessons from the Ber E 1 Model system

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In order to study the early "sensitization phase" we have used Ber e 1, the major allergen from Brazil nut seeds (Bertoletaexcelsa) and the related but weak allergenic SFA8 from sunflower seeds (Helianthus annus) as model systems. We showed that the purified recombinant proteins can mimic their native counterparts in structure, biophysical characteristics and immunoreactivity. We demonstrated that these proteins were highly resistant to thermal/chemical-induced denaturation and proteolytic digestion. Ber e 1 is the only intact brazil nut seed protein that withstands peptic digestion, making it a prime candidate for recognition by the gut immune system. By double labellingber e 1 (15N/13C) we established the 3D structure (NMR) in solution and successfully mapped the ige binding structural epitope using chimaeric proteins. BALB/c mice i.p. Sensitised with Ber e 1 did not show any significant antibody production, even in the presence of alum. The induction of a Th2 type response with the presence of Ber-specific anaphylactic antibodies was only restored when specific Brazilnut lipid fractions at 1:1 stoichiometry were added. NMR experiments showed that Ber e 1 possesses a hydrophobic site able to accommodate one lipid molecule. When incubated with murine bmdc/T-cell Ber e 1 induces a Th2 immature DC phenotype whilst the control SFA8 shows a mature Th1 response with high levels of IL-12 and no involvement of Toll-like receptors. Preliminary experiments with CD1 KO animals and T-cell clones from sensitive patients strongly suggested a involvement of inkt cells on the allergenicity of Ber e 1. In conclusion, our results demonstrated that endogenous natural lipids from Brazilnut are essential to the in vivo allergenicity of Ber e 1.

SOP-2: Effect of boiling on aggregate formation and on the allergenicity of Ara h1 and Ara h2/6 from peanut

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Background: Before consumption, peanuts are often boiled or roasted which could alter the structure of the peanut proteins and therefore result in altered allergenicity. Aim: Within the europrevall-project, we investigated how boiling affects the allergenic properties of major peanut allergens. Method: Both Ara h 1 and Ara h 2/6 were purified from either raw (N) or roasted (R) peanuts. N-Ara h 1 and N-Ara h 2/6 were boiled for 15 min at 100°C and 110°C respectively, both in the absence (H) or presence of glucose (G). Results: Both H-Ara h 1 and G-Ara h 1 showed partial loss of Ara h 1 secondary structure and formation of rod-like branched aggregates, while denatured R-Ara h 1 showed compact, globular aggregates, and no evidence of glycation. Both H-Ara h 2/6 and G-Ara h 2/6 were partly denatured, hydrolyzed and aggregated, while R-Ara h 2/6 retained its native structure. Thermal treatment of Ara h 1 and Ara h 2/6 resulted in both a reduced ige-binding capacity and an impaired ability to induce mediator release. In contrast, both R-Ara h 1 and R-Ara h 2 and 6 retained the ige binding capacity. In addition, thermal processing of both Ara h 1 and Ara h 2/6 did not affect the proliferation capacity and Th2 cytokine secretion of allergen stimulated PBMC. Conclusion: The data presented here, support the hypothesis that boiling, as opposed to roasting, may reduce the allergenicity of peanuts and might provide a novel form of antigen for use in allergen-specific immunotherapy.

KNA-3: Toll like receptors based adjuvants: towards specific induction of protective immune responses

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For humans and animals, vaccination has been the most successful medical intervention for the prevention of disease in the twentieth century. Nonetheless, there is no easy way for a vaccine in some infectious diseases, cancer or allergic disease. One of the most important reasons for this is the lack of induction of the appropriate immune response in each case. With the development of new vaccine antigens, from molecular recombinant technologies, also comes the need for more effective adjuvants that will facilitate the induction of appropriated adaptive immune responses. The majority of vaccines comprise two components, the antigen of therapeutic interest and an adjuvant. Adjuvants are fundamental in vaccines because: 1. Lower antigen doses in vaccine. 2. Enable complex combination vaccines, overcoming antigenic competition. 3. Overcome limited immune response in immunocompromised populations – such as young children, chronic diseases, etc 4. Increase antibody titers and effector T cell responses 5. Induce appropriate protective responses and more rapidly. 6. Extend the duration of response by enhancing memory B and T cell responses. Thus, the need for qualitatively specific induction of immune responses (Th_1, Th_2, Th_{17}, Treg, etc…), implies the need for tailor-made adjuvants for specific vaccines in each disease that are also safe, and immunologically potent. The importance of a correct adjuvant is exemplified in the actual successful Malaria vaccine in human clinical trials that use exactly, the same malaria antigen, previously shown to be ineffective with combined with a different adjuvant.

The search of adjuvants is now recognized as a bottleneck of vaccine development. There is a need to develop new, more powerful and especially specific adjuvant that redirect the immune response to the required protective type. The induction of one or another type of the immune response depends on cells of the type innate system activated. This activation depends of pattern recognition receptors (PRR) that pathogen interacts with. Probably, the best-known types of PPR are Toll-like receptors (TLR). There are 10 tlrs signalling different from one to another. Depending on which TLR become activated by the pathogen, different specific inflammatory, antiinflammatory, Th1, Th2, Th17, etc immune responses are generated. This property of tlrs of linking innate with adaptative Immune response makes them very attractive to design "intelligent vaccines". For these reasons, TLR ligands have become a focus in therapeutic studies for their potential use as adjuvants in vaccine formulations with the systematic development of vaccines that coordinately engage the innate and adaptive immune systems by incorporating a TLR ligand into the vaccine construct.

Currently, the major use for compounds that activate TLR2 are as adjuvants. Because of their ability to induce strong Th1 responses, TLR4 and TLR9 agonists are being developed for treatment of allergic rhinitis. In collaboration with Diomune S.L, a biotech company, specialized in research and development of new immunology treatments oriented to human and animal health areas in infectious, inflammatory and autoimmune diseases we have developed several new TLR2/4 inverse agonists, that promote the development of a Th1 innate immune response, which have interesting immunomodulating properties and does not have adverse effects. When combined with appropriate antigens, they trigger a recall type 1 response. Furthermore, they induce the secretion of IL-12 and a moderate Th1 response characterized by the secretion of IFN-gamma. Those compounds can activate macrophages and induces the secretion of proinflammatory cytokines, such as TNF but 100-1000 times less strong than full TLR agonists as pathogenic lpss from many Gram negative bacteria, as *Salmonella* or *E. Coli*. Owing to its immunomodulator effect, those TLR2/4 ligands affect the immune response of several parasites. We have proved its adjuvant ability in *Leishmania* and in influenza infections. Thus, a soluble extract of *Leishmania* (SLA) alone induce a Th2 response (not protective) but when combined with IVI303 it changes to a Th1 response and completely protects animals. This makes those ligands ideal adjuvants in any vaccine trial that required the induction of a moderate (not toxic) Th1 response.

C-15: Effects of different adjuvant formulations on cellular migration, maturation and antigen trafficking from the site of vaccination

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The recent recognition of innate immune activation as a critical driver of the adaptive immune response has major implications for vaccine development, and adjuvant research in particular. Using a unique and well established sheep model of pseudoafferent lymphatic cannulation, we investigated the kinetics of antigen transport and the innate inflammatory response in the afferent lymph draining the site of vaccination. Our studies demonstrated, for the first time, the in vivo effect of aluminium hydroxide adjuvant on soluble antigen flow and its enhancement of particulate antigen uptake by dendritic cells (dcs) at later time points^1. In contrast, monophosphoryl lipida (MPL) enhanced neutrophil and monocyte recruitment but had no effect on antigen uptake by dcs^2. We have expanded this analysis to other classes of adjuvant including oil in water, and liposomes with/without the immunomodulators poly I:C and cpg. Both dramatic and subtle differences were observed in antigen uptake by afferent lymph cells over time and in the cell types recruited into the lymph following injection of the different adjuvant formulations. Next generation sequencing of mrna derived from afferent lymph cells has revealed in detail the effects of novel adjuvant systems on gene expression over time, indicating that distinct molecular profiles can be generated for different adjuvant formulations. Further exploitation of this unique model will allow us to home in on the different cellular and molecular pathways that are activated by different adjuvants in vivo, and how they determine the physiological and immunological responses that impact on vaccine efficacy and safety.

C-16: Combination adjuvants signaling through tlr9

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Small oligodeoxynucleotides (ODN) containing unmethylated cpg dinucleotides (cpg ODN) induce strong inflammatory responses upon TLR9 ligation giving rise to a Th1 biased immune response upon vaccination. However, there are potential safety concerns associated with the use of cpg ODN as adjuvant in humans owing to the strong inflammatory response induced by cpg ODN that could theoretically lead to harmful non-Ag specific inflammatory disorders. Furthermore, high doses of cpg-ODN are often needed for immunostimulatory effects in humans and larger animals that may enhance the risk of excessive inflammatory response. To circumvent these limitations combination of TLR9 agonist and other immunomodulatory substances have recently been considered with promising results. Few examples are: cpg-odns combined with the human adjuvants alum and MF59, IC31®, a two component adjuvant consisting of an antibacterial peptide (KLK) and an ODN without cpg motif designed to bypass possible systemic side effects of cpg-ODN and conjugation of cpg-ODN to a completely non-toxic mutant of the B subunit of cholera toxin (CTB). The presentation will give an overview of some of the combination adjuvants stimulating through TLR9 and the beneficial roles they could have especially for induction of mucosal immunity, as well as elevation of vaccine-induced immune responses in low responder groups such as neonates, HIV positive and the elderly.

C-17: Virus LikeParticles as adjuvant

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The list of effective vaccine covers just a small fraction of the human microbial pathogens. The existing vaccines are mainly limited to the microorganisms we are able to culture and produce and/or to those whose killing is mediated by humoral response (antibody mediated). It has been more difficult to develop vaccines capable to induce functional cellular response needed to prevent or cure chronic diseases. Several adjuvant and formulation developed in the last twenty years could facilitate intracellular antigen processing and activate antigen presenting cells. Nevertheless, the adjuvant toxicity among others limitation may it difficult for new adjuvants to fulfil the increasing regulatory standards. With understanding of immunological system together with the achievements at preclinical levels a great interest appeared on therapeutic vaccination to treat chronic diseases. Several results suggest that specific enhancement of T-cell responses is possible in persistently infected patients. New strategies should be taken into account in the improvement of cell-based immune responses in order to control or eventually clear the virus or tumor cells. This work is aimed to present preclinical and clinical results with vaccine candidates developed using as a vaccine platform virus like particles (vlps). Able to stimulate mucosal as well as systemic immunity. Vlps based on envelope, membrane or nucleocapsid microbial proteins induce a strong immune response after nasal or systemic administration in mice, non human primates and humans. In addition, the immune response obtained was biased in a Th1 sense. The vlps were also able to immunoenhance the humoral and cellular immune responses against several viral antigens as measured by LPA and IFN-g ELISPOT assays. Studies in animals and humans with nasal and systemic formulations evidenced that it is possible to induce functional immune response against HBV, HCV and dengue.

C-18: Liposomes as immunoadjuvants: immunopotentation with encapsulation of Sticholysin (st), a pore-forming protein (pft) of the sea anemone *Stichodactyla helianthus* and contribution of B-1 cells

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Liposomes (LP) are attractive antigen carriers due to their ability to enhance humoral and cellular immune responses. Our group has studied a promising strategy to improve the antigen-specific cytotoxic T CD8+ lymphocyte (CTL) response based on the co-encapsulation of St and ovalbumin (OVA) into LP (LP/OVA+St). LP/OVA+St significantly enhanced an OVA-specific CTL activity in comparison with LP/OVA treatment in C57BL/6 mice. Moreover, LP/OVA+St conferred a higher protection to mice challenged with OVA-expressing tumor cells than LP/OVA. Interestingly, the inclusion into LP of a mutant St forming a reversible-inactive dimer stabilized by a disulphide bond also induced a similar antigen-specific CTL response, indicating the effectiveness of this safer alternative. On the other hand, the most frequent specificities of the antibody repertoire of peritoneal B-1 cells are phosphatidylcholine (PC) and phosphocholine. LP containing PC have been extensively studied as adjuvants. However, the role of B-1 cells on the immunoadjuvant properties of LP has not been explored. We demonstrated the participation of these cells in the generation of OVA-specific antibodies immunizing BALB/xid and BALB /c mice with OVA encapsulated into PC-containing LP. Transfer of PC-specific antibodies to BALB/xid mice partially restored the response induced by the liposomal preparation in these mice. The adjuvanticity of LP was further demonstrated to be dependent on PC, no enhanced anti-OVA response was induced when the antigen was encapsulated into phosphatidylglycerol-containing liposomes.
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C-19: Saponins from *quillaja brasiliensis* form ISCOM-type particles

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Current design of vaccines requires the use of more effective and safe adjuvants. Saponins are natural glycosides some of which have proven to be effective as adjuvants. These compounds are widespread in the plant kingdom, however, only saponins from the specie Quillaja saponaria Molina have been extensively studied and employed as adjuvant, even though the disadvantage of their toxicity. Colloidal nano-structured formulations containing saponins called iscoms (Immune Stimulating complexes) have been developed, which are less toxic than the pure ones. These complexes formed by self-assembly of saponins, cholesterol and phosphatidilcholine also require lower antigen doses to generate a protective immune response, what represents a substantial advantage when it comes to producing a vaccine. We hereby present the study of nano-structured formulations based on saponins from the tree species Quillaja brasiliensis, as an alternative to the use of Quillaja saponaria. The ternary system was formulated using the ethanol injection method and different mass ratios of each component. Analog formulations prepared using Quil A saponins were used as controls. The micelles type, size, Z-potential and homogeneity present in the resulting colloidal solutions were analyzed by negative staining Transmission Electron Microscopy and Dynamic light scattering, showing the formation of relatively heterogeneous dispersions of diverse colloidal structures, being remarkable the formation of ISCOM-type particles. The ISCOM-type particles yield and the preparation homogeneity were significantly improved by the use of a defined saponin fraction prepared from the aqueous saponin extract by chromatographic methods. These results show the possibility to prepare this type of nano-structured colloidal solutions from new natural sources, in particular saponins from the South American species Quillaja brasiliensis.

C-20: Anchor characteristics of artificially membrane anchored cytokines applied as 'natural adjuvants' bound to virus-like particles are key to their optimal biological function

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Background: Decoration of Virus-like particles (VLP) with functionally active, immunomodulatory molecules enhances particle immunogenicity and promotes selective immune recognition of antigens they bear.Aims: We sought to investigate the influence of anchor characteristics of artificially membrane-bound cytokines to improve the immunogenicity of VLP co-expressing viral antigens. **Methods:** One (1Ig), two (2Ig) or four (4Ig) immunoglobulin(Ig)-like domains of CD16b were inserted between the model cytokine IL-2 and the minimal GPI-anchor acceptor sequence of CD16b (GPI). **Results:** We identified a membrane anchor – '2iggpi' – conferring an up to tenfold increase in targeting IL-2 onto VLP, when compared to the minimal GPI-anchor acceptor sequence. A resulting increase in co-stimulatory potential was particularly prominent in situations where additional molecules were targeted onto VLP. When co-expressed on VLP with H2-Db presenting the immunodominant lymphocytic choriomeningitis virus glycoprotein(LCMV-GP)33-41 peptide, IL-2::2iggpi was superior to IL-2::GPI to induce proliferation of primary CD8+ LCMV-GP-specific P14 TCR transgenic T-cells in vitro, particularly when antigen was limited. Correspondingly, the proportion of ifngamma producing and CD107a+ antigen-specific T cells was increased. Upon in vivo challenge of mice with antigen-specific VLP co-expressing IL-2::2iggpi, larger proportions of proliferating LCMV-specific CD8+ T-cells could be isolated from blood, lymphnodes and spleens of immunized mice when compared to IL-2::GPI. **Conclusion:** Anchor characteristics critically influence the potency of developed IL-2 implemented as co-stimulatory molecule to affect VLP immunogenicity. VLP are currently used as promising immunization platforms, thus leading to further understanding of the mechanism of VLP action, may lay the ground for the development of even more advanced immunization strategies. The research was funded by the Austrian Science Fund (FWF): SFB4609-B19 and SFB F1816-B13, the Austrian Research Promotion Agency Bridge grant: 812079 & Biomay AG and the Christian Doppler Laboratory for Immunomodulation.

C-21: New developments using probiotic spores as alive adyuvants in vaccines

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Over the last few decades, the academies and pharmaceutical industries have identified a large number of potential new antigens for vaccine development. However, the successful of these developments relies on delivery systems able to prolong the stability of the vaccines after manufactured and avoid the rapid loss of their biological activity once administrated to the people. The bacterial spore is a tremendously resistant dormant cell that has been proposed as vaccine vehicle of new antigens to mucosal surfaces. Traditionally, Bacillus spores have been used as probiotics both in humans and animals. In particular, spores of Bacillus subtilis are safe for human consume (GRAS category) and easy to genetically manipulate in order to clone, express and display heterologous antigens on the spore surface as well as on the vegetative cell after spore germination at the mucosal sites. The intrinsic immunostimulating properties of bacterial spores, their interaction with APC and the induction of cytokines make spores attractive for their use as adyuvants for current and developing vaccines. Here, we will present the newest developments using probiotic spores as delivery vehicles, with intrinsic adyuvant capacity, of polyvalent antigens to mucosal surfaces after oral, nasal and/or vaginal administration. The importance of the high stability (heat-stability) and reproductive capacity, to increase immunogenicity, of the recombinant probiotic spores as alive adyuvants for vaccines as well as for the delivery of antigens and biotherapeutic molecules will be discussed.

C-22: Understanding adjuvant immunotoxicity mechanisms for improve the safety evaluation during vaccine development

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In spite of the existence of several regulatory guidelines regarding to vaccines and adjuvants, there are limitations for the prediction through the conventional non-clinical test, being recommended toxicological designs on a case-by-case basis. Taking in to count that the great majority of adverse reactions induced by vaccines are immunotoxic, here are discussed the present knowledge relating to the various parameters and reactants of the immune system at the cellular as well as molecular level that are believed to play a key role in immunotoxicity of adjuvants, addressing limitations of the current non-clinical models and new strategies that can be included during the process of research and development. Although many of the reactions discussed here were obtained from preclinical studies of experimental adjuvants, sometimes under extreme conditions, this information is of great value as it may facilitate the understanding of the toxicity mechanisms, aid the design of standardised models for the assessment of adjuvant safety and the possible application to new candidates for human vaccines.development.

C-23: Toll-like receptors in experimental asthma

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Mouse models of asthma have been developed to study the inflammatory mechanisms of asthma. For this, mice are sensitized and challenged with different kinds of allergens, being ovalbumin (OVA) the most widely used antigen. However, the OVA model of allergic lung disease does not represent the scenario to which asthmatics are normally exposed throughout their life. Thus, the murine model of asthma-like inflammation has been adapted to environmental airborne allergens such as those derived from house dust mites. Many patients have positive skin prick tests to allergens derived from the mites *Blomia tropicalis* or *Dermatophagoides pteronyssinus* and sensitization to these mites is associated with asthma. Although the allergen nature represents an essential component of asthma, other environmental factors such as endotoxin must also be considered in asthma development. Endotoxin is high molecular complex derived from the cell wall of Gram-negative bacteria constituted mainly by lipopolysaccharides (LPS) that activate immune cells via Toll-like receptors. LPS is ubiquitous in the environment and it is often present in household dusts. Epidemiological studies claim that endotoxin exposure during childhood could protect against the development of asthma later in life while other studies indicate that endotoxin exposure is a risk factor for asthma. Experimental data obtained with the murine OVA model are in line with the dual role of LPS in asthma development. It is now clear that the route, concentration, timing and duration of LPS exposure can determine whether LPS down- or up-regulates Th2-mediated allergic responses. Using the OVA model we have previously shown that a Th1/Th17-prone (LPS) adjuvant, administered subcutaneously with a Th2-prone (alum) adjuvant during OVA priming, inhibited Th2 responses in a dose dependent manner without inducing a Th1-associated lung inflammation. These results indicate that tlrs agonist could be used as vaccine in allergy. In order to extend these observations we determined the effect of tlrs agonists on allergic responses induced by *Blomia tropicalis* allergens and for comparison we used the OVA model. I shall present the results with tlrs agonists that activate TLR4 and TLR2 (conventional LPS), TLR4 (ultra pure LPS) or TLR2 (Pam3CSK4, a synthetic tripalmitoylated lipopeptide that mimics the acylated amino terminus of bacterial lipoproteins) and discuss the main differences obtained between the OVA and *Blomia tropicalis* model of allergic lung disease.

C-24: Adjuvants and vector systems for the sublingual route

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There is a strong interest in identifying adjuvants acting either on dendritic cells (dcs) or T cells which could elicit allergen-specific CD4+ Th1 and regulatory T lymphocytes in patients with established Th2 responses. Such adjuvants could eventually be administered in association with conventional allergen extracts prepared from natural sources, but also with recombinant allergens. In addition, mucoadhesive particulate vector systems can also be considered to improve AIT by better addressing the allergen(s) to tolerogenic antigen presenting cells. Among molecules impacting DC function to favour the induction of T Reg and Th1 responses we identified dexamethasone plus 1,25-dihydroxy vitamin D3, bacterial probiotic strains (eg Lactobacillus plantarum, L. Helveticum, Bifidobacterium bifidum) as well as TLR2 (Pam3CSK4) and TLR4 (monophosphoryl lipid A, synthetic lipid A analogs) ligands. All those molecules were able to enhance antigen-specific tolerance in a murine model of established asthma. Various vector systems including polymerized maltodextrin, chitosan and the adenylate cyclase protein from *Bordetella pertussis* were also shown to enhance tolerance induction during SLIT in mice with OVA-induced asthma, as a consequence of a specific addressing of the allergen to oral myeloid dcs. Some of those positively charged carbohydrate polymers yield mucoadhesive particles capable to bind efficiently to negatively charge epithelial cells, thus enhancing the duration of allergen contact with the mucosa. Collectively, such adjuvants and vector systems could help increasing the clinical efficacy of sublingual allergy vaccines while simplifying immunization schemes.

C-25: Preclinical development of tlr-ligand allergen fusion proteins as allergen vaccines

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Toll-like receptor (TLR) agonists are considered as suitable TH1-promoting adjuvants for the treatment of allergy. TLR ligands can either be mixed with allergen extracts or conjugated to allergens. In line with this non-methylated cpg motifs from bacterial DNA (TLR9 ligand) have been chemically conjugated to Amb a 1, and the S-layer protein sbsc from Gram+ bacteria (TLR2 ligand) was genetically fused to recombinant Bet v 1. Objective of our study was to investigate the immune modulatory properties of Listeria monocytogenes-derived TLR5 ligand flagellina (flaa) applied as mixture or genetically fused to the target allergen. Using Ova as model allergen we showed a recombinant flaa:Ova fusion protein to activate both, human and murine TLR5. Flaa:Ova induces up-regulation of TLR5 on myeloid dendritic cells (mdc) and represses TH1 as well as TH2 cytokine secretion from Ova-specific DO11.10 CD4 T cells by inducing IL-10 secretion from mdc. Furthermore, flaa:Ova was able to suppress Ova-induced cytokine secretion from TH2-polarized CD4 T cells derived from Ova/alum-immunized BALB/c mice. Using myd88-/- mice flaa:Ova-mediated suppression of cytokine secretion from Ova-specific T cells was shown to be dependent on TLR-signalling. The activation of mdc by flaa:Ova was suppressed by inflammasome and proteasome inhibitors. Prophylactic vaccination of BALB/c mice with flaa:Ova, but not the single components or the mixture of both proteins, was sufficient to prevent Ova-induced intestinal allergy. Prevention of clinical symptoms was accompanied by suppression of Ova-specific ige and induction of Ova-specific igg2a antibody production. In summary, flaa:Ova showed superior immune modulating capacities compared to the mixture of equimolar amounts of flaa and Ova. The immune modulating properties likely are determined by simultaneous administration of adjuvant and antigen to antigen presenting cells. Therefore, TLR ligand containing fusion proteins are promising vaccine candidates for intervention of TH2-biased allergic immune responses.

C-26: Clinical trials with tlr-ligands for treatment of asthma and allergic diseases

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This presentation will provide the clinical audience with an overview of the clinical trials that have been performed with specific immunostimulatory adjuvants including immunostimulatory cpg-oligonucleotides [synthetic oligonucleotides containing a cpg motif in specific sequence] that act through TLR-9 pathways and the MPL construct [an immunostimulatory adjuvant derived from detoxified LPS originating from the gram negative bacterium *Salmonella Minnesota* (Monophosphoryl Lipid A)] that acts through the TLR-4 receptor ligand. The clinical strategies of using an allergen complexed with an adjuvant, the adjuvant combined (mixed) with the allergen, or the adjuvant alone to induce immune modulation of allergic respiratory conditions including allergic rhinitis and asthma will be discussed.

C-27: MPL® as an adjuvant in allergy vaccines

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Monophosphorolyl Lipid A (MPL®) is a detoxified form of lipopolysaccharide (LPS) and is a selective immunostimulant which enhances the immune response to a co-delivered antigen. MPL is recognized by Toll-like receptor 4 (TLR4) on antigen-presenting cells (apcs) and stimulates APC maturation and migration to local lymph nodes and drives a Th-1 type response. This stimulation and re-direction of the immune system makes MPL suitable for use in allergen immunotherapy where it has been used in Pollinex Quattro® (MATA MPL) since 1999. Immunological studies in mice with allergoid + MPL formulations have confirmed allergen specific immune-stimulation in favour of Th-1 responses. In vitro studies with human peripheral blood mononuclear cells (pbmcs) from pollen-allergic patients have shown that, compared with placebo, MPL-adjuvanted allergoids raise interferon-γ and suppress IL-4 and IL-5 that MPL-mediated immunomodulation is IL-12 dependent. Additionally, lymphocyte stimulation following challenge with allergoids + MPL was more pronounced than challenge with allergoids alone. In clinical studies, the immunological benefit of MPL can be seen in the stimulation of igg4 and, more importantly, in clinical outcome. Ragweed MATA MPL showed a 42% reduction in total symptom score compared to 31% for Ragweed MATA (non-MPL) and a responder rate of 63% compared to 40% (p=0.018) respectively.The use of MPL in allergen immunotherapy has enabled the development of ultra-short course subcutaneous immunotherapy (uscit). Initial clinical study has also indicated that a shift towards a Th-1 pattern of immune response with corresponding clinical benefit may also be achievable with short-course sublingual immunotherapy with MPL.

C-28: Proteo-Cochleate adjuvant platform for allergic vaccines

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Background. TheAllergies affect from 17 to 45% of the population in different countries. The Phl p5 of *Phleum pretense* and Der s1of *Dermathophagoides siboney* are major allergens in Europe and Cuba, respectively. Long Immunotherapy treatment are frequent in allergy field and adjuvants as determinant vaccine component could be useful to shortest this treatment. They immunopotentiate (IP) the innate immunity, delivery (DS) the antigens/allergens to desired places, and polarized (Pz) the adaptative immune response to desire one. **Aims.** To show the potentialities of Proteo-Cochleate, Finlay adjuvant platform (FAP), particularly for allergy vaccines. **Results.** The FAP consists of a family of detergent-extracted outer membrane vesicles called Adjuvant Finlay Proteoliposome (afplx®) and their transformation into Cochleates (Co) named and afcox. In addition, PL non-derive Co was also develop. Afplx® and afcox contain similar protective proteins (which permit to be use as specific vaccine) and several synergistic microbial-associated molecular patterns (MAMP, which permit to be use as vaccine adjuvant). The most studied were AFPL1® and afco1 derived form *Neisseria meningitidis* serogroup B. They contain: LPS, Porins, lipoproteins, and trace of bacterial DNA as main synergistic IP; non-living DS based in lipids; LPS as main Pz driving to Th1 and CTL immune responses; and particles as nano- or micro-particles that could be processed by Proteasome. They: induce long-lasting memory response; and could be used with diverse antigens and by parenteral or mucosal routes. The adjuvation of Der s1 absorbed onto aluminum hydroxide by i.m or s.c route polarized the anti allergen immune response to a Th1 pattern with the induction of igg2a and IFN□ and a significant reduction of specific ige response. The airborne allergen challenge maintains this shift form Th2 to Th1 results. This approach was also effective in animal previously immunized with AFPL1® and allergic mice and passed the stability and animal toxicity test to go soon to clinic. The rphl p5 was explored also by mucosal route. Similar shift from Th2 to Th1 (igg2a and ige) pattern than Der s1 was obtained particularly by i.n route. **Conclusion:** FAP seems to be very promissory for allergen vaccine.

SOP-3: IgG/IgE Shift and th1 response induced by a *Dermatophagoides siboney* vaccine using the AFLP1® combination adjuvant, in sensitized mice

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Introduction: An important trend in allergen-specific immunotherapy is to investigate new adjuvants with immunomodulatory properties. Previous work has shown the pro Th1 adjuvant effect of *Neisseria meningitidis* proteoliposome (PL). **Objective:** To assess the immunogenicity and non-clinical safety profile of a novel anti-allergic vaccine candidate based on allergens from *Dermatophagoides siboney* and a combination adjuvant containing PL and Alum, known as AFLP1®in sensitized mice. **Methods:**In a therapeutic experimental setting C57/Bl6 mice were first sensitized administering *D.siboney* allergen by IP route and exposing mice to allergen aerosols. Later, allergic mice were treated with 3 doses of the AFLP1-adjuvanted vaccine containing 2µg of Der s1 allergen by subcutaneous route. Further, mice were subjected to inhalation allergen challenge. Ige, igg1, and igg2a allergen-specific antibody response was measured by ELISA, as well as the specific cellular response (IL-13, IL-10 and INF-γ). **Results:** As in the previously tested preventive model, the AFLP1 adjuvanted vaccine was able to induce a mixed igg1/igg2a antibody response and moderate levels of IFN-γ, although together with Tr1 (IL-10) and Th2 (Il-13) cytokines. Moreover, after the allergen challenge, it was noted a significant increase ($p<0.05$) of the igg/ige ratio, with a decrease in bloods eosinophils and allergic inflammation in lung tissues as compared to allergic controls. **Conclusions:** The adjuvanted vaccine does not exacerbate the allergic response nor promote Th1 inflammation, supporting a satisfactory safety profile for further clinical trials in humans. This immunomodulatory effect suggests clinical benefits both in cellular and blocking antibody responses.

SOP-4: A model of subcutaneous priming plus sublingual boosting using two nano-particulate adjuvant formulations for immunotherapy of respiratory allergy

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Background: Novel adjuvants are being increasingly investigated in order to improve conventional allergen-specific immunotherapy, regarding efficacy, shrinking of treatment duration and safety. **Objective:** To evaluate the immune modulating effect of a novel experimental nano-particulate vaccine formulations in a therapeutic murine model of respiratory allergy, using a priming-boosting strategy. **Methods:** Adjuvanted vaccine formulations were prepared by combining purified native allergens of the mite *Dermatophagoides siboney* (Der s 1 and Der s 2) with immune modulating molecules in nano-proteoliposomes (npl) and nano-cochleates (nch). C57/Bl6 mice were sensitized administering *D.siboney* allergen by IP route and exposing mice to allergen aerosols, and then, treated with the experimental formulations, first with subcutaneous priming with npl-Ds and later, with sublingual daily doses during 4 weeks (boosting) with nch-Ds. Finally, mice were subjected to inhalation allergen challenge. **Results:** Subcutaneous priming induced a moderate pro-Th1 allergen-specific response with mixed igg1 and igg2a antibodies, moderate amount of IFN-gamma, besides Th2 and Tr1 cytokines. After the boosting intervention by sublingual route, igg2a values, similar to those of healthy animals, were achieved. In treated mice subjected to allergen challenge, ige antibodies showed a decrease, together with an increase of the igg/ige ratio, increase of IL-10 and CD4+foxp3+ cells, as well as, decrease of local and systemic eosinophilia and peribronchial inflammatory infiltrate and mucus secretion, as assessed by lung histology. **Conclusions:** This novel vaccine formulation, using the priming-boosting strategy by systemic and mucosal routes, showed promising results by modulating the allergic response first to a Th1 cytokine profile and later to a regulatory and tolerogenic pattern.

C-29: Dose-response effect in allergen IT (Task Force Paper EAACI) & add comments

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Abstract: For a century, allergen-specific immunotherapy (SIT) has proven to be an effective treatment for allergic rhinitis, asthma, and insect sting allergy. However, as allergen doses are frequently adapted to the individual patient, there are few data on dose-response relationship in SIT. Allergen products for SIT are being increasingly required to conform to regulatory requirements for human medicines, which include the need to demonstrate dose-dependent effects. This report, produced by a Task Force of the EAACI Immunotherapy Interest Group, evaluates the currently available data on dose-response relationships in SIT and aims to provide recommendations for the design of future studies. Fifteen dose-ranging studies fulfilled the inclusion criteria and twelve reported a dose-response relationship for clinical efficacy. Several studies also reported a dose-response relationship for immunological and safety endpoints. Due to the use of different reference materials and methodologies for the determination of allergen content, variations in study design, and choice of endpoints, no comparisons could be made between studies and, as a consequence, no general dosing recommendations can be made. Despite recently introduced guidelines on the standardization of allergen preparations and study design, the Task Force identified a need for universally accepted standards for the measurement of allergen content in SIT preparations, dosing protocols, and selection of clinical endpoints to enable dose-response effects to be compared across studies.

C-30: Clinical proof of concept for SQ-Standardised house dust mite allergy immunotherapy tablets in patients with respiratory allergy

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Background: House dust mite (HDM) allergy immunotherapy tablets (AIT) are under development. The tolerability of the ALK HDM allergen immunotherapy tablet was investigated in two trials including adult and paediatric patients. Aim: To investigate dose-response, efficacy and safety of the HDM AIT in adult patients with HDM induced respiratory allergic disease. Methods: A total of 604 subjects, age ≥ 14 years, with mild to moderate HDM-induced asthma and rhinitis were randomised to 3 different dose groups of active treatment; 1, 3 and 6 development units (DU) or placebo. Prior to treatment initiation, the use of inhaled corticosteroid (ICS) was standardised and tapered to the lowest dose providing asthma control. Symptomatic medication was provided as rescue medication to all treatment groups. Results: The trial met its primary endpoint demonstrating a statistically significant difference in the reduction of ICS between the 6 DU group and placebo ($p = 0.004$). In a subgroup of patients on low ICS dose with rhinitis symptoms and/or medication intake, a statistically significant difference in total combined rhinitis score was demonstrated between the 6 DU group and placebo ($p = 0.02$). The most frequently reported treatment-emergent adverse event was oral pruritus. Two serious aes, migraine (1 DU) and dizziness (3DU), respectively, were assessed as possibly treatment-related by the investigator. Conclusion: The ALK HDM allergy immunotherapy tablet was well tolerated in all three dose groups. Effect on asthma and rhinitis after one year of treatment was demonstrated for the 6 DU group relative to placebo by a statistically significant reduction in the use of ICS and total combined rhinitis score.

C-31: Defining the optimal biological dose: the NGCGM3/VSSP cancer vaccine, a case study

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Two Fase I/II clinical studies were conducted with the ngcgm3/VSSP cancer vaccine in metastatic breast cancer and melanoma patients. The main objective of these trials was the definition of the Optimal Biological Dose (OBD) for a new formulation of the vaccine, applied by SC route and without emulsification in Montanide ISA 51. Noteworthy the 900 ug dose emerged as the OBD, selected in the two distinct trials, accordingly to the established criteria (immunogenicity, safety and efficacy).

C-32: Allergen imunotherapy, a 2011 practice parameter third Update

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These parameters were developed by the Joint Task Force on Practice Parameters, representing the American Academy of Allergy, Asthma & Immunology (AAAAI); the American College of Allergy, Asthma & Immunology (ACAAI); and the Joint Council of Allergy, Asthma & Immunology".The objective of "Allergen Immunotherapy: A Practice Parameter Third Update" is to optimize the practice of allergen immunotherapy for patients with allergic diseases. This parameter is intended to establish guidelines for the safe and effective use of allergen immunotherapy, while reducing unnecessary variation in immunotherapy practice. An annotated algorithm in this document summarizes the key decision points for the appropriate use of allergen immunotherapy. The section on efficacy summarizes the evidence demonstrating that allergen immunotherapy is effective in the management of properly selected patients with aeroallergen and stinging insect hypersensitivity. This document also contains recommendations for optimizing the efficacy and safety of allergen immunotherapy, including specific recommendations on prevention and management of adverse reactions and a uniform classification system for grading systemic reactions. Specific recommendations guide the physician in selection of those patients for whom allergen immunotherapy is appropriate. Aeroallergen immunotherapy should be considered for patients who have symptoms of allergic rhinitis/conjunctivitis or asthma with natural exposure to allergens and who demonstrate specific ige antibodies to the relevant allergen or allergens. There is also some evidence that patients with atopic dermatitis with aeroallergen sensitivity might benefit from allergen immunotherapy. Candidates for immunotherapy are patient whose symptoms are not controlled adequately by medications and avoidance measures or those experiencing unacceptable adverse effects of medications or who wish to reduce the long-term use of medications. Allergen immunotherapy is recommended for patients with a history of a systemic reaction to *Hymenoptera* stings who demonstrate *Hymenoptera*-specific ige antibodies. There is evidence that venom immunotherapy (VIT) might be effective in reducing large local reactions (llrs) that might cause significant morbidity and impair quality of life. The focus of this parameter is on allergen immunotherapy practice in the United States. Although several studies have demonstrated the efficacy of sublingual immunotherapy (SLIT), there is no FDA-approved formulation for SLIT, and this treatment route is considered investigational in the United States. Oral immunotherapy and SLIT for food hypersensitivity are also considered investigational. This lecture outlines the key highlights, new developments and modifications of previous practice parameter updates.

C-33: Subcutaneous immunotherapy practice parameters

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Allergen immunotherapy by subcutaneous inoculation (SCIT) was described more than 100 years ago. Efficacy has been established in allergic rhinitis, allergic asthma, insect allergy and possibly atopic dermatitis, though debate continues as to the relative effectiveness with the use of mixed, multi-allergen vaccines in contrast to single or cross-reactive allergen vaccines. The major risk of SCIT is systemic, allergic reactions or anaphylaxis. The occurrence of fatal systemic reactions from SCIT, based upon surveys performed by the American Academy of Allergy Asthma and Immunology and the American College of Allergy Asthma and Immunology, is one (1) per 2-2.5 million injections. Our charge as physicians overseeing immunotherapy is to reduce the occurrence of anaphylaxis if possible, to identify systemic reactions to SCIT and to intervene promptly to avoid life-threatening anaphylaxis.

Anaphylaxis is a syndrome and is not easily defined to facilitate consistent recognition and assessment. Anaphylaxis may involve a single organ or tissue and be very severe, involve multiple organs or tissues and be mild and anything in between. Some guidelines require the presence of significant respiratory compromise or hypotension before classifying as anaphylaxis. The challenge in assessing reactions to SCIT is that intervention is more likely to be effective if initiated early rather than waiting for more severe manifestations, particularly since SCIT is administered to individuals with significant allergy and increased risk of anaphylaxis.

The World Allergy Organization sponsored the composition of a paper describing a practical assessment and scoring strategy for systemic reactions following SCIT. This is a useful approach and is more likely to be helpful than debating criteria necessary to label a reaction as anaphylaxis versus a systemic reaction without severity sufficient to be categorized as anaphylaxis. The concept is that by identifying reactions as systemic and assigning a severity score, clinicians may be enabled to consistently communicate experience, identify risk factors and judge the effects of prevention strategies and of early interventional treatment compared to delayed treatment. This classification is summarized as follows:

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
One organ or tissue signs or symptoms without lower airway or cardiovascular system affected (Example: generalized itching)	More than one organ system affected OR change in lower airway function OR GI symptoms OR uterine cramps (Example: decrease in peak expiratory flow OR nasal congestion AND generalized itching)	Lower airway obstruction not responsive to bronchodilator or edema of upper airway with or without stridor	Respiratory failure or hypotension with or without loss of consciousness	Death

This classification system also includes notation of time of onset of symptoms or signs and time before administration of IM epinephrine if given. This is based upon the evidence that the more the onset of symptoms or signs after the allergen injection the more likely the reaction will be severe. There is also evidence that delay in the administration of epinephrine is associated with more severe reactions and death. This observation supports the early utilization of epinephrine even when the severity of the systemic reaction is a low grade, especially if the onset of symptoms or signs is in close proximity to the administration of the SCIT injection. Using this classification schema, 4% of 773 patients, 0.1% of total injections, treated over the course of one year in a multi-physician, specialty practice experienced a systemic reaction. Fourteen subjects with systemic reactions were Grade 1, 13 Grade 2 and two individuals were Grade 3 and Grade 4 respectively. No patient fatalities were reported. Ninety-one percent received IM epinephrine, 84% received systemic corticosteroids, and 94% were treated with oral H1 antagonists. There were no delayed reactions. The most common symptoms and signs of systemic immunotherapy reactions were generalized pruritus (34%), upper airway pruritus (28%), cough (25%) and shortness of breath (22%).

The treatment of anaphylaxis is epinephrine, administered intramuscularly to attain a more rapid and consistent blood level. The dose of epinephrine is 1:1000 0.01 ml/kg up to a dose of 0.5 ml. This should be repeated as necessary without waiting a specific time period, with the patient under close observation. Total dose of epinephrine should usually not exceed 1.5 ml (1.5mg) as fatalities from anaphylaxis, not associated with immunotherapy, have been attributed to an overdose of epinephrine. Lower doses may be considered if the severity of the systemic reaction seems modest but the clinician would prefer not to delay treatment, resulting in greater potential risk. Any improvement reported or observed following epinephrine would argue for the systemic reaction being anaphylaxis.

The differential diagnosis of anaphylaxis in subjects with systemic complaints following SCIT includes vasovagal reactions and hyperventilation with anxiety. Administration of epinephrine in these situations would either result in some improvement or aggravation of anxiety. This adverseeffect will be evident with close observation and would warrant alternative therapies such as atropine or reassurance.The safety of epinephrine administration when supervised by a physician argues for the early use of parenteral epinephrine when the initial symptoms or signs occur without necessitating a delay until more severe manifestations develop, which may place the patient at greater risk. Lower doses of epinephrine, e.g. 0.1 ml IM, should be considered if the manifestations are not definitely anaphylaxis, and doses can be repeated if the response is improvement with residual manifestations.

Complete prevention of systemic reactions to allergen immunotherapy is a worthy goal but not likely achievable. Nevertheless, all reasonable efforts should be made to minimize risk. Increased risk is associated with immunotherapy recipients with asthma, particularly unstable;with initial injections from new vials;with subjects treated during a seasonal exposure to a significant allergen; with administration without supervision by a health professional; with rush or accelerated protocols; with subjects having an increased basal serum tryptase; and with concomitant beta blocker therapy, which most likely increases severity of reactions but probably does not increase frequency or prevalence of anaphylaxis. Human error or dosing errors are also responsible for anaphylaxis from immunotherapy. Angiotensin blockers have been associated with an increase in systemic reactions or anaphylaxis when subjects are treated with insect venoms, and this risk likely applies to angiotensin receptor blockers. Pretreatment with H1 antihistamine has not been proven to decrease systemic reactions but may reduce local reactions.

The risk of anaphylaxis associated with immunotherapy can be satisfactorily minimized by using the correct criteria for selecting subjects to treat and avoiding individuals with unstable asthma, observing patients for at least 30 minutes in clinic and treating the initial manifestations of any systemic reactions and informing treated subjects of symptoms or signs that would warrant a return to medical facilities. Providing self-injectable epinephrine is a consideration but is not a necessity.

C-34: The diagnosis is the first step of any allergen specific immunotherapy: are we measuring what we should?

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Pending Abstract

C-35: IgE-binding epitopes: facts and speculations.

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Pending Abstract

C-36: Allergen-specific CD4+ T cell responses in healthy, allergic individuals and during immunotherapy

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Surrogate biomarkers of efficacy are needed in support of allergen specific immunotherapy. The aim of this study was to relate changes in peripheral CD4+ T cell responses to clinical efficacy during sublingual immunotherapy (SLIT). CD4+ T cell responses were assessed in pbmcs from 89 grass pollen allergic individuals enrolled in a double blind placebo-controlled SLIT study conducted in an allergen exposure chamber. Surface phenotype, proliferative responses, cytokine production and gene expression were analyzed in coded samples at baseline, and after 2 and 4 months of SLIT in pbmcs after in vitro allergen stimulation or in MHC class II/peptide (pmhcii)-tetramer reactive CD4+ T cells. The four-month SLIT course induced a 29.3% improvement of the average rhinoconjunctivitis total symptom score in the active group, when compared to the placebo group. In parallel, only minor changes in Th1 (CCR5), Th2 (crth2, CCR4) and Treg (CD25, CD127, Foxp3) markers were detected in CD4+ T cells from patients in both active and placebo groups. A down-regulation of IL-4 and IL-10 ($p<0.001$) gene expression and IL-10 secretion ($p<0.01$) were observed, as well as a decrease in potential "pro-allergic" Th2A cells from patients receiving active tablets ($p<0.01$), but without any correlation with clinical benefit. Pmhcii-tetramer analysis of Phl p 1- and Phl p 5-specific CD4+ T cells confirmed that clinical responses are not associated with dramatic alterations in T lymphocyte polarization. In conclusion, changes in patterns of peripheral CD4+ T cells are not a marker for the early onset of efficacy during SLIT.

C-37: Immunosomes for modulation of allergen-specific immune responses

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Background: Adaptive T cell immune responses are initiated by antigen-presenting cells (APC). For that purpose, APC present nominal antigen bound to major histocompatibility molecules (pmhc) to the specific antigen receptors of T cells. To achieve sustained T cell activation several co-stimulatory and adhesion molecules specifically expressed on the surface of APC have to synergize with pmhc in a concerted fashion. Soluble factors secreted during T cell/APC interaction contribute to an even more complex milieu. Aims: Create a reductionist approach for antigen-presentation by applying enveloped virus-like nanoparticles (VLP) as the common platform. Use C-terminal introduction of glycosyl phosphatidyl inositol acceptor sequences to efficiently target immune receptors of interest to VLP. Results: We could show that cell-free VLP are regularly formed, can be purified and stored over prolonged periods of time and are able to activate CD8+ and CD4+ T cells independently of accessory cells. Depending on the set of co-expressed molecules, VLP were found to be able to profoundly modulate the function allergen-specific T cells. Conclusions: VLP represent valuable tools, which will help to elucidate the function of antigen-specific T lymphocytes in more detail. Moreover, they might open new avenues to modulate pathological T cell responses, such as those initiating and driving allergic diseases.

C-38: Parasite infections and allergen sensitization

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The evolution of ige responses is influenced by the environment and provides important information about the natural history of atopy and allergic diseases. Allergen sensitization is influenced by exposure but also by concurrent sources stimulating the immune system. Ascaris lumbricoides induces a strong response, involving components of the immune system beyond ige synthesis or Th2 response. It is expected that, under these particular conditions, sensitization to environmental allergens and clinical expression of allergy may be different to that observed in societies where parasitic diseases have been eradicated. Cross reactivity between Ascaris and house dust mites (Blomia tropicalis and Dermatophagoides pteronyssinus) makes difficult to define the primary sensitizer because both sources are frequent in the tropics. Birth cohort studies may allow analyzing the patterns and interactions of the ige responses to Ascaris and mite allergens, as well as the impact of these responses on total ige levels and clinical phenotypes. Based on the FRAAT birth cohort study (Acevedo N, et al. BMC Pulmonary Medicine 2012, 12:13) we have explored this dynamics, evaluating the sensitization (at four age ranges) to Ascaris, Bt and Dp extracts, and to Asc l 1, Asc l 3 and ascgst recombinant allergens. The prevalence of sensitization was high and starts very early for both Ascaris and dust mite allergenic sources and by three years of age reach adults' levels. Total ige increases parallel to specific ige levels. Sensitization to Bt was associated with wheezing. Sensitization to Asc l 1 is a good early marker of Ascaris infection.

C-39: The effect of single and multiple infections by parasites, bacterium and viruses on atopy and wheeze in a setting of latin american children

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Background: The current epidemic of asthma and atopy has been explained by alterations in immune responses related to reduction in childhood infections. However the findings of epidemiological studies investigating the association between infection with atopy and asthma have been inconsistent. Infections, particularly in developing countries and low income populations rarely come alone, making important the study of co-infections, with species of a range of taxonomically distinct pathogens to investigate their association with atopy and allergic diseases. Objective: to investigate the effect of single and multiple infections on atopy and wheeze in urban children from Latin America. Methods: allergen-specific ige in serum (sige) and skin-prick-test (SPT) reactivity for the most common local allergens were measured in 1128 children aged 4 to 11 years. Data on wheezing and potential confounders were collected by questionnaire. Infections by nine pathogens were assessed by serology and stool examination. Associations of wheeze and atopic outcomes with single and multiple infections were analyzed by multivariate logistic regression. Results: in the whole population negativity for Toxoplasma gondii was associated with a higher prevalence of sige (≥0.70 ku/L), while negativity for Ascaris lumbricoides, T. Gondii, Toxocara sp, Herpes simplex and Epstein-Barr viruses were associated with a higher prevalence of SPT. Children with three or fewer infection markershad a higher prevalence of sige and SPT compared with those with four or more infection markers. However, isolated infections or pathogen burden were not associated with the prevalence of atopic or non-atopic wheeze. When stratified by age, the association between pathogen burden and prevalence of atopy was stronger in the oldest age group. Conclusion: The findings provide support for the idea that the mechanism proposed for the 'hygiene hypothesis' is operating in an urban Latin American context but it expression is so far restricted to the atopic status of individuals and not on the perceived asthma symptoms.

C-40: The role of fatty acid binding proteins in allergy

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The fatty acid binding proteins (FABP) are cytosolic proteins that facilitate the transport and solubility of fatty acids. They are found in different organisms from invertebrates to human and have highly conserved tridimensional structure and biological functions. The group 13 of mite allergens belongs to this protein family. Nucleotide and amino acid sequences homologues to these mite allergens have been indentified in other allergen sources such as cockroaches, shrimps and mosquitoes, however molecular and immunological characterization of these proteins remains to be done. Frequency of sensitization to recombinant allergens from B. Tropicalis (Blo t 13), and D. Farinae (Der f 13) from 11 to 53% in mite-asthmatic allergic patients in different populations is reported, with strong ige response in a number of those sensitized patients. Similar lipid binding activity was demonstrated for these two allergens which accommodate in the lipid binding pocket different fatty acids including docosahexaenoic acid and arachidonic acid. A moderate ige cross- reactivity between Blo t 13.0101 and one isoform of Der f 13 indicated the existence of cross reactive and specie specific antibodies. In some asthmatic allergic patients ige reactivity against human FABP was detected by ELISA, and low degree of cross-reactivity between human and mite fabps was demonstrated by cross-inhibition, suggesting that reactivity to endogenous FABP may be induced by sensitization to mite FABP. In conclusion: allergens belonging to FABP show the characteristic of pan allergen, and the lipid binding properties might be involved in the type of immune response induced in atopic individuals.

C-41: Neural pathways in allergy

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The prevalence of allergic diseases is continuously increasing. It is estimated that approximately one-third of the general population is affected by allergic disease. Accumulating evidence indicates bi-directional interactions between the immune and nervous systems, but little is known about the consequences of allergic processes on behavior and neural activity. Allergic food aversion, a phenomenon firstly described in 1994 by Cara et al, is a direct evidence of behavioural changes as consequence of a food allergic process. Food aversion is observed when allergic mice, differently from control animals, avoid drinking the solution containing the allergen to which they are sensitized. It was shown that OVA-sensitized mice orally challenged with the allergen present increased levels of anxiety and activation of emotionality-related brain areas, such as paraventricular nucleus of the hypothalamus (PVN) and central nucleus of amygdala (cea). Parallel studies investigated allergic aversion behavior in an experimental model of allergic lung disease. Using a dark-light box, it was shown that OVA-sensitized mice, differently from control animals, hesitated entering the attracting and supposedly safer, dark chamber in which the allergen had been previously nebulized, preferring the lit (usually aversive) side of the box. Increased activity of the PVN and cea was also observed in OVA-sensitized mice following a nasal OVA challenge [47]. Altogether, these results reinforce the fundamental role of an allergic response on the brain activation associated with avoidance behavior towards the allergen exposure.

C-42: Bioavaliability of allergen vaccines

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Like conventional vaccination, where the success is measured by the induction of high levels of neutralising antibodies, allergen preparations were thought to present similar mechanisms, that is, induction of (blocking) antibodies that will interfere with the sige-antigen interaction. In such a case, the higher the amount of antigen the better, being only limited by the adverse event profile of the particular allergen dose. Despite the small amount of antigen compared with conventional vaccination, the previously mentioned design criteria have governed the design of allergen preparations.There is no head-to-head comparison between unaltered and modified extracts or between aqueous and adjuvanted preparations. In recent animal studies the active dose has been diluted up to twenty fold, maintaining the adjuvant dose, without losing its immunological effect. On the other hand, a fixed allergen dose combined with decreasing aluminium content showed a progressive decline of the immunological response along with the adjuvant dilution. These mouse data suggest that the effect of aluminium salts in allergen vaccination might have been underestimated and indicate that the rational design of allergen preparations should be based on the (minimum) dose that is able to maintain a clinical effect, rather than on the maximum tolerated dose, that has become a cornerstone of our current understanding of the dose response relationship during SIT and has driven the design of preparations in recent years. Moreover, a review of the published literature shows that there is no properly designed dose-effect study performed with subcutaneous allergen preparations, testing different allergen doses with a constant amount of adjuvant like aluminium salts.

C-43: Safety aspects of allergen subunit vaccines

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Background: The surface of Adeno-associated viruses (AAV) consists of 60 capsomers, suitable for high density display of vaccine peptides. AAV-like particles (AAVLP) can be generated via assembling recombinant AAV-2 capsid fusion proteins for vaccination. Aims: The aim of this study was to evaluate the immunogenicity and safety aspects of an AAVLP-displayed B-cell epitope, taking ovalbumin (OVA) as a model antigen/allergen. Methods/Results: An OVA-derived linear B-cell epitope and, for control purposes, OVA-non related peptide TP18 (derived from rabbit cholesterol ester transfer protein) were inserted into capsid protein AAV-2. When we immunized BALB/c subcutaneously, sera of AAVLP-OVA immunized mice showed similar titers of OVA-specific igg1 compared to mice immunized with OVA protein. However, in OVA immunized mice high OVA-specific ige levels could be recorded, whereas immunizations with OVA-AAVLP rendered background ige levels only. In accordance, sera of OVA mice permitted mast cell degranulation upon OVA trigger in a specific β-hexosaminidase release assay, whereas sera of OVA-AAVLP mice did not contain anaphylactogenic antibodies. In an in vivo anaphylaxis experiment, upon intravenous OVA challenge OVA-immunized mice reacted with a significant drop of body temperature, whereas AAVLP-OVA mice remained unaffected. Conclusion: We conclude that aavlps are suitable for the display of antigenic peptides and for inducing specific immune responses in the mouse model. Further, in sensitized individual possibly local, but no systemic anaphylactic side effects may occur. This may be important for the design of allergy vaccines.

C-44: Pharmacokinetics and standardization of mPEG modified allergens

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Background: mpeg allergens were developed to suppress ige sensitization a concept that failed. Aims: To document mpeg modified allergens for subcutaneous immunotherapy (SCIT). Methods: To control and document the degree of pegylation, document the presence of allergen after pegylation, study the release from the injection site, and the distribution to lymphoid organs, and finally document the degree of clinical efficacy in relation to non-pegylated material. Results: The degree of pegylation could be controlled. All aa were present in the final product, mpeg-modified allergens were shown to have a prolonged circulation time, were deposited in lymph nodes, the spleen and liver, the clinical effect was superior to that of the non-pegylated product. Conclusions: The production of mpeg modified allergens: Production is controlled, contain the aa present before pegylation, are directed towards lymphoid tissues, induce less side effects and have better effect than the original product. Thus, mpeg modified products are well suited for SCIT.

C-45: Using mass spectrometry for determination of major allergens in allergen standardization

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Background: Products for specific diagnosis and immunotherapy of ige-mediated allergies are currently based on natural extracts. Quantification of major allergen content is an important aspect of standardization as important allergens particularly impact vaccine potency. Standardization requires the application of scientifically documented methods for routine batch release, and it can be improved by introducing new and better methods as they are developed. Aims:The aim of the study was to develop a mass spectrometry (MS) based assay for absolute quantification of Timothy (Phleum pratense) pollen allergens Phl p 1 and Phl p 5 in P. Pratense extract. Methods: High resolution and accurate mass (HRAM) MS provided by Orbitrap technology was selected for its ability to detect peptides with high selectivity and mass accuracy. Isotope labeled internal standard peptides were used for absolute quantification of specific isoallergens of Phl p 1 and Phl p 5 at low fmol level in P. Pratense extract. Results: Robustness and linearity of the method was demonstrated with intra day precision ≤ 5 % (n=3). Phl p 1.0102 was shown to be 5 times less abundant than its variant Phl p 1.0101 andphl p 5.02 was shown to be 9 times more abundant than the Phl p 5.01. Conclusions: Manufacturing of consistent allergen products requires detailed knowledge on essential major allergens. Use of Mass Spectrometry (MS) for qualitative and quantitative determination of major allergens improves quality as it is independent of the use of antibodies as reagents.

C-46: Potency tests for modified and alum-adsorbed modified products

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Chemically modified allergens adsorbed to aluminium are widely used as vaccines for immunotherapy. According to the guideline on allergen products these vaccines are to be characterized for potency and need to be monitored for stability. Ige based potency assays are well-established and validated for demonstrating the reduction of ige binding potencies of allergoids. These assays, however, are not suitable for modified allergens (allergoids) and vaccines (aluminium adsorbed allergoids) since their very essence is negligible ige reactivity. There are no well-established tests available that can be used for measuring potency of allergoids. Furthermore, an additional difficulty of testing alum-adsorbed allergoid is aluminium, which disturbs performance of immunoassays. Like the ige based potency tests for allergen extracts, it would be desirable to have antibody-based assays for both allergoids and alum-adsorbed allergoids. This presentation will show results of recently developed antibody-based assays which can be used for determining potency of both allergoids and alum-adsorbed allergoids.

C-47: Quality and allergen composition of mite allergen source materials

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Background: Traditionally, the growth medium for the house dust mites Dermatophagoides pteronyssinus and Dermatophagoides farinae has been human dander / yeast. The last 10 years Citeq uses a semi-synthetic growth medium for the house dust mites to minimize non-mite proteins and avoid human and animal components and allergens from plant origin. Aims: To optimize in process controls in the production-phase of whole culture and mite bodies. To show the biochemical parameters of final products. To define the der p 1 and der f 1 allergens of the cultures of D. Pteronyssinus and D. Farinae with MS analysis. Results: Optimal harvest-time of whole culture is determined by ELISA, specific growth parameters and visual parameters. For the production of mite bodies growth parameters give the most control. The biochemical parameters show that mite products must be kept under strict conditions. Large temperature fluctuations decrease der p 2 concentrations considerably and lower der p 1 concentrations as determined by ELISA. The MS-data are obtained with MALDI-TOF. The spectra show to which group of isoforms der p 1 and der f 1 belong. Der p 1 is present in 2 isoforms at amino acid position 124, in 2 separate batches and in purified der p 1. Conclusion: Effective in process controls assure healthy cultures and determine the optimal harvest-time, both for whole cultures and mite bodies. Trend analyses are important for the quality of both the analysis and the final product. The MS-data show that der p 1 exists in 2 isoforms in 2 different batches and in purified der p 1.

C-48 Development of BM32 - a Recombinant Vaccine for Immunotherapy of Grass Pollen Allergy: from R&D to first clinical trials

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Background:
BM32 – a recombinant vaccine for specific immunotherapy (SIT) of grass pollen allergy - is the first member of a new generation of allergy vaccines based on a "Peptide/Carrier" concept. It is a combination of four active ingredients (APIs) containing peptides derived from grass pollen allergens. BM32 has been designed to minimize side effects and allow establishment of a convenient immunotherapy schedule

Aims:
During the pharmaceutical development of BM32, preclinical pharmacology and toxicology studies, and development of GMP production and formulation processes were carried out to pave the way for first clinical trials.
A skin test study (N=60) was conducted as the first-in-man trial in 2011 followed by an immunotherapy study (N=70) which is currently being evaluated.

Results:
Preclinical studies in different rodent and non-rodent species demonstrated that immunization with BM32 induces strong allergen-specific IgG responses. In preclinical safety studies a dose of 80µg per API (i.e. 320µg of total protein), was established as No-Observed-Adverse-Effect-Level (NOAEL) after 9 s.c. applications of BM32.
In the course of the skin test study BM32 was found to be safe and well tolerated up to a concentration of 100 µg/ml per API component using skin prick methodology and atopy patch testing.

Conclusion:
The results of the preclinical and early clinical studies encourage us to proceed with the clinical development of BM32. If successful, it is expected that BM32 will dramatically simplify SIT as currently practiced. The target is to substantially reduce the number of annual injections, and make SIT similar to standard vaccination procedures.

C-49: GMP manufacturing and quality control of recombinant allergy vaccines

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Background: Recombinant allergy vaccines for clinical studies and for market supply must be produced according to the regulations of Good Manufacturing Practice (GMP). This contribution covers specific topics of process development, manufacturing and quality control for recombinant allergy vaccines, from the view point of the GMP manufacturer.Aims: The goal was to GMP-manufacture the drug product BM32, a recombinant vaccine against grass pollen allergy, which is currently investigated in a clinical phase iib study. BM32 is based on a peptide-carrier approach and consists of four individually manufactured recombinant proteins. Methods and Results: Case study data will be shown to discuss the generic and individual aspects of the four different active pharmaceutical ingredients of BM32. For development of a recombinant vaccine, the selection of a suitable expression system (E. Coli) was the first important step. In order to obtain a high fermentation yield, state-of-the-art high cell density cultivations were applied by using fed-batch technology. After cell lysis by either freeze-thaw cycles or high pressure homogenization, the recombinant products were purified by applying chromatographic separation techniques. Buffer formulation, adsorption to the adjuvant (aluminium hydroxide) and aseptic filtration and filling were the concluding tasks towards the final pharmaceutical vaccine. To discuss aspects of quality control, data of release testing, In-process control and stability testing will be presented. Conclusion: By addressing generic and individual features of the four proteins of BM32, it could be shown that GMP manufacturing can be set up and finalized in a short period of time. BM32 product quality, yield and stability were assessed to be promising with respect of the planned clinical development plan.

C-50: Advances of cuban dengue vaccine candidates. Adjuvant uses

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Dengue represents a major, growing public health problem with an estimated of 2.5 billion people at risk of infection and about 50 millions of cases. Vector control is the only way for controlling dengue transmission, however, in spite of efforts, an expanded geographical extension of the vector and viruses is observed. Consequently there is an urgent need for a safe and effective dengue vaccine. A dengue vaccine should induce long-lasting simultaneous protection to the four viruses avoiding the immune enhancement of viral infection. Domain III of the dengue envelope protein has been implicated in receptor binding being also target of specific neutralizing antibodies. This motif has emerged as a promising region for a subunit vaccine candidate. Our group expressed in *E. Coli*, domain III of the four serotypes fussed to p64k of *Neisseria meningitides.* Monovalent recombinant proteins of each serotype induced neutralizing antibodies and protection after viral challenge in mice. DENV-1 and DENV-2 vaccine candidates were immunogenic and induced total or partial protection in monkeys. Finally, the immune response induced by the recombinant protein both in mice and monkeys was highly serotype-specific In addition, vlps from the recombinant DENV-2 capsid protein as well as a chimeric protein comprising domain III and the capsid of the same serotype were also obtained. Evaluation of both vaccine candidates in immunized mice showed significant protection after viral challenge as well as a strong cellular immune response. To improving the immunogenitiсy of our candidates, several adyuvants have been tested. Here we summarized results obtained in the evaluation of some of the formulations in mice and monkeys.

C-51: Virus like particles based on the recombinant capsid protein of dengue-2 enhance the immune response against recombinant proteins

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The most advanced Dengue (DEN) vaccine candidates until now are based on attenuated viral strains but they have the risk of reactogenicity and the return to virulence. In this regard, vaccine candidates based on protein subunits are advantageous despite the need to use potent adjuvants or immunopotenciators. Virus like particles (VLP) have been described as inducers of potent immune responses principally due to their property of been efficiently taken up by dendritic cells. In this work VLP composed of the capsid protein of Dengue 2 virus (C-VLP) were evaluated as enhancers of the immune response against chimeric proteins formed by a fragment of the envelope protein of DEN-1 and DEN-4 viruses fused to protein P64K from *N. Meningitidis* (PD10 and PD24). After immunization, the balb/c mice were bled and analyzed for the production of antiviral antibodies (Abs) by ELISA. Mice immunized with PD10/C-VLP and PD24/C-VLP in aluminum hydroxide showed the higher titers compared with mice immunized with the recombinant proteins alone. One month after the last inoculation, mice were challenged with lethal DEN-1 and DEN-4 and observed during 21 days. These results show for the first time the enhancer capacity of VLP of the capsid protein of Dengue virus, rendering it an attractive alternative to protein subunit vaccine candidates.

C-52: Recombinant H1N1/2009 pandemic influenza vaccine containing advax™ polysaccharide adjuvant: randomizsed clinical trial of immunogenicity and safety

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Access to vaccines is critical during influenza pandemics. A recombinant hemagglutinin (rha)-based vaccine could overcome production hurdles of conventional technology but has not previously been tested in a real-life pandemic setting. The aim of this study was to determine the efficacy of a recombinant vaccine during a period pandemic infection,and whether its immunogenicity could be enhanced by an inulin-based novel polysaccharide adjuvant (Advax™). Methods: 281 adults aged 18-70 years were recruited,andrandomisedin a double blinded, parallel-group study of rha H1N1/2009 vaccine with or without adjuvant. Immunizations were at 0 and 3 weeks with rha 3, 11 or 45 ug. Serology and safety were followed for 26 weeks. Results: At baseline, only 9.1% of subjects (95% CI = 6.0 - 13.2) had seroprotective H1N1/2009 titers. Seroconversion rates varied by rha dose, presence of adjuvant, subject age and number of immunizations. Eighty percent (95% CI = 52 - 96) of 18 - 49 year olds who received rha 45ug with adjuvant developed titres consistent withseroprotection at week 3, representing a 11.1-fold increase in antibody titers from baseline. Advax™ adjuvant increased seroprotection rates by 1.9 times after the first and 2.5 times after the second immunization when compared to rha alone. Seroprotection was sustained at 26 weeks and the vaccine was well tolerated with no significant complicaitons or safety issues. Conclusions: The study confirmed the ability to design, manufacture, and release a recombinant vaccine within a remarkably short time from the start of a real life influenza pandemic. Advax™ adjuvant significantly enhanced rha immunogenicity and provided antigen dose-sparing. (Australia New Zealand Clinical Trial Registry: ACTRN12609000674235).

C-53: AFCo1 and AFPL® Overcome the thymus-independent stimuli of unconjugated polysaccharide antigens

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Bacterial polysaccharides are T-cell independent antigens type 2 (TI-2) that do not induce immunological memory and are poor immunogens in infants. Bacterial polysaccharide conjugate vaccines are the gold standard used to confer protection by inducing humoral immune responses against TI-2 antigens. Adjuvants have been used to improve the immune response to vaccine antigens. In these works we explored the efficacy of a mucosal adjuvant like afco1 (Adjuvant Finlay Cochleate) and different AFPL® (AF Proteoliposome) to elicit high levels of specific antibodies response, subclass pattern, affinity maturation against capsular polysaccharide Vi of *Salmonella Typhi* or *Neisseria meningitidis* serogroup A after a primary response. Also, we evaluated the antibodies response, B, T cell immunity, and memory responses after a booster dose with plain polysaccharide. Vigorous specific B and memory B cell responses after booster dose were induced. Effector memory and central memory T cell responses were developed. The combined formulation of capsular polysaccharides with a mucosal adjuvant like afco1 or AFPL provides an improved immunogenicity, in particular with regard to cellular responses and long lasting and rapidly expanded cells.

C-54: On the role of vaccine delivery for the induction of functional immuneresponses by TB vaccines

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It is known that protection against tuberculosis is mediated primarily by Th1 cells and that Th1 priming by TB vaccines is vitalfor their efficacy. With the first novel vaccines now being in clinical trials, IFN-γ has therefore emerged as a surrogate marker for the protection conferred by the trial vaccine. The CAF01 adjuvant, consists of cationic liposomes based on dimethyldioctadecylammonium (DDA) and synthetic mycobacterial cordfactor (TDB) that promotes a C-type lectin mediated immune activation through the MINCLE receptor.CAF01 promote high levels of TH1 responses that are stably expressed as longlived memory responses and furthermore induce strong humoral responses to various vaccine antigens.The DC targeting/activation patterns required to elicit Th1 responses remain undefined but one known requirement is that of a physical association between antigen and adjuvant. I will discuss the importance of avoiding the targeting of dcs by antigen without their simultaneous activation by the in order to induce a powerful Th1 response and how the formation of a vaccine depot at the site of injection plays a major role for the ability of CAF01 to induce a Th1 response. Finally I will present recent data from a clinical trial with CAF01 as an adjuvant for a TB vaccine.

C-55: Adjuvants modulate the immune response to proteoliposome of *micobacterium smegmatis* in mice

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Proteoliposomes of *M. Smegmatis* were obtained after a bioinformatic study demonstrating its potential as vaccine candidate against tuberculosis. In order to evaluate its immunogenicity and the induction of immune responses against *M. Tuberculosis* in mice proteoliposomes were administered with Alum or Incomplete Freund Adjuvant. With the use of both adjuvants were elicited specific and cross reactive immune responses against *M. Tuberculosis* at the humoral and cellular level. The intensity, pattern of subclasses and recognition of different antigenic components were modulated by the adjuvant used.

C-56: DNA Vaccines for the prophylaxis of tuberculosis

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Plasmid DNA vaccination is a powerful and easy method for the induction of strong humoral and cell mediated immune responses in mice. By virtue of its strong capacity to induce CD4+ mediated Th1 and CD8+ mediated CTL responses, this vaccine approach is particularly attractive for the prophylaxis of intracellular pathogens, such as M. Tuberculosis and other pathogenic mycobacteria. DNA vaccines appear to be particularly effective for priming immune memory and subsequent boosting with recombinant protein, recombinant pox-viruses or with M. Bovis BCG vaccine are particularly promising for future applications. We have previously shown that priming of mice with pdna encoding the promising vaccine candidate Ag85A (Rv3804c) prior to BCG vaccination can increase the protective efficacy of the BCG vaccine, as indicated by significantly prolonged survival time of mice challenge with virulent Mtb H37Rv. Nevertheless, as the BCG vaccine is routinely administered to neonates, priming with DNA before BCG is not a realistic option for the clinic. We are therefore analyzing whether simultaneous administration of BCG and pdna-Ag85A could be an alternative approach. These studies give a proof of concept for additional experiments in which BCG will be combined with plasmid DNA encoding dormancy (dosr) regulon encoded proteins against which the BCG vaccine induces only a poor immune response. The most likely formulation that may eventually reach clinical testing, may be a combination of plasmid DNA –encoding protective (fusion)-proteins with the existing M. Bovis BCG vaccine. Vaccines protecting at the same time against primary (post-infection) tuberculosis and against reactivation of latent tuberculosis will undoubtedly have the biggest impact on the control of this infectious disease.

C-57: Mimotope vaccines against cancer to induce IgG or IgE

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Background: Generally, antibodies belong to the most successful defense strategies of the immune system. Except for ige, which we know from its deleterious function in allergy, but are still confused about its original defense purpose. We propose that ige may have its natural function in the defense against cancer, and term the new research direction "allergooncology". There are several arguments supporting this theory: 1.) Allergies are epidemiologically inversely correlated with several types of cancer and natural anti-tumor antigen ige exists; 2.) Ige has adjuvant functions in anti-tumor responses; 3.) Preclinical studies using recombinant anti-cancer ige antibodies have convincingly demonstrated the superor tumoricidity of the ige class. Aims: For epitope specific immunotherapy of HER-2 expressing breast cancer we have previously selected mimotopes from a phage display library using the well known clinically applied antibody trastuzumab (Herceptin ®) for selection. Coupled to KLH these mimotopes were able to induce specific igg of the same biological activity as the trastuzumab antibody itself. For proof of concept, the aim of the presented studies was to turn the immune response against this HER-2 mimotope from igg towards ige. Results: The immunization strategy for directed ige induction was based on the fact that oral antigens may turn to allergens when persisting gastric digestion. Therefore, we applied the above trastuzumab mimotope/KLH to BALB/c mice orally in the context of anti-acid medication. Indeed, mice formed ige which was able to arm RBL cells, and mediator release could be triggered in a highly specific manner by HER-2 overexpressing SKBR-3 tumor cells. More importantly, the supernatants from these experiments were acting cytotoxic on the cancer cells. Conclusion: Based on the evidence we strongly believe that ige could be applied for the benefit of cancer patients in passive or active immunotherapy form. Being complementary to igg it may attract a distinct and very potent panel of tumoricidic effector cells.

C-58: Towards an adjuvant for immunocompromised scenarios

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Certain therapeutic vaccines are designed for individuals suffering immunosuppresive conditions or treatments (cancer, HIV, other chronic viral infections), while adjuvants are usually developed for preventative vaccines in normal immune scenarios. In our project we hypothesized that probably these two situations are quite differents and specific adyuvants for immunocompromised hosts deserve intentioned actions and experimentation. Very Small Size Proteoliposomes (VSSP), a clinically tested nanoparticulated adjuvant formulation, seems to be preferentially active in such immunocompromised scenarios. In this presentation data will be provided showing how VSSP are able to circumvent the effects, over the specific cellular immune response, of the suppressive effect of tumor stimulated mdscs and CT associated leukopenia.

SOP-5:Co-administration of CR3 with core and surface hepatitis B antigens induces a TH2-TH1 shift of the HIV-Specific response

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Background: After HIV infection, a Th1 to Th2 cytokine-profile shift is observed and it has been related to disease progression. One of the possible benefits of a therapeutic vaccination might be to counterbalance this phenomenon allowing viral replication control by a Th1-type immune response. TERAVAC-HIV is a multiantigenic formulation vaccine candidate against HIV-1. It comprises a recombinant protein of HIV (named CR3) that contains Th and CTL epitopes, and the surface (S) and nucleocapsid (C) antigens of HBV. Previous results showed that both VLP of HBV provide a Th1 adjuvant effect. Immunization of CR3 alone in alum results in a Th2-type response. Aim: In this work we addresses if a Th2-type response induced against CR3 could be reversed to a Th-1 pattern by subsequent administration of the TERAVAC formulation. Results: After a first phase, a CR3-specific Th2-type response was verified in animals inoculated with CR3 or viral lysate in alum by induction of IL-4 and IL-10 secretion with negligible levels of IFN-gamma and igg2a antibodies. However, after subsequent TERAVAC immunization the same groups of animals showed high levels of IFN-gamma secretion and production of igg2a antibodies in serum, in accordance with a Th1-profile of cytokine secretion. **Conclusions:** A successful generation of a Th1-type response was demonstrated under an ongoing-Th2 response after TERAVAC immunization. This result suggests a therapeutic benefit of this vaccine candidate in the restoration of the Th1-type HIV-specific cellular response in seropositive patients.

C-59: Status of allergen products in USA

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The Laboratory of Immunobiochemistry regulates allergenic extracts and other biological products that are used for the diagnosis and/or therapy of allergic diseases. Regulation of these products includes lot release testing of allergenic extracts that are standardized for potency and oversight of clinical studies that evaluate the safety and efficacy of new products for allergy immunotherapy. The speaker will review the process of regulation of allergenic products in the United States and then will discuss three specific regulatory issues relevant to the regulation of allergenic biologics: 1) a planned change in the potency assay for standardized short ragweed pollen and cat allergen extracts from the radial immunodiffusion assay to an ELISA; 2) the use of environmental exposure units in pivotal Phase 3 studies; and 3) the FDA review of non-standardized allergenic products for safety and efficacy.

C-60:Product regulation approach and batch release in Germany

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The first allergen products have been authorized in Germany in the 1970ies. In parallel, non-standardized named patient products (npps) have been used for SIT. Allergens frequently causing allergic diseases and previously marketed as npps are now subjected to obtain market authorization (MA) according to the German "Therapy Allergens Ordinance" (TAO). This was made to ensure that products for treatment of highly prevalent allergies have a MA. For products within the scope of the TAO, manufacturers were required to notify (1) which of these products were to be sold during a transition period to finalize ongoing patient treatments or (2) for which products applications for marketing authorizations were to be submitted. A transition period of 3 years was allowed during which the products were still marketable. For the latter notifications, the products are allowed to be marketed until a decision on the marketing authorization has been made. Allergen extracts have to be characterized by comparison with in-house references using protein profiling methods. Sera pools made of sensitized human donors are to be established as standards. Protein content and biological potency have to be within specifications redefined by the European Pharmacopeia monograph. Npps subjected to the TAO have to pass a nonclinical assessment and clinical trial program to obtain MA demands on quality, safety, efficacy. Placebo-controlled studies will increase the evidence-based knowledge of SIT and the comparability of clinical trials (Kaul, S. Et al., Allergy 2011). Allergen products are subject to official batch release by the competent German Federal Agency, the Paul-Ehrlich-Institut (PEI).

C-61: Current situation of allergenic extracts registration in Brazil

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Pending Abstract

C-62: Progress in regulation of therapeutic vaccines and biological products in Cuba

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Scientific development and the use of new technologies are impacting the pharmaceutical regulatory environment with increasing licensing requirements. Therefore, the Drug Regulatory Authorities have internationally developed a greater control on the different pharmaceuticals products that are marketed in countries, including the so-called therapeutic vaccines. One of the most remarkable achievements of the Cuban national biopharmaceutical industry has been the development of several therapeutic vaccines for the treatment of chronic diseases such as cancer, arthritis, cardiac dysfunctions, AIDS and allergy through the induction, modification or amplification of the host immune response. The aim of this paper is to give a brief overview about national and international procedures related to this topic. We will emphasize on strengthening the legal basis which is established by our Regulatory Agency, to face this challenge during the last two decades, through the incorporation of new approaches and specific considerations in the regulatory system according to typical characteristics, flexibility and the current scientific knowledge of the clinical development for these biological products. Besides, it would provide information on major deficiencies that were found in inspections of Good Clinical Practices (GCP) arising from National Program of Inspections to Clinical Investigators, to ensure protection of subjects and timely access to market of those substances with quality, safety, efficacy and reliable information for its rational use.

C-63: Design of novel sublingual and subcutaneous immunotherapies in Latin American countries

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Rational design of immunotherapy requires an understanding of target demographic. This includes an assessment of allergen prevalence and the distribution of the target population.

To support treatment of prevalent allergies across countries in Latin America substantial formulation design and product characterisation has resulted in the development of two novel product platforms. These two product platforms include a subcutaneous dosage form (Tyrovac) and sublingual dosage form (Acarogen).

For each dosage form, product formulation and design has considered characterisation and standardisation of allergen profile with biochemical, chemical, microbiological, pharmacological and toxicological assessment. A solid stability profile and well established clinical use is also important in product design and has been considered for both novel forms.

These parameters will be discussed in the context of novel product design in Latin America.

C-64: Introducing allergen immunotherapy into the public healthcare sector: the Cuban experience

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Allergen Immunotherapy (AIT) is the only therapeutic approach capable of modifying the underlying immunological basis of allergic diseases. Thus, AIT has advantages over conventional symptomatic treatments from the point of view of cost-effectiveness and long-lasting effect, a very relevant aspect for the management of allergic diseases by a public health care sector. However, implementation of wide programs of AIT is hampered by the limitation of its prescription by allergy/immunology specialists, and the risk of systemic reactions. The sublingual route, which is gaining in worldwide acceptance and scientific support, has the potential to allow the expansion of AIT, since the possibility of self-administration by the patient and better safety profile. This work describes the Cuban experience regarding the development of allergen vaccines of three prevalent species of House Dust Mites, and its countrywide introduction into the public healthcare system. Allergen vaccines of *Dermatophagoides pteronyssinus*, *D. Siboney* and *Blomia tropicalis* were developed as biopharmaceutical products following requirements compatible with European regulations. The manufacturing process was able to achieve consistent results at industrial level. The efficacy and safety in allergic asthma was assessed in 9 clinical trials, either by subcutaneous injection or by sublingual route. Overall, treatment was effective for 76% of patients. Asthma symptoms and medication declined 60% ($CI_{95\%}$: 51-69) by SCIT or 56% (CI: 43-69%) by SLIT, as compared to placebo. Sublingual route showed no adverse systemic reactions and a lower frequency of local reactions. VALERGEN® vaccines were licensed for therapeutic use, becoming the first registered allergenic products in Cuba, and were introduced country-wide into the public healthcare sector. An on-going prospective Pharmacovigilance study, performed in 1719 asthmatics patients in 24 allergy services is confirming the efficacy and safety of these products in the routine clinical practice. The number of patients under treatment has been increased over 20 000 in a period of 4 years, achieving an estimated coverage of 5% of the target patients. A training program is in place for Allergy specialists, paraclinical personnel and family doctors for expanding further the sublingual route. Availability of high quality standardized allergen vaccines becomes a valuable and cost-effective tool for expanding the etiological approach for asthma management.

C-65: Management of occupational allergy

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Occupational asthma may be defined as disease characterized by eather or both a variable airflow limitation and airway hyperresponsiveness due to causes and conditions attributable to a particular working environment and not to stimuli encountered outside the workplace. For diagnosis and therapeutic management purposes must be always considered the following points: 1.-Assessement the relationship of the workplace and the development of asthmatic symptoms. 2.-Investigation of the workplace in order to locate the responsible offending agent. 3.-Demonstrate that the suspected offending agent located is really the responsible one. 4.-Treatment of the patient. Removal from the workplace is the most important recommended procedure. This is the best treatment. An early diagnosis relative to the onset of asthmatic symptoms is the most important goal for outcome in order to prevent the persistent morbidity and disability for asthma. Immunotherapy performed with high molecular weight non toxic agent have been carried out in some cases in which removal from workplace has not been possible. We have obtained clearly improvement in patients with occupational asthma due to Obeche or African maple wood sawdust as well as in wheat flour induced asthma.

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RTD-1:The impact of allergic conditions on children with speech and language disorders

R. Creticos

Abstract: The round table discussion will focus on the effects of allergies on speech and language disorders in children. This will include a discussion of voice related problems, including voice quality (hoarseness), resonance (denasality), and breath control. We will also discuss the impact of middle ear fluid (due to allergies) on articulation (speech sound) development and the development of language skills in young childeren.

KNA 4: Vaccine adjuvants designed for veterinary and human use

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In a context where antigens are more and more defined and purified, adjuvants are essential to enhance immune response induced by vaccination. The development and selection of new adjuvant concepts shall take into account different parameters such as recipient target, route of immunization, type of immune response desired, duration of immunity and safety. The objectivation of adjuvant formulations in small animal models allow to optimize the adjuvantal properties for antibody profiles, pro-inflammatory chemokines and cytokines environments, and cellular activations. Reproduced and confirmed results in targeted animals field tials or human clinical trials reinforce further steps of development and use. Among all technologies developed in the past years some have demonstrated their interest for use into commercial vaccine. Associated higly refined surfactants and oils, that are present in Montanide@ ISA adjuvants, can be used to obtain very potent emulsions that can be water-in-oil, oil-in-water or double emulsions (water-in-oil-in-water). These formulations are developed since many years and can present optimised properties and fine specifications in order to be intimately mixed with the antigen for improved stability and efficacy of the vaccine. Montanide@ IMS are new concept of aqueous adjuvants, described as Immunosol, and are mixing amphiphilic properties of surfactants with specific immunostimulating compounds, to render very fluid adjuvants in safety oriented profile. Injectable polymers such as calibrated spheric micronic gel particles of sodium polyacrylate are used to enhance cellular mediated immunity. Montanide@ Gel is moreover very well tolerated and can be advised for companion animal vaccination. Industrial development ease and scale-up feasibility of each adjuvants as well as cost of production are important parameters. In a constant quality improving requirement environment the GMP standards have to be applied to all steps of production in order to fulfil reproducibility, traceability and specifications. This is a key parameter for an adjuvant in order to be included into human clinical trials. Thus water-in-oil rendering adjuvants, Montanide@ ISA 51 VG and Montanide@ ISA 720 VG, are integrated into Seppic's quality system and participate to the clinical development of very potent and promising therapeutic vaccines in cancer strategies.

C-66: Use of efficiency model in the aquiculture experimentation.

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A multifactor efficiency model is offered to evaluate to the use and effects of new drugs, probiotics, vaccines and methods for the improvement freshwater aquaculture systems. The model allows assessing results of new methods as well as to discriminate between several parameters that affect the culture and to determine the critical points to be corrected subsequently. Using as information source the harvest summary emitted at the final and partial culture cycle ending determines partial efficiency for alewife waste, yield; final fish weight; culture timing and food conversion rate as well as the whole process multifactor efficiency. Model validation was made with information gathered from three Villa Clara African freshwater catfish fattening farms showing the utility in the analysis of the behavior of this culture in 2011 from a single pond, a farm or higher level. Comparison between determined efficiency for each parameter allows discriminating where the primary effects of chemists appear and variations respect to witness. The application of the model is suggested for the analysis of research results in this and other cultures.

C-67: Fish diseases and vaccines, probiotics and other pharmaceuticals in aquaculture

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An update of the situation of world aquaculture is presented with emphasis on the main fishes, crustaceans, and mollusks species. The emphasis on the intensification of aquaculture on recent years had made that factor like crowding and worsening of environmental conditions determinate the optimal conditions to the increase of diseases in the cultures. An overview of the main diseases and the control and preventive methods is presented with remarks on the use of vaccines, coadjutants, probióticos, and other pharmaceuticals and denoting the new application field in diseases prevention and control for the aquaculture development in Cuba and the world.

C-68: Immunochemotherapy using nitrovynilfuran compounds entrapped in immunoactive cochleate structures

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Cochleates are supramolecular structures that have been developed either as vaccine adjuvants or as drug carriers. The main difference relays on the kind of phospholipids used to form the cochleate and the active compound that is entrapped. Cochleates used as drug carriers generally consists of phosphatidylserine entrapping an active drug molecule; while vaccine adjuvant cochleates are based on lipopolysaccharides and other microbial associated molecular patterns that entrap the antigens or act themselves as immunomodulators. The present work deals with a combination of both kinds of cochleates in which molecules with antibacterial and antifungal activity were entrapped into cochleates previously developed as vaccine adjuvants due to their immunoactive effect. Such cochleates have proved to be particularly effective for the treatment of fowlpox in young pigeons, for the prevention of infectious diseases in African sharptooth catfish (*Clarias gariepinus*) and studies are in course to evaluate their potential as antileishmanial therapeutic agents. A synergistic (or at least additive) effect was found between the nitrovynilfuran compounds and the family of afco cochleates. Results suggest that this line of research opens new perspective for the prevention and treatment of infectious diseases of varied etiology.

C-69: Methodology to design and optimize stable oily formulations using montanide 888 VG

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The emulsions are used in biotechnology in the formulation of a large number of immunogens. Efforts have been devoted to study and understand these systems, but the complexity of the emulsifiers, the interaction forces and active ingredients that are used mean that there are inaccuracies in the prediction of stability. In the present work a study was conducted to determine the influence of phase composition, and ionic strength on the behavior of an emulsion using the Montanide 888 VG as emulsifier. Using a mixture experimental design mathematical model was adjusted, describing the influence of emulsion composition on the mechanical stability, the thermal stability, viscosity and particle size. A numeric factor for ionic strength was included. Mechanical stability was determined by centrifugation and thermal stability by accelerated test at 37 °C. Viscosity was determined using a rotational viscometer by Ostwald de Weale law. The simulation and optimization models were developed using Matlab 7.0.1 software. Emulsifier concentration had a significant influence in the emulsion cost and in the emulsion stability. To minimize the emulsion cost, it was observed that employing a composition of 50% aqueous phase, 47.5% mineral oil and 2.5% emulsifier Montanide 888 VG can be obtain a stable emulsion for the formulation of veterinary immunogens.

SOP-6: Adjuvant use in production of tetanous antitoxin in horses

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The horse tetanus antitoxin immunoglobulins have been mainly used as heterologous serums in human, however it is of highlighting used as standard serum for the production of the corresponding vaccine and in addition to tetanus immunization of horses to protect until the protection was established. Obtention of horse antitetanus anatoxin hyperimmune plasma with high antibody concentration and biological activity constitutes a problem to solve. The use of adjuvant systems is of essential importance in antisera production, and its currently selection in tetanus antitoxin preparation has become a main issue in terms of animal welfare as well as economics. In order to generate high and durable antibody response to anatoxin antigen following a single immunization and to produce potent tetanus antitoxin in minimum possible period, evaluation was made for water in oil (w/o) emulsion immunopotentiation capacity and safety in donor animals. Certain characteristics including water/oil ratios- ranging from 60/40 to 40/60 (w/w(%))- and mixing shear (rpm) were analyzed. W/O emulsions were formulated and evaluated in immunized mares for magnitude of *in vivo* antibody formation and local reactions. High oil content proved most effective for antibody response formation, formed droplets by low mixing shear evoked higher antibody responses. They were applied several multi- site immunization protocols with low dose of immunogen in low volumes. The antitoxin titers did not vary significantly from each to other immunization protocol. The antitoxin titers increased quickly and reached a plateau around the 6 weeks for three groups of animals. The antibody titers were significantly higher in the fourth group of animals, reaching their activity to 2000 Lf-eq/ml. The adjuvant system used could be a possible alternative for immunization in tetanus antitoxin preparation considering its immunopotentiation capacity and safety in donor animals.

SOP-7: Obtain of aqueous and oily vaccines against paramyxovirus type 1 infection in pigeons and assessment of immune response

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Newcastle disease is a highly contagious viral infection of poultry, caused by an avian Paramyxovirus serotype 1 extended in the world. Paramyxovirus type 1 infection in pigeons produces clinical respiratory, digestives and neurotropics sign. This work had the objective of obtaining different vaccines for pigeon against Paramyxovirus type 1, elaborated with "La Sota" chicken's classical strain. Four candidates inactivated were elaborated: two using Incomplete Seppic Adjuvant (ISA)-70 and one ISA-50 as oily adjuvant. Another vaccine was formulated with Aluminium's Hydroxide. The formulations contain the same antigen but in different proportions and it has the requirements of quality established in the European Pharmacopoeia. 2 dose of 0,2ml by subcutaneous road in neck given 4 weeks apart were applied. We studied the response of antibodies in pigeons, showed in the Haemagglutination Inhibition test (HI). The appearing of tissues or systemic damages was evaluated. After a single dose in seronegative pigeons, the candidate with gel of Hydroxide of Aluminium promoted superior Geometric Mean (GM) of the antibody titres (GM=4,3) inciting studies of dose for this alternative of production. The GM of all candidates after twenty eight days of revaccination were between 4.9 and 6.6 but the one belonging to the candidate elaborated with ISA 50 was significantly superior (GM=6,6). With the scheme of 2 dose given 4 weeks apart, the 4 vaccines promoted high levels of antibodies HI in seronegative pigeons in correspondence with the results of the commercial vaccine Colombovac PMV (GM among 3 and 5), without developing tissues or systemic damages.

SOP-8: Comparison of the adjuvant activity of aluminum hydroxide and calcium phosphate on the antibody response towards the Bothrops asper snake venom

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Immunological adjuvants are compounds that have the ability to enhance the immune response towards co-injected antigens. Adjuvant activity is showed by substances such as mineral salts, oil emulsions, immune stimulating complexes, bacterial derivatives, carbohydrates, liposomes, cytokines, virus-like particles and polymeric microparticles. These substances acts by mechanisms such as immunomodulation, antigen presentation, targeting, induction of CD8+ cytotoxic T-lymphocyte responses and depot generation.The aim of this work was to study the adjuvanticity of aluminum hydroxide and calcium phosphate on the antibody response towards the venom of the Bothrops asper snake. It was found that at in vitro conditions, with exception of some basic phospholipases which are better adsorbed by calcium phosphate, most of the venom proteins are similarly adsorbed by both mineral salts. After injection, none of the adjuvants allowed a fast release of the venom proteins at interstitial conditions. Leukocyte recruitment induced by the venom was similarly enhanced when it was adsorbed on both mineral salts, but venom adsorbed on calcium phosphate induces a higher antibody response towards all tested HPLC fractions of the venom. On the other hand, co-precipitation of venom with calcium phosphate decreases the capacity of the salt particles to couple venom proteins at in vitro conditions, but increase the venom ability to induce leukocyte recruitment and antibody response towards venom proteins. These finding suggest that in addition to chemical nature, physical properties of the particles forming the immunogen depot could play an important role on the adjuvant activity of mineral salts.

C-70: Primary endpoints, validation, and clinical efficacy in trials with subcutaneous and sublingual immunotherapy

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It is my plan to discuss the primary endpoints that validate clinical efficacy in clinical trials of immunotherapy. The purpose of this presentation is to show the different approaches that have been used to design successful clinical trials, and likewise to identify likely reasons for unsuccessful clinical trials. This talk will highlight those specific study design components that positively impact upon clinical trial outcomes. The emphasis of this talk will be on providing an understanding of the pertinent clinical trial design elements that are necessary to successfully complete Phase 1 (Safety), Phase 2 (dose determination; mechanistic studies), and Phase 3 clinical trials where adequate demonstration of efficacy and safety are paramount for regulatory approval. In order to do this, I have selected specific clinical trials that highlight the relevant components that define successful outcomes. I have chosen selected studies of classic SCIT, modified SCIT with incorporation of an adjuvant, and studies with sublingual IT.

C-71: Disease modifying effect of a sq-standardised grass pollen allergy immunotherapy tablet

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Background: The clinical development program for the ALK grass allergy immunotherapy tablet (AIT) is a comprehensive program of 20 on-going or completed, randomized, controlled clinical trials including a total of over 4500 patients in active treatment documenting tolerability, safety and efficacy of AIT treatment in adult and paediatric patients suffering from grass pollen induced allergic disease. Aim: The aim of the long-term GT-08 study was to investigate disease modifying effect, safety and efficacy of the ALK grass AIT in adult patients with grass pollen induced allergic rhino-conjunctivitis. Results: A total of 634 subjects, age ≥ 18 years, with moderate to severe allergic rhino-conjunctivitis inadequately controlled by the use of symptomatic medications were randomised to receive active treatment or placebo. The trial was originally planned for 1 year and extended in the process. A representative subset of 351 participants continued in the extension period. Daily treatment was continued for a total of 3 years followed by a 2 year follow-up period. Two years after completion of treatment, the active group showed a mean 27% reduction ($p \leq 0.003$) in combined symptom and medication score. The level of efficacy on all endpoints was similar to the previous 2 seasons. No safety issues were reported during the follow-up period; however, the treatment did cause local application site-related adverse events, which were generally mild and resolved soon after initiation of treatment. Conclusion: Two years after completing a 3-year treatment period with the SQ-standardised grass AIT, a sustained, significant, disease-modifying effect was observed. This trial is the first large-scale, randomised, placebo-controlled trial showing long-term disease modification after grass AIT treatment.

C-72: Recombinant allergens for allergen specific immunotherapy

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Recombinant allergens can be produced in highest quality as chemically defined molecules with consistent physical, chemical and immunological properties. Whereas the concentration of individual allergens varies from batch-to-batch and from allergen to allergen in natural extracts, recombinant allergen preparations offer the possibility to design a pharmaceutical product with a defined composition and exact concentrations of all components in optimal doses. The challenge is the selection of the right allergens that are able to replace a whole extract. In some instances the source material contains only one relevant major allergen, such as Bet v 1 from Birch pollen, Fel d 1 from cat or Amb a 1 from ragweed. In other cases, such as grass pollen and mites, several allergens must be considered. Recombinant grass and birch pollen preparations have already been shown to be clinically effective and further clinical development programs are in progress. For the treatment of grass pollen allergy we developed a cocktail of 5 major grass pollen allergens that are structurally and immunologically equivalent to their natural counterparts. For the treatment of birch pollen allergy we developed a hypoallergenic folding variant of Bet v1 (rbet v 1-FV) with a random coil structure that is unable to refold to the native conformation. This folding variant exhibits reduced ige-binding, but preserved T-cell reactivity. This hypoallergenic mono-component preparation was initially compared with an unmodified birch pollen extract in an open, randomized, comparative controlled study.The promising results of this study encouraged a following investigation of rbet v 1-FV in a large DBPC phase III study. This study showed a significant and clinically relevant reduction of the combined SMS as primary endpoint in comparison to placebo after treatment for 18 months. In an open follow-up study, the active group received a third year pre-seasonal treatment. A long-term follow-up 2 and 3 years after completion of the treatment shows a sustained clinical benefit for the patients receiving recombinant Bet v 1-FV. Conclusion: Recombinant allergens / hypoallergens can be produced as fully standardized pharmaceutical products and have been shown to be clinically effective

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C-73: Hypoallergenic Bet v 1 derived contiguous overlapping peptides are well tolerated in human and lead to sustained immunological responses

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Allergen specific immunotherapy with whole pollen extract is of long duration and may induce anaphylaxis. A novel immunotherapy based on Contiguous Overlapping Peptides (cops) derived from Bet v 1, the major birch pollen allergen was tested in a randomized, placebo-controlled phase I/iia clinical trial. A mixture of three cops (allert™, Anergis SA, Switzerland) spanning the whole Bet v 1 molecule was selected for its inability to bind ige in vitro. Prior to the pollen season, allert was injected subcutaneously to 15 adult volunteers at D0, D7, D14, D21 and D51 at a cummulative dose of of 440 ug, equivalent to about three years of SCIT, with Aluminum hydroxide as adjuvant. Control volunteers (n=5) received the adjuvant only. No immediate allergic reaction, no serious side effect or treatment discontinuations occured, whereas few mild and reversible delayed systemic reactions were observed. Allert induced a vigorous Bet v 1 specific immune response associated with INF-gamma, IL-10 and anti-Bet v 1-specific igg4 increases. Moreover, two months after the second season post treatment, specific igg4 response was still markedly increased as compared to pre-treatment values or placebo. Thus immunotherapy with a mixture of three cops derived from Bet v 1 (allert) is safe and immunogenic, and leads to long term immunological memory. The cops technology is now applied to further allergies, including Ragweed and House dust mites.

KNA 5: IgEantibody responses: understanding the interaction between adjuvants and route of exposure

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Measurement of ige antibodies become possible shortly after the discovery of this isotype (Wide et al Lancet 1967). At first, the units were arbitrary but with definition of the international units for ige it became possible to define units of ige antibodies and to relate them to total ige. Using crude extracts on the ige assay solid phase it has become clear that the titer of ige antibodies is an important determinant of the risk for asthma (see Soto-Quiros et al JACI in press 2012). It is also clear that high titer ige antibodies to mite, cat, and grass pollen are a dominant feature of the changes that occur with moving to modern levels of hygiene. In the last few years, there has been a progressive expansion of the analysis of ige antibody responses to individual proteins. These analyses have provided insight into the cross reactivity (e.g. Tropomyosins), the significance of different peanut proteins (e.g. Ara h 2 and Ara h 8) and also into the route of induction of ige responses to different epitopes: (e.g. Fel d 1, Fel d 2 and Fel d 5w or alpha-gal) (see Commins et al AJRCCM 185:723, 2012). Within these findings is increasing evidence that the route of exposure can have a major influence on the production of ige antibodies. Thus the oral route is normally tolerogenic, but even when responses occur they are generally low titer (see Erwin et al Annals of Allergy 104:496, 2010). By contrast, ige antibody responses to peanut or alpha-gal are often present in very high titer and current evidence suggests that they are induced through the skin. The question is whether the route of exposure implies a different mechanism for the induction of ige plasma cells. There is excellent evidence that ige B-cells induced inside a germinal center undergo apoptosis. Because of this, any adjuvant or dose of antigen that induces germinal centers will switch off ige responses. By contrast inhalation of allergens or entry through the skin can lead to direct switch from igm to ige (see Aalberse et al JACI 113:983, 2004). These ige B-cells become plasma cells and produce ige in protected sites within the bone marrow. There is now real potential for analyzing adjuvants and routes of exposure for their effects on long term ige responses and also for understanding how the oral route can alter subsequent ige antibody responses.

P1.1 Role of B-1 cells in the immune response against an antigen encapsulated into phosphatidylcholine-containing liposomes

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B-1 lymphocytes comprise a unique subset of B cells that differ phenotypically, ontogenetically and functionally from conventional B-2 cells. The most frequent specificities of the antibody repertoire of peritoneal B-1 cells are phosphatidylcholine (ptc) and phosphocholine. Liposomes containing ptc have been studied as adjuvants and their interaction with dendritic cells and macrophages has been demonstrated. However, their interaction with B-1 cells has not been explored. In the present work, we studied the contribution of B-1 cells to the humoral response against ovalbumine (OVA) encapsulated into DPPC- and cholesterol-containing liposomes. BALB/xid mice, which are devoid of B-1 cells, showed quantitative and qualitative differences with respect to wild type animals in the OVA-specific antibodies induced with this immunogen. Transfer of ptc-specific antibodies induced in wild type animals to BALB/xid mice partially restored the response induced by the liposomal preparation in the former mice. This response was further enhanced in BALB/xid mice reconstituted with B-1 cells. These cells were demonstrated to internalize ptc-containing liposomes and to migrate from peritoneal cavity to spleen. The adjuvanticity of liposomes was further demonstrated to be dependent on ptc, for no enhanced anti-OVA response was induced when the antigen was encapsulated into phosphatidylglycerol and cholesterol-containing liposomes. In conclusion, we have demonstrated for the first time the central role of B-1 cells for the immunostimulatory properties of DPPC-containing liposomes. Our results showed the participation of both the anti-ptc antibodies and the B-1 cells themselves, probably through their function as apcs, in the antibody response against a model antigen.

P1.2 Liposomes encapsulating Sticholysins with immunoadjuvant properties to stimulate an antigen-specific cellular immune response

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Background: Cytosolic delivery strategies of antigens in order to improve the antigen-specific cytotoxic T CD8+ lymphocyte (CTL) response are currently crucial in the development of anti-tumor vaccines. Liposomes (LP), lipid-vesicles widely used as adjuvants, nowadays are an attractive alternative for antigen delivery into the cytosolic space of antigen-presenting cells. In order to improved the antigen delivery from liposomes, promising strategies have employed the co-encapsulation into LP of bacterial pore-forming toxins with the target antigens. Sticholysins I and II (Sts, sti/II) are pore-forming toxins produced by the sea anemone *Stichodactyla helianthus* extensively studied in our lab. **Aims:** Due to the functional homology of Sts with bacterial pore-forming toxins, we studied the stimulation of antigen-specific CTL-mediated immune response by LP co-encapsulating Sts with ovalbumin (OVA) as model antigen (LP/OVA+St). **Results:** LP were prepared using a lipid composition where Sts do not exhibit pore-forming ability. *In vivo* CTL assays were carried out using C57BL/6 mice. Immunization with LP/OVA+St enhanced significantly an OVA-specific CTL activity in comparison with LP/OVA treatment. Moreover, LP/OVA+St conferred a higher protection to mice challenged with OVA-expressing tumor cells than LP/OVA. Interestingly, the inclusion into liposomes of a sti mutant forming a reversible-inactive dimmer stabilized by a disulphide bond also induced a similar antigen-specific CTL response. Besides, the CTL activity induced by LP/OVA+St was independent of CD4+ T cells help. **Conclusions:** Our results suggest the potentialities of LP encapsulating Sts as a novel and promising tool for priming cellular immune responses, which are keys in the immunity against tumors.

P1.3 Aluminum phosphate as adjuvant improves the immunogenicity of monovalent conjugates against serotypes 1, 14 and 19Fof*Streptococcus pneumoniae*

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Streptococcus pneumoniae is a pathogen that causes bacterial pneumonia, bacteremia, meningitis and otitis media. The vaccination with capsular polysaccharides (PS) is ineffective in toddlers and young infants, due to his T-independent nature. The success of conjugated vaccines against Neumococco is due to PS are become immunogenic for these groups of risk, because they are processed as T-dependent antigen by immune system. The Center for Biomolecular Chemistry is working on develops an heptavalent conjugated vaccine against Neumococco. Frequently, aluminum adjuvants are included on vaccine formulations to improve the immunogenicity of antigens. Herein, the effects of aluminum phosphate (alpo4) adjuvant on the immunogenicity of three monovalent conjugates against serotypes 1, 14 and 19F were evaluated. Balb/c mice were immunized with three doses of conjugates 1-TT, 14-TT or 19F (2 µg PS/dose) with and without alpo4 adjuvant. The serum and mucosal antibody response, the antibody avidity and inmunoglobulin isotope expressed were evaluated by ELISA. The conjugate 1-TT adjuvated induces antibodies igg with higher avidity and promote affinity maturation compare with non-adjuvated, but the adjuvant doesn't improve the levels of antibodies igm or igg anti-PS in serum or mucosa. For conjugate 14-TT, adjuvant induces higher levels of igg anti-PS after second dose, marked increment of igg1 in serum and igg in respiratory mucosa compare with the same conjugate without adjuvant. The conjugate 19F-TT adjuvated improve the levels of igg anti-PS and his avidity for polisacaridical antigen after second dose. In conclusion alpo4 as adjuvant improves the immunogenicity of monovalent conjugates 1-TT, 14-TT and 19F-TT.

P1.4 Synthesis and evaluation of fresh aluminum phosphate for ITS possible use as adjuvant

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Adjuvants are traditionally used for enhanced the immune response to several antigens for vaccination strategies. Aluminum salts are the most commonly used in human vaccines, mainly aluminium phosphate. The Center for Biomoleculoar Chemestry and the Finlay Institute are carrying out the project "Development of a pneumococcal conjugate vaccine". This vaccine includes capsular polysaccharides of seven serotypes conjugated to tetanus toxoid, adsorbed on aluminum phosphate (alpo4). There are commercial variants of alpo4 as Adjuphos®, but it is known that fresh synthesized aluminium phosphate has better adsorptive properties and allows a better consistency in the process of antigen adjuvation. Therefore, we developed a process for the synthesis of aluminium phosphate in small laboratory quantities in order to evaluate it as vaccine candidate adjuvant. The procedure consists of two main steps: synthesis and washing of the obtained gel. The synthesis was performed at room temperature, maintaining a constant ph and agitation. Washings were performed by cycles of dilution-centrifugation-decantation. The product was characterized by X-ray spectroscopy, refractive index and scanning electron microscopy. Also, it was determined the ratio aluminum: phosphorus, chloride content and the adsorption capacity. We compare the adsorptive properties of fresh aluminium phosphate and commercial Adjuphos®. Our results show better adsorption capacity of fresh aluminium phosphate with respect to commercial. The fresh alpo4 characterization demonstrates that we have a product with the required quality to be used as adjuvant in a pneumococcal conjugated vaccine.

P1.5 Comparison of freshly prepared aluminum phosphate and commercial Adju-phos adsorption capacity

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Aluminum phosphate has been used as an effective vaccine adjuvant and include in vaccines for children like some Haemophilus influenzae type b and pneumococcal conjugate vaccines. Aluminum based adjuvant becomes more ordered during aging at room temperature, accompanied by a decrease in protein adsorption capacity. That is why during the development of a vaccine, the adjuvant can be change from a commercial aluminum phosphate (Adju-phos) to an in-house produced aluminum phosphate. Also, to prepare vaccines, adjuvants must be sterile. A frequently employed sterilization technique is autoclaving. This method accelerates the transformation to a more stable polymorphic form; reduce the surface area of aluminum phosphate and therefore the protein adsorption capacity. The goal of this study is to compare the effect of sterilization on the adsorption of aluminum phosphate synthesized under good manufacturing practices conditions and commercial like adjuvant. We compared the adsorption capacity of freshly prepared alpo4 with Adju-phos. As a model protein were use tetanus toxoid (TT), the Cuban vaccine candidate against pneumococcal and Hib-TT. The results show superior adsorptive properties for the in-house synthesized adjuvant than the commercial Adju-phos.

P1.6 The hen's egg test on chorioallantoic membrane (HET-CAM), useful in vitro assay for assessing irritation properties of immunoadjuvants formulations

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Context: The assessment of local tolerance of immunoadjuvants are performed within the repeat-dose toxicity study, but under this strategy is difficulty to assume the existence of primary irritant effect, because inflammation and others local side effects detected at the end of the experiment can be due to other causes like the activation of danger signals not derived from the disruption of the tissue integrity for direct irritation. In this work is proposed an in vitro method named hen's egg test on chorioallantoic membrane (HET-CAM) as a tool to be used together with in vivo methods for a better characterization of local effects of adjuvants. ***Methods:*** Seven adjuvants were evaluated with HET-CAM and an in vivo local tolerance test to detect different levels of irritation. ***Results:***Although there was a significant correspondence among both methods evaluated by chi-squared test ($p < 0.001$), there were also subtle differences, not marking an absolute correspondence (detected by Contingency coefficient and Lambda coefficient), interpreted by the ability of many adjuvants to recruit inflammatory cells in the absence of tissue irritation. ***Conclusion:***HET-CAM, is an useful in vitro assay for assessing irritation properties of immunoadjuvants formulations.This first result can encourage a more extensive validation study that reveals the utility of this in vitro method as a tool during non-clinical evaluation of immunological adjuvants.

P1.7 Molecular mimicry between mayor proteins from neisseria meningitidis b and self-proteins is insufficient condition for triggering early post-vaccination autoimmunity in mice and rats vaccinated with OVM-derived adjuvants

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Background and Aims: The concern that certain vaccines may induce autoimmune disease has been conjectured and one of the proposed mechanisms is molecular mimicry (Mm) between vaccine antigens and self-structures. We investigated if Mm for T epitopes of major proteins from *Neisseria meningitidis* B contained in afco1 and AIF-nch (OVM-derived adjuvants**)**, and self-proteins, are able to trigger early autoimmunity reactions in mice and rats vaccinated. **Methods:** Mm between *N. Meningitidis*porb, , and human/mouse proteinswas investigated using the bioinformatic tools: SWISS-PROT/trembl SYFPEITHI and FASTA data bases. C57BL/6 and DBA mice, and Sprague Dawley rats, were immunized intranasally or intramuscularly with AIF-nch, afco1 or vehicle using different treatment protocols. Clinical signs were recorded daily and body weight, every 15 days. Animals were sacrificed at the end of experiments, and full necropsy was performed including microscopic studies, leukocyte count, T CD4+ and TCD8+ cell quantification in local lymph nodules, biochemistry and anti-dsdna antibody levels in sera by ELISA were determined. **Results:** Mm was foundin several self-proteins from: blood, liver (including fetal liver), skin, brain, lungs, and testicles (human and mouse). Significant alterations of the endpoints evaluated were not detected in any vaccinated mouse, except some lymphoid follicles with germinal centers in the draining lymph node, due to lymphocyte activation induced by the formulation adjuvant. **Conclusion:** Despite the existence of Mm between porb and self-proteins there was no evidence of organic damage in mice vaccinated under our experimental conditions.

P1.9 A recombinant vaccine of H5H1 Ha1 fused with foldon and human IgG fc induced complete cross-clade protection against divergent H5N1 viruses

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Introduction: Development of effective vaccines to prevent influenza, particularly highly pathogenic avian influenza (HPAI) caused by influenza A virus (IAV) subtype H5N1, is a challenging goal. **Methods**: In this study, we designed and constructed two recombinant influenza vaccine candidates by fusing hemagglutinin 1 (HA1) fragment of A/Anhui/1/2005(H5N1) to either Fc of human igg (HA1-Fc) or foldon plus Fc (HA1-Fdc), and evaluated their immune responses and cross-protection against divergent strains of H5N1 virus. **Results**: Results showed that these two recombinant vaccines induced strong immune responses in the vaccinated mice, which specifically reacted with HA1 proteins and an inactivated heterologous H5N1 virus. Both proteins were able to cross-neutralize infections by one homologous strain (clade 2.3) and four heterologous strains belonging to clades 0, 1, and 2.2 of H5N1 pseudoviruses as well as three heterologous strains (clades 0, 1, and 2.3.4) of H5N1 live virus. Importantly, immunization with these two vaccine candidates, especially HA1-Fdc, provided complete cross-clade protection against high-dose lethal challenge of different strains of H5N1 virus covering clade 0, 1, and 2.3.4 in the tested mouse model. **Conclusion**: This study suggests that the recombinant fusion proteins, particularly HA1-Fdc, could be developed into an efficacious universal H5N1 influenza vaccine, providing cross-protection against infections by divergent strains of highly pathogenic H5N1 virus.

P1.10 Adjuvant effect of bacterial outer membrane vesicles with penta-acylated lipopolysaccharide on antigen-specific T cell priming

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Background: Outer membrane vesicles (OMV) are nano-sized spherical blebs shed by Gram-negative bacteria and have been utilized in vaccine development. **Aims**:In the present study, we evaluated T cell adjuvant activity of OMV with strictly penta-acylated LPS produced by msbb/pagp mutant of non-pathogenic Escherichia coli W3110 (momv) compared to OMV with hexa-acylated LPS produced by wild-type E. Coli W3110 (womv). **Results**:Penta-acylation of LPS renders momv less endotoxic than womv in in vitro and in vivo toxicity assays. In mice, momv has adjuvant activity on T cell priming not only in KLH protein immunization but also in SIINFEKL peptide immunization. The T-cell adjuvant activity of momv was comparable to that of womv and LPS and was abrogated in TLR4 K/O mice. In innate immunity, momv stimulated bmdcs to up-regulate co-stimulatory and antigen-presenting molecules and to produce pro-inflammatory cytokines in a TLR4-dependent manner. Of note, momv induced cytokine production at a significantly less extent compared with womv. **Conclusion**:Taken together, we propose that momv with penta-acylated LPS is a safe vaccine adjuvant for T cell priming and can be used in vaccine development against viral diseases and cancer.

P1.11 Preliminary results of AFCo1 and AFPL1 co-administered with rPhl p5 by parenteral and mucosal route in mouse

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Background: Timothy grass (*Phleum pratense*) is one of the most common airborne pollens with Phl p5 as major allergens in Europe. Seasonal allergic rhinitis is characterized by events that are under regulation of T helper 2 (Th2) immune response pattern. Mucosal administration of soluble antigens is generally recognized to lead to induction of an antigen-specific immunological hyporesponsiveness, termed mucosal tolerance, and treatment with allergen peptides containing major T cell epitopes have proved to be highly effective in preventing allergic disease. We have two adjuvants, the proteoliposome (PL) of *Neisseria meningitidis* serogroup B (AFPL1®) and the cochleate (afco1). They have been shown potentialities as adjuvant for several immunization routes. **Aim:** To evaluate the immunogenity induced by rphl p5 adjuvanted with afco1 or AFPL1 administered by different routes in mice. **Results:** Female Balb/c mice were used and immunized by: three intranasal (i.n) or oral or 2 subcutaneous (s.c) doses with afco1+ rphl p5, AFPL1+ rphl p5, or rphl p5 alone. In the s.c route an additional rphl p5-Alum was included. A booster dose was given. We determined the anti rphl p5 igg1, igg2a, igg2b subclasses and ige immunoglobulin class. Low or not anti rphl p5 ige responses were detected. A significant anti-rphl p5 igg1 response was obtained in all adjuvanted groups and routes, although the i.n route showed the highest values. Higher titles of igg2a were observed in group that received the adjuvanted rphl p5 by i.n or s.c routes than control groups. A booster anti rphl p5 igg2a response was obtained by i.n route in adjuvanted formulation. **Conclusion:**A **s**hift of Th2 (ige and igg1) to Th1 (igg2a) and the useful of mucosal immunization was obtained. Additionalcellular studies are ongoing.

P1.12 Diverse adjuvant activity of saponins from the South American flora

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Saponins are natural glycosides, widespread within the plant kingdom, composed by a triterpenoid or steroidal aglycone moiety, linked to one or more sugar units. They show high structural diversity and are amphiphilic, thus they act as tensoactives. Some of them, notably those isolated from *Quillaja saponaria*, are very active as immunization adjuvants; however, their haemolytic and/or cytotoxic activity limits their use in humans. Both the haemolytic/cytotoxic and adjuvant activities are very variable within the group. With the purpose of identifying new saponins potentially useful as adjuvants, we evaluated the toxicity and characterized the adjuvant capacity of saponin preparations isolated from South-American plants. We report the characterization of pure saponins from *Ilex* spp. And *Passiflora alata* and of a saponin fraction from *Q. Brasiliensis*. We studied the humoral (total igg and igg isotypes) and cellular (DTH reaction) responses of mice immunized with the assayed samples using either tetanus toxoid or a viral antigen preparation (bohv-5). Alum and saponins from *Q. Saponaria* were included as control adjuvants. All assayed saponins were found to be less toxic than Quil A in *in vitro* assays. Some saponins from *Ilex* and *Passiflora alata* presented an activity similar to that of alum, when used as adjuvants of tetanus toxoid. In contrast, the saponin mixture from *Q. Brasiliensis* elicited strong humoral and cellular responses, similar to those produced by Quil A, when used as adjuvant of a preparation of bohv-5; this response included the generation of neutralizing antibodies and the production of Th1-type cytokines.

P1.15 IMS4112 and VLP of HBV as adjuvants for a quimeric protein of HIV-1

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Background: Nowadays, most vaccine candidates against HIV-1 are focused on the induction of an anti-viral Th1-type response. In this regard, we have developed a vaccine candidate formulation named TERAVAC-HIV. It includes three antigens: the recombinant HIV-quimeric protein CR3 comprising Th and CTL epitope rich regions from different viral proteins and the nucleocapsid (C) and surface (S) VLP of HBV. The adjuvant effect of VLP of HBV allows the induction Th1 HIV-specific cellular response. Aim: In the present work, the immunogenicity in mice of TERAVAC-HIV was compared with another formulation based on CR3 adjuvated with the IMS 4112 nanoparticle (Seppic, France). Results: The anti-CR3 cellular response was elicited after intranasal (i.n.), subcutaneous (s.c.) And i.n./s.c. Immunization schedules with both formulations. However, the advantage of the HBV VLP over the IMS 4112 was demonstrated in terms of cellular response kinetics after i.n./s.c. Co-administration. In this sense, after five doses, only mice immunized with TERAVAC-HIV elicited CR3-specific IFN-□-secreting cells. This result was accompanied by proliferation of CR3-specific CD4+ and CD8+ T lymphocytes. Conclusion: These results in mice suggest that HBV VLP are better adjuvants than the Seppic nanoparticle IMS 4112 to generate an anti-CR3 Th1 cellular response.

P1.16 Influence of the adjuvant in the immune response to proteoliposomes and liposomes from non-pathogenic mycobacteria in mice

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Proteoliposomes from BCG and *M. Smegmatis* and liposomes from *M. Smegmatis* were obtained and their humoral and cellular antigenicity was evaluated with samples from tuberculosis patients and healthy controls PPD+ and PPD-. The immunogenicity and induction of cross reactive responses in mice against *M. Tuberculosis* were evaluated using different adjuvants. The antigenicity of the different vaccine candidates was demonstrated in humans. All the candidates demonstrated immunogenicity in mice and induced cross reactive responses against *M. Tuberculosis* antigens. The profile of the immune responses obtained differed according the combination of experimental vaccine candidate and adjuvant used.

P1.17 Comparison of the adjuvanticity of different oil/water emulsions prepared with Freund´s adjuvant on the antibody response towards snake venoms

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Administration of snake antivenoms is the only clinically validated treatment for snakebite envenomation. Antivenoms are formulations of immunoglobulins purified from plasma of immunized animals towards snake venoms coupled to adjuvants. Nowadays, among existent adjuvants, Freund's adjuvant induces the highest production of antibodies towards snake venoms. This adjuvant is a formulation of mineral oil (that may contain dead Mycobacterium bacilli) used to produce a W/O emulsion, which has an aqueous phase where the venoms are dissolved. In the present work, the ability of different emulsions prepared using the Freund's adjuvant to increase the production of antibodies towards venoms from the most important venomous snakes in sub-Saharan Africa: Echis ocellatus, Bitis arietans y Naja nigricolis was compared. It was found that in the case of the three venoms, a simple emulsion prepared at a ratio of 50:50 has higher adjuvant effect, than emulsions prepared at ratios of 25:75 or 75:25. However, in the case of the Naja nigricolis venom, a multiple emulsion increased the production of antivenom antibodies similarly to a simple emulsion, but with diminished local injure. It was concluded that for some antivenoms, damage associated to the Freund's adjuvant administration could be significantly reduced, without affecting the antibody production, by using multiple emulsions.

P1.18 Very small size proteoliposomes derived from *Neisseria meningitidis*: an effective adjuvant for antigen-specific cytotoxic T lymphocyte response stimulation under leukopenic conditions

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Leukopeniais a severe condition resulting from both pathological processes and some treatments, like chemotherapy in cancer patients. However, the activation of the patient immune system is required for the success of immunotherapeutic strategies, as cancer vaccines. In this regard, leukopenia constitutes a major hurdle to overcome, mainly due to the impairment of cytotoxic T lymphocyte (CTL) responses. Adjuvants are basic components of vaccine formulations, which might be useful to stimulate immunity under this immunosuppressed condition. To this aim, we tested the capacity of a novel nanoparticulated complex, very small size proteoliposomes (VSSP), to promote CTL even in a leukopenic scenario. Noteworthy, we observed that a VSSP-based OVA vaccine induced a normal antigen-specific CTL response in mice rendered leukopenia by the administration of high doses of the chemotherapeutic agent cyclophosphamide (CY), while under the same conditions the OVA antigen formulated in the TLR-3 agonist polyinosinic-polycytidylic acid (P(I:C)) was ineffective. Moreover, an appropriate combination of VSSP with the P(I:C) vaccine was able to restore the CD8+ T cell effector function in leukopenic mice. VSSP induced not only a faster repopulation of immune cells in CY-receiving animals, but also enhanced the recovery of memory T lymphocytes and myeloid dendritic cells (dcs) while simultaneously abrogated the immunosuppressive capacity of myeloid-derived suppressor cells (mdscs). Our results suggest that VSSP could be a particularly suitable immunomodulator to be used in CTL-promoting active immunotherapy strategies operating in severe immune compromised scenarios.

P1.19 Results of repeated dose intramuscular administration of the adjuvant combination Montanide ISA51 VG/VSSP in Sprague Dawley Rats

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Introduction: In the design of vaccines is common to use adjuvants as immune response enhancers. The Montanide ISA 51 VG is a water/oil emulsion with immunomodulatory activity. VSSP adjuvant is a very small size proteoliposome derived from the combination of outer membrane proteins of *Neisseria meningitidis* with GM3 ganglioside. **Objectives:** With the aim of assess the effects of the combination Montanide ISA51 VG/VSSP, we performed repeated dose administration (intramuscular) for 14 days in Sprague Dawley rats. **Methods:** Rats were inspected daily for clinical signs. Body weight was weekly evaluated and rectal temperature was measured before and after each administration. Blood samples were collected for haematological and serum biochemical determinations at the beginning and at the end of experimentation, and complete necropsy were performed with macroscopic and microscopic analysis of organs. **Results:** The assay ended with a 100% survival. The Montanide ISA51 VG/VSSP group showed hardening and swelling at the administration site. Body weight and rectal temperature were not affected. The Montanide ISA51 VG/VSSP administration induced increase in polymorphonuclear cells, with relative lymphopenia conditioned by primary neutrophilia. The pathological analysis showed cysts, granulomas and proliferation of round cells, fibroblasts and fibrocytes in the application site. **Conclusions:** It could be concluded that the administration of the mixture Montanide ISA51 VG/VSSP causes changes apparently caused by the inflammatory reaction at the site administration, being similar to those described when administered Montanide ISA51 VG alone.

P1.20 Interleukin 2 mutein as adjuvant for anticancer therapies

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Interleukin-2 is a member of a common gamma chain cytokine family with potent capacity for stimulating T cell proliferation in vitro. In vivo IL-2 is necessary for expansion of antigen specific T effector cells and for induction of effective memory response. On the other hand is responsible for development and maintenance of Treg cell . IL-2 has been used for treatment of metastatic melanoma and renal cell carcinoma, but only 15- 20% of patients experienced any clinical benefit. The low efficacy of the IL-2 based therapy seems to be related with expansion of Treg cells during treatment, and Treg cells in turn, dampen any antitumor response. Looking for improvement on efficacy, IL-2 has been assayed as adjuvant for therapeutic vaccines for cancer or in combination with other therapies. In the CIM, we have developed an IL-2 mutein using bioinformatic tools. This new molecule behaves as an agonist of IL-2 in vitro and in vivo, which is able to expand NK cells and CD8 memory phenotype lymphocytes but not Treg cells. The mutein has been assayed in combination with anticancer vaccines in different murine tumor models and has been effective boosting the immune and antitumor responses induced by the therapies assayed.

P1.21 From *neisseria meningitidis* serogroup B vaccine to Potent Adjuvants

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Introduction: Outer membrane vesicles (OMV) from microorganisms have been demonstrated to be a good target for vaccine development. A meningococcal BC vaccine (VA-MENGOC-BC®) has been used in Cuba and elsewhere since 1988 with more that 60 million doses delivered without severe adverse events observed. Adjuvants are essential vaccine components that immunopotentiate (IP) the innate immunity, deliver antigens to desired places, and polarize (Pz) the adaptive immune response to the desired level. Adjuvants are not licensed as such and are mainly kept by companies who develop their own vaccines thus avoiding competitors. Furthermore, currently there are not adjuvants capable of enhancing immune responses at mucosal surfaces where most of infectious agents enter or establish in the host. Therefore, adjuvantdevelopment is mandatory to any pharmaceutical company dedicated to vaccine development, particularly for mucosal use. **Aims:**Demonstrate and characterize the adjuvant effect of OMV as supporting of the Finlay Adjuvant Platform (FAP). **Results:** The FAP consists in a series of detergent-extracted OMV called Proteoliposome (PL) because of their high protein content in addition to lipids and their transformation into Cochleates (Co) named afplx and afcox, respectively. In addition, afco3x not-derived from Proteoliposome was also developed. The former (i.e. Afplx and afcox) contain several protective proteins (which permit it to be used as a specific vaccine candidate) and several synergistic microbial-associated molecular patterns (MAMP, which permit it to be used as a vaccine adjuvant). The most studied are AFPL1 and afco1 derived form *Neisseria meningitidis* serogroup B. They contain: LPS, Porins, and traces of bacterial DNA as the main synergistic IP; non-living delivery systems based on lipids; LPS as a main Pz driving to Th1 and CTL immune responses; and nano- or micro-particles. They induce long-lasting memory response and could be used with diverse antigens by both parenteral and mucosal routes. The afco3 contains particulate MAMP, which reduces the cost and has possible uses in veterinary vaccines. Last but not least, finding a good mucosal adjuvant will permit the development of Single Time Vaccination Strategies which combine parenteral and mucosal vaccination simultaneously. **Conclusion:** FAP seems to be very promising in vaccine development.

P1.22 Physicochemical and biological characterization of 1E10 Idiotypic Vaccine

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Background: 1E10 monoclonal antibody is a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been tested as an anti-idiotypic cancer vaccine, adjuvated in Al(OH)3, in several clinical trials for melanoma, breast, and lung cancer. During early clinical development this mab was obtained in vivo from mice ascites fluid. Currently, the production process of 1E10 is being transferred from the in vivo to a bioreactor-based method. Results: Here, we present a comprehensive molecular and immunological characterization of 1E10 produced by the two different production processes in order to determine the impact of the manufacturing process in vaccine performance. We observed differences in glycosylation pattern, charge heterogeneity and structural stability between in vivo-produced 1E10 and bioreactor-obtained 1E10. Interestingly, these modifications had no significant impact on the immune responses elicited in two different animal models. We also investigate the mechamisms by 1E10 adsorbs to alum in order to modulate the adsorption in the final formulation. Conclusions: Changes in 1E10 primary structure like glycosylation; asparagine deamidation and oxidation affected 1E10 structural stability but did not affect the immune response elicited in mice and chickens when compared to 1E10 produced in mice. The driving force of absorption of 1E10 to alum is ligand exchange rather than electrostatic interactions.

P1.23 Consistency of the manufacturing process for active pharmaceutical ingredients for gangliosides vaccines (NGCGM3/VSSP AND NACGM3/VSSP) in BioCen

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At the Center of Molecular Immunology (CIM) was designed and described a new immuno-adjuvant for use in vaccines in different clinical trials (melanoma, renal and breast cancer). These very small proteoliposomes, called VSSP (very small size proteoliposomes), combined in its structure the ganglioside GM3 (ngcgm3/VSSP or nacgm3/VSSP), for which properties have been described suppressor of the immune system and outer membrane vesicles Neisseria meningitidis, with demonstrated immunostimulatory properties.

At the Active Pharmaceutical Ingredients Plant on biocen, is performed the production of the apis for gangliosides vaccines (nacgm3/VSSP and ngcgm3/VSSP). During a first period was transferred to dialysis process technology from the CIM to biocen, running all the necessary adjustments to move from a batch size of 400 mg to 2000 mg. Subsequently, and to ensure better compliance with the GMP and the scale-up of the process, was carried out jointly the introduction and development of the process by ultrafiltration technology. The aim of this paper is to show the consistency of the manufacturing process of these apis, with an analysis of consecutive 44 batches produced during the period 2008-2011. They show no differences between batches produced by the two methods transferred, in addition to meeting the quality requirements of all manufactured apis and the satisfactory result of the real-time stability studies at 3 and 6 months of these products.

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P1.24 Influence of phosphate salts and alum content on to the immunogenicity of a *Dermatophagoides siboney* AFPL1-adjuvanted vaccine

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Introduction: Allergen adsorption can be relevant for clinical efficacy and safety of alum-adsorbed allergen vaccines, particularly, for the novel experimental vaccine containing the combination adjuvant AFPL1®. Phosphate ions interfere with adsorption of Dermatophagoides allergens to alum hydroxide. On the other hand, alum is associated to formation of granulomas at the site of injection, so its content should be minimized. **Aim:** To evaluate the immunogenicity of formulation variants of a Dermatophagoides siboney AFPL1-adjuvanted vaccine, using different content of phosphate salts and alum hydroxide. Methods: A 23 experimental design was used for evaluating the effect of phosphate (0-8.5mmol/L) and alum content (0.5-2mg/ml) on to adsorption of Der s1, as measured by ELISA. Six variants were further selected for immunogenicity testing in Balb/C mice, regarding allergen-specific igg, igg1, igg2a, and ige antibodies and cytokine levels in broncho-alveolar lavage (BAL) in challenged mice with allergen aerosol. **Results:** Best variants regarding Der s1 adsorption were in the range 0-4.26mmol/L of HPO4 or 0-2.5mmol/L of H2PO4, achieving values up to 99.9%. Decrease of Al(OH)3 content to 0.5mg/ml did not significantly ($P>0.05$) influenced adsorption. All variants induced igg1 and igg2a allergen-specific antibodies, after three immunization doses. However, the low alum variant showed slightly significant reduction of both igg1 and igg2a, although with less ige and eosinophils in challenged mice. Phosphate content did not significantly influence the response. Moreover, the cytokine levels in BAL did not changed significantly among formulation variants, although, all were superior to the Th2 control, regarding the antiallergic inflammatory local effect. **Conclusions:** Reduction of alum and phosphate content could be considered as pharmaceutical improvements with no disadvantages regarding immunogenicity of this experimental antiallergic vaccine.

P2.1 Usefulness of a murine model of respiratory challenge test for the preclinical pharmacological evaluation of novel allergen vaccines

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Introduction: Murine allergy models are commonly used in basic immunological research. Allergen exposure by the inhalant way has been used for sensitization process or for challenging allergic mice. Safety pharmacology is a novel concept introduced for preclinical assessment of pharmaceutical products. **Aim:** To establish a murine model of respiratory challenge test with house dust mite allergen, useful for preclinical evaluation of novel allergen vaccines. Methods: Balb/C or C57/BL6 mice of both genders were used. The challenge test was standardized using an allergen extract of Dermatophagoides siboney with an allergen concentration of 500 µg/ml, exposing mice daily for 30 min during 5 consecutive days with a further exposure 48 h later. The challenge test was used both in a preventative or therapeutic models, after administering different vaccine candidates. **Results:** The challenge exacerbated the allergic response, showing features both of the immediate response (raise of total and allergen-specific ige levels) as well as the Th2 inflammation (raise in IL-5, IL-13, blood eosinophils) at local level in bronchoalveolar lavage, with typical histological alterations in lung tissues, namely, peribronchial cell recruitment, goblet cell hyperplasia, mucus secretion and eosinophilia. No major differences were noted between Balb/C or C57/BL6 strains or between genders. The challenge test allowed good discrimination of groups administered with different treatments, allergic or negative control groups, both in the preventative model (non-sensitized mice) or in the therapeutic model (allergic mice). **Conclusions:** The murine respiratory challenge test proved to be a useful tool for assessing the pharmacological effect and the preclinical safety profile of allergen vaccines under development.

P2.2 Experimental model of asthma to *Blomia tropicalis* recombinant antigens

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Introduction: The mite Blomia tropicalis is the more frequent sensitizer agent in tropical regions, including in cities of Northeastern Brazil. Immunotherapy for the treatment of allergy to mites is indicated, but the use of total extract of the allergen agents has been contraindicated due to possible anaphylactic reactions. **Aims:** We obtained recombinant antigens of *B. Tropicalis* with the purpose of developing a useful experimental model for investigating the use of hypoallergenic recombinant antigens in immunotherapy of allergy to this mite. **Methods:** The sequences encoding the antigens of B. Tropicalis (Blo t 5 and 21) were obtained from their deposits in the database genbank. The proteins were expressed in Escherichia coli and purified by affinity chromatography. Groups of A/J male mice were sensitized with B. Tropicalis extract, rblo t 5, rblo t 21 or both intraperitoneally. The challenge was carried out by intranasal instillation. In 22 days, animals were anesthetized to obtain the samples and analysis of the following parameters: total white cell count in the BALF, measurement of the eosinophil peroxidase activity in BALF and macerated lungs, serum levels of specific ige antibodies. **Results:** Animals sensitized with *B. Tropicalis*, rblo t 5 , rblo t 21 or both, showed an infiltrate of inflammatory cells in BALF and increased levels of specific ige antibodies. The groups sensitized with *B. Tropicalis*, rblo t 5 and rblo t 5 plus rblo t 21 showed an increase of peroxidase activity in eosinophilic lung macerate. **Conclusion:** The rblo t 5 and rblo t 21 sensitization induced an airway inflammatory profile in A/J mice strain similar to those caused by the total mite extract. However, further studies are needed to evaluate and to standardize the experimental model of allergy to B. Tropicalis recombinant antigens.

P2.3 Potential of Brazilian Natural products as immunomodulators of allergic diseases

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Introduction: Allergic asthma has emerged as an important public health problem of urban populations in developed countries. Very often herbal medicine is used to treat this widespread disease, due to the lack of efficacy and the important side effects related to the classical drugs in use. Using an ethnopharmacological approach, we examined some natural products popular used by folk medicine of Northeast Brazil in an experimental model of allergic disease against *Blomiatropicalis* L. **Methods:** We examined so far 4 different species named: *Cissampelossympodialis* and its alkaloid Wariftein, *Ocimumgratissimum* L and its polyphenols rosmarinic acid and eugenol,*Allium cepa* L. And its flavonoid, quercetin, *Cymbopogoncitratus* and its main volatile oils, citral and geranial. The respiratory allergy was induced in A/J mice by administration of Bt extract and the treatment was done using different doses of extracts and its compounds, then were evaluated the changes induced by these drugs on immunological parameters related to the allergic process, which are up-regulated in this allergic model. **Results:** All 4 species used by folk medicine in Brazil presented immunomodulatory effects on our murine model. Some parameters evaluated were number of total cells in bronchoalveolar lavage (BAL); activity of eosinophil peroxidase (EPO) on the lung; serum level of specific ige; levels of IL-4, IL-5, IL-13 on BAL and spleen; histopathological changes in the lung and production of nitric oxide (NO) by peritoneal macrophages. **Conclusions:** The ethnopharmacological approach is an important strategy in order to potentially identify new molecules to treat pathologies. Additional experiments are in progress in our laboratory in order to further understand the exact mechanisms whereby these molecules exert their anti-allergic activity.

P2.4 Effects of 4-Alil-2-Metoxifenol in murine model of allergic respiratory disease induced by *Blomia tropicalis* mite

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Introduction: Asthma has emerged as an important public health problem of urban populations in developed countries as well as Latin America.Although current treatments are able to control symptoms and improve lung function in most patients, severe acute exacerbations still occur and contribute significantly to morbidity and mortality of asthma in all age groups.Moreover, the most common drugs, corticosteroids, have high rates of side effects. Therefore, new weapons to the therapeutic arsenal need to be developed being natural products an important source to obtain that. We decided explore the anti-allergic effect of 4-Alil-2-Metoxifenol oreugenol (EUG) in the present study because this is the main molecule of the essential oil from *Ocimum gratissimum L*. Which we and other authors have described its immunomodulatory potential.In this context, the objective of this study was to evaluate the therapeutic potential (anti-inflammatory and bronchodilator) of EUG on an asthma experimental model. **Methods**: To this end, AJ mice were sensitized (100 µg per animal, s.c.) And challenged (10 µg per animal, i.n) with *Blomia tropicalis* (*bte*) mite extract. Sensitized animals were treated or not with EUG (80 or 40mg/Kg) and were analyzed the following parameters: number of total cells in broncho-alveolar lavage (BAL); activity of eosinophil peroxidase (EPO) on the lung; serum level of specific ige; levels of IL-4, IL-5, IL-13 on BAL and spleen; histopathological changes in the lung and production of nitric oxide (NO) by peritoneal macrophages. In addition, the capability EUG in relaxing tracheal smooth muscle was also evaluated. **Results:** Treatment of animals with EUG led to statistically significant reduction of airway inflammation, reducing the cellular infiltrate, EPO and mucus in the lungs, production of Th2 cytokines and NO. We also observed a dilator effect of EUG by the relaxation of isolated trachea. **Conclusion:** These results suggest that EUG has a potential effect as an anti-asthmatic drug with both bronchodilator and immunomodulatory properties, which can be a candidate to compose the therapeutic weapons to treat this pathology.

P2.5 Toxicity assay in repeated doses of *Dermatophagoides siboney* allergen extract in mice

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Introduction: Allergen extracts are used for hyposensitivity and immunotherapy treatments, reducing significantly clinical symptoms of allergic disease.Objetive: Because of its wide use in immunoallergen therapy, we evaluated the Dermatophagoides siboney allergen extract to establish potential toxicity following repeated subcutaneous dosing in Cenp:NMRI mice. **Materials and Methods:** Animals were randomly distributed into two groups, control (vehicle) and treated (166.6 UB/animal), and were observed daily for clinical signs of toxicity following treatment. Body weight was weekly measured. At the end of the study, blood samples were collected for hematology and serum chemistry analysis and animals were euthanized for gross necropsy and histological examination of tissues. **Results:** There were no significant differences in body weight or hematology parameters between control and treated animals. Differences were noted in uric acid, blood urea nitrogen and glucose; however, these alterations were not considered to be of biologic relevance. Pathology evaluations demonstrated hemorrhagic and inflammatory lesions at the administration site in both experimental groups. **Conclusions:** We conclude that repeated dosing of 166.6 UB did not cause significant toxic effects in the mouse model.

P2.6 Enzymatic activity in native and polymerized mite allergen extracts

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Background: The proteolytic nature of some mite allergens is well established. This activity can be associated with an increase in allergenicity due to a shift towards a Th_2 immune response. In *Dermatophagoides pteronyssinus*, serine proteases (Der p 3, Der 6 and Der p 9) and cysteine protease (Der p 1) have been described. **Objectives:** To investigate the proteolytic activity of native and glutaraldehyde modificated (allergoids) extracts of *D. Pteronyssinus*. **Methods:** Total proteolytic activity was measured using the SIGMA protease fluorescent detection kit and the Azocoll method. The specific cysteine protease E-64 inhibitor was used to verify the specificity of the enzymatic activity. Serine and cysteine protease activities were assayed using the synthetic peptides S-2288 (D-Ile-Pro-Arg pna) and L-1460 (Pyr-Phe-Leu-pna), respectively. Der p 1 and Der p 2 levels were measured by ELISA. Two types of unmodified mite extracts (with more or less fecal material) were used. **Results:** Allergoids show a significant reduction in their proteolytic activity when compared with unmodified native extracts (1.12 ± 0.28 vs. 50.16 ± 22.79 BAEE units/mg of total protein; $p < 0.05$). Moreover, there were significant differences between native extracts with more or reduced amount of fecal particles (50.16 ± 22.79 vs. 0.38 ± 0.14 BAEE units/mg of total protein; $p< 0.05$). This total enzymatic activity could be partially removed after adding E64 (31.63 ± 8.54 vs. 10.11 ± 3.29 BAEE units/mg of total protein; $p< 0.05$). Serine protease assays showed that complete native extracts presented a higher proteolytic activity than extracts prepared with only bodies with a reduced amount of fecal particles, or allergoids (422.1 ± 179 vs. 39.10 ± 36.01 vs. 42.56 ± 31.96 BAEE units/mg of total protein, respectively; $p = 0.0005$). Similar results were obtained in cysteine protease activity (L-1640 peptide) (1,090 ± 220 vs. 230 ± 100 vs. 240 ± 220 units/mg of total protein, respectively; $p = 0.0005$). Heating the extracts significantly reduced the proteolytic activity. **Conclusions:** The glutaraldehyde polimerization process significantly decreases the proteolytic activity of *D. Pteronyssinus* extracts. Native extracts prepared with mites bodies and some fecal particles and have less proteolytic activity than extracts containing whole bodies and a large amounts of fecal particles.

P2.7 Beneficial effect of hydrolyzed Egg in allergy

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There is clinical evidence to recommend the use of partially hydrolyzed infant formulas (HA-IF) for at risk children as an option for prevention of allergic diseases. As cow's milk allergic infants are at risk of developing allergy to newly introduced foods at weaning, we aimed to extend the concept of HA-IF to egg, another potentially allergenic food. Hydrolyzed egg (HA egg) was produced from whole egg. Characterization and reproducibility of the product was assessed and residual antigenic proteins were quantified using ELISA. Allergenicity of the HA egg was tested both in vitro (serotonin release assay) and in vivo (rat model of allergy to ovalbumin). The capability of HA egg to induce oral tolerance to ovalbumin was assessed in both rat and mouse models. HA egg production was shown to be highly reproducible in terms of peptide profiling, residual allergenic proteins and in vivo benefits. Composition of HA egg, as compared to whole egg showed a clear shift towards peptides <1000 Da. The allergenicity of HA egg was also highly reduced in vitro and almost no allergic response (assessed by RMCPII quantification in sera) was observed in rats preliminary sensitized to ovalbumin and orally challenged with HA egg. Moreover, we showed that HA egg was able to induce oral tolerance to ovalbumin. In conclusion, we have shown that the concept of "low allergenicity linked to induction of oral tolerance" can be applied to other food allergens than cow's milk. A small human SOTI trial with HA eggs is being launched.

P2.8 Histological analysis as a tool for the preclinical pharmacological evaluation of therapeutic allergy vaccines

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Introduction: Murine models of respiratory allergy are useful for immunological research. Use of clinical important allergens with intrinsic allergenic activity would allow reproducing manifestations of clinical allergy in these models, particularly in the lower respiratory track. **Aim:** To establish the basis for the histological analysis of respiratory features of the allergic response in a murine therapeutic model of respiratory allergy as a preclinical evaluation tool for novel allergy vaccines. **Methods:** A therapeutic mice model was used. C57/Bl6 mice were first sensitized administering Dermatophagoides siboney allergen extract by IP route and exposing mice to allergen aerosols, and then, treated with three experimental vaccine formulations, by subcutaneous injection or sublingual route. Finally, mice were subjected to inhalation allergen challenge. Samples were taken from lung tissue and placed entirely in formalin 10%. The specimens were embedded in paraffin, and 0.5µm sections were cut. Slides were stained with hematoxiline-eosine and examined under microscopy. **Results:** After the sensitization procedure, mice showed histological alterations, resembling the typical features of the respiratory allergic response in humans. Among them: peribronchial accumulation of inflammatory cells, particularly eosinophils, airway thickening, goblet cell hyperplasia with intensive mucus production and prominent bronchiectasis. Also, prominent vascular and perivascular inflammation were seen in mice, a feature that is not usually relevant in human asthma. These effects were attenuated by the administration of experimental vaccines, both by injection or sublingual routes proving the pharmacologic effect of these products. **Conclusions:** Histological analysis proved to a useful tool for assessing the pharmacological therapeutic effect and safety profile of allergen vaccines.

P2.9 Dose repeat toxicity and local tolerance of a novel nano-liposome adjuvanted allergen vaccine

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Introduction: Novel adjuvants and delivery systems are being increasingly used in order to improve conventional allergen-specific immunotherapy, regarding efficacy and safety. Liposomes are particularly attractive for this purpose. Nano-scale liposomes can be easily processed by antigen presenting cells mimicking virus particles. A nano-liposome allergen vaccine formulation containing allergens of Dermatophagoides siboney (Der s 1 and Der s 2) is under development in Cuba. **Aim:** To evaluate the dose-repeat toxicity and local tolerance of a novel nano-liposome adjuvanted allergen vaccine of Dermatohpagoides siboney. **Methods:** A therapeutic murine model of respiratory allergy was used. C57/Bl6 mice were previously sensitized administering D.siboney allergen by IP route and exposing mice to allergen aerosols, and then, treated with the experimental formulations by subcutaneous route, with 6 doses 2µg Der s 1 per dose. Systemic toxicity was assessed measuring body weight and performing macroscopic and histological examination of several organs. The injection site was inspected by macroscopic examination and histological analysis. **Results:** No significant differences ($p<0.05$) between body weights in treated mice and negative control group was observed. Differential leukocyte count was also unchanged. Normal histology was observed, excepting some alterations in the spleen (reactive hyperplasia with secondary lymphoid follicles), linked with immunologic stimulation. The injection sites showed normal histology in contrast with previous findings with the use of alum. **Conclusions:** The results support the safety from the toxicological point of view of this novel allergen vaccine formulation.

P3.1 Clinical evolution of allergic patients under immunotherapy with Valergen mite vaccines

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Introduction: Specific immunotherapy is an effective treatment for allergic diseases, and it a distinguishing feature of the Allergy Specialty. The use of standardized allergen extracts is essential for its reliability. **Aim:** To assess the clinical evolution of allergic patients with asthma or rhinitis under immunotherapy with industrially manufactured mite allergen vaccines (VALERGEN) by subcutaneous route. **Methods:** A descriptive study of 107 adult allergic patients with positive Skin Prick Tests to Dermatophagoides pteronyssinus, D. Siboney or Blomia tropicalis subjected to SCIT with VALERGEN products (BIOCEN, Cuba), was performed. General clinical assessment was carried out at 6, 9 and 12 months of treatment. The presence and classification of adverse reactions was recorded. **Results:** 57% of patients showed a satisfactory clinical evolution at 6 months of treatment. At 9 months this number raised to 94% and at 12 months, 97% of patients showed this positive outcome. Only one patient presented a systemic adverse reaction (frequency 0.9%) classified as Grade III. **Conclusions:** The standardized VALERGEN extracts have showed its efficacy regarding positive clinical evolution of patients under treatment, by subcutaneous route. The frequency and severity of systemic adverse reactions was low in this study.

P3.2 Adherence and tolerability of mite allergen sublingual immunotherapy with different dosing schedules in asthmatic children

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Introduction: Specific allergen immunotherapy with mite allergens is effective both in rhinitis and asthma. However, injection immunotherapy carries a high risk of systemic adverse events, so, the sublingual route (SLIT) is being used more frequently. Efficacy of SLIT in asthma has been widely studied, however, in Cuba, the therapeutic effect of mite vaccines of *Dermatophagoides pteronyssinus* (Dp), *D.Siboney* (Ds) and *Blomia tropicalis* (Bt) has been demonstrated only in adults, but not children. **Objective:** To determinate adherence and tolerability of SLIT with mite allergen vaccines using different treatment schedules in asthmatic children. **Methods:** Thirty children (2 to 15 years) with mild to moderate asthma were selected for each treatment schedule. Three dosing schedules were used: (1) maximum of 3 daily drops; (2): max. 5 drops and (3) max. 10 drops, all by sublingual route. Incremental updosing was used for 3 weeks. A maintenance dose of 2000 BU was used once a week for schedule 1 and twice a week for schedules 2 and 3, during 2 months. Standardized House Dust Mite allergen vaccines of Dermatophagoides pteronyssinus, D. Siboney and Blomia tropicalis were employed (VALERGEN, BIOCEN, Cuba). **Results:** Adherence to treatment was above 90% for the three schemes with no statistically significant difference ($p>0,08$) among them. The patients tolerated the different dosing schedules without having to change the planned scheme. Only two patients reported local adverse reactions, classified both as grade I (mild tongue itching without medication). **Conclusions:** All children tolerated the three treatment regimens similarly. It is suggested that future clinical studies explore doses well above the current ones using more concentrated products.

P3.3 Efficacy and safety of a novel ragweed allergy immunotherapy tablet (AIT) during peak season in north america

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Rationale: Ragweed-allergic patients suffer most when allergen levels in the environment are at their highest (peak season). During that period, traditional pharmacotherapy may not control symptoms adequately. We report findings from an investigation conducted to evaluate the peak-season efficacy and safety characteristics of 2 strengths of a short ragweed allergy immunotherapy tablet (AIT) (Ambrosia artemisiifolia) in allergic rhinoconjunctivitis (ARC) subjects with or without asthma. **Methods:** Adults with ragweed-pollen induced ARC were randomized 1:1:1 to daily ragweed AIT (6 Amb a 1-U, 12 Amb a 1-U) or placebo for approximately 16 weeks before, throughout, and following ragweed pollen season for a total of approximately 52 weeks. Subjects in all treatment groups had access to predefined, open-label allergy rescue medications to use in a step-wise manner during ragweed pollen season if a prespecified ARC symptom threshold was met. Symptoms and rescue medication use were recorded daily in e-diaries. Efficacy endpoints included daily symptom score (DSS), daily medication score (DMS), and the total combined daily symptom and medication score (TCS) during the peak ragweed pollen season. Safety was monitored through adverse event (AE) reporting and by an external monitoring committee. **Results:** 565 subjects were randomized, and 560 were included in the data analysis. The majority of subjects (85%) were multisensitized, and 22% had asthma. Ragweed AIT 6 and 12 Amb a 1-U groups showed 21% and 27% improvement in TCS versus placebo plus rescue medication [PBO(+R)] with significant between-treatment differences of −1.8 and −2.2 (P<.05). DSS and DMS for 6 Amb a 1-U (14% and 34%) and 12 Amb a 1-U (17% and 45%) were superior versus PBO(+R) with significant between-treatment differences (P<.05). Peak season efficacy was comparable to the entire season results. Most treatment-related aes were mild, local, application-site reactions with no reports of systemic allergic reactions. Both active doses demonstrated similar safety profiles. **Conclusions:** Findings from this phase 3 clinical trial indicate that once-daily ragweed AIT is effective and well tolerated when administered pre-and co-seasonally in multisensitized North American adults allergic to ragweed.

P3.4 Adverse events related to allergen immunotherapy with standardized house dust mite vaccines in asthmatic patients.

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Background: Allergen-specific immunotherapy is the only therapeutic approach capable to modify the natural course of the allergic disease, inducing long-lasting change of the immune response. Nevertheless, the risk of systemic reactions is still a serious limitation for its use. **Objective:** To characterized Adverse Events (AE) related to immunotherapy with House Dust Mite VALERGEN extracts in asthmatic patients. **Method:** A longitudinal descriptive study was carried out in 406 asthmatic patients (147 children and 222 adults) from September 2010 to October 2011, in Bayamo, an inland city in eastern Cuba. Vaccines of D. Pteronyssinus (90 patients), D. Siboney (114), and Blomia tropicalis (101) were administered; 101 patients received allergen mixes of Ds+Bt or Dp+Bt; 43.6% of patients received SLIT and the rest SCIT. Interviews were accomplished to adults or to children's tutors, checking administration and reaction data. **Results:** After one-year treatment, 37 patients reported AE; 15 of them were classified as systemic reactions and 22 were local. Of systemic reactions, 15 were by subcutaneous route (frequency per-injection 0.31%) and one was severe (grade IV), reported in an adult. Only 5 systemic reactions were reported by sublingual route, 4 classified as Grade I and one Grade II. Thus, for SLIT, the frequency of systemic AE per administration was 0.025%. Most AE with SCIT were reported with allergen mixes (OR:4.00, $p<0.01$). **Conclusion:** It was confirmed that SLIT is safer regarding the risk of systemic reactions. Although, the frequency of systemic reactions in SCIT was relatively low, precautions should be taken and the personal should be prepared to face severe allergic reactions.

P3.5 Effectiveness and impact on quality of life of sublingual inmunotherapy with house dust mite vaccines in allergic conjunctivitis

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Introduction: Ocular allergy involves the inflammatory process of the eye´s surface. Nowadays, it is an on-growing global entity with an incidence between 25% and 40% in general population. **Purpose:** To evaluate the effectiveness of sublingual immunotherapy for treatment of allergic conjunctivitis and its impact for the patient life´s quality. **Methods:** A longitudinal prospective study was achieved for evaluating permanent allergic conjunctivitis of 30 patients. The variables to be studied were gathered from the personal history chart. An initial evaluation was made at the first consultation and a second one took place at 6 months after treatment. The results were evaluated by means of an analog visual scale for symptoms and signs. The patients answered a Quality of Life questionnaire (EAPIQ) before and after treatment. Sublingual immunotherapy was performed with standardized allergen extracts of Dermatophadoides siboney, D. Pteronyssinus and Blomia tropicalis (VALERGEN, biocen, Cuba). **Results:** Ocular symptoms (redness, edema, tearing, secretion, photophobia and visual acuity impairment) significantly decreased after administrating sublingual immunotherapy. Comparing both pre and post questionnaires a statistically significant reduction was observed in regards to patients´ daily activities limitation and ocular symptoms. A 0,7% of patients referred some adverse events, none was serious and the 100% of patients were able to reduce oral medication. **Conclusion:** The VALERGEN sublingual immunotherapy demonstrates tolerability and effectiveness for all the symptoms of permanent allergic conjunctivitis in clinical practice, observing improvement in the patient's quality of life.

P3.6 Adverse reactions to allergen immunotherapy using house dust mite vaccines in asthmatic patients.

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Introduction: The risk of adverse allergic reactions during allergen immunotherapy (IT) is well known. These reactions can be local and systemic. In Allergy services in Cuba, there is a growing use of standardized allergen vaccines of house dust mites for asthma treatment. **Aim:** To assess the frequency and severity of adverse reaction during IT in asthmatic patients under subcutaneous or sublingual IT. **Methods:** The sample was 100 patients with mild to moderate asthma attending the allergy service of our hospital in Havana during 2011 and the first three months of 2012, receiving either SCIT or SLIT with VALERGEN extracts of D. Pteronyssinus, D. Siboney or Blomia tropicalis (BIOCEN, Cuba). All patients received high maintenance doses, above 1000 BU. A standard questionnaire was filled by the doctor. Statistical tests (Chi2) were applied. **Results:** 15 (15%) patients presented adverse reactions, of them 5 (33%) were mild, 4 (27%) moderate and 6 (40%) severe. 9 (60%) reactions were classified as local and 6 as systemic of different grades. 80% of AR were caused by the subcutaneous route and the frequency was significantly greater ($p<0.05$) as compared to sublingual route. No systemic reactions were reported by SLIT. Most treated patients showed clinical improvement either by SCIT or SLIT. **Conclusions**: It is recommended to maintain a strict surveillance on asthmatic patients receiving SCIT with mite allergens and to follow all the precautions indicated for this treatment.

P3.7 Clinical response to subcutaneous immunotherapy (SIT) in polysenzitised profiline positive patients is reflected by the modification of allergen-specific IgE, IgG4, IgE/IgG4-ratio levels measured by microarray technique (ISAC 112)

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Background: Poly-sensitized patients and associated OAS pollinosis are usually not an indication for subcutaneous immunotherapy (SIT). The clinical response to allergen specific immunotherapy usually does not correlate well with allergen-specific ige levels analyzed by standard immunoassay. **Aims:** Efficacy of SIT of 10 commercially different extracts of SIT in 10 poly-sensitized patients all sensitive to profilines is studied. Correlation between clinical response to SIT and biological modification during SIT is studied. **Results:** Median improvement evaluated by the physician was 74% for the pollinosis symptoms' score and 71.5% for the food's symptoms' score. A specific ige ratio for grass profilin before SIT is the most relevant in the two clinical cases were SIT was adapted for the melon, the most frequently implicated food. After SIT, this specific ige ratio for grass profilin is the most elevated in the clinical case with the lower pollinosis clinical score improvement after SIT (55%). This specific grass profilin ratio in igg4 after SIT is the most elevated in one of the clinical case with the highest clinical score improvement for pollinosis after SIT (100%). During SIT, the specific ige levels and the ige/igg4 ratio are decreased whereas igg4 levels are increased (ISAC measurements). **Conclusions**: Poly-sensitized profilin patients with pollinosis and associated OAS can benefit from an individual SIT based on biological evaluation of ige and igg4 level by a new microarray-based assay (Immuno Solid phase Allergen Chip, ISAC 112) allowing a component resolved individually diagnosis leading to a component resolved individually adapted treatment.

P3.8 3-years course of allergen-specific immunotherapy (ASIT) in children with allergic diseases

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Background: 10-40% of the population suffers from various form of allergy. Especially essential increasing of the allergy prevalence is observing during last decade among children of different ages. ASIT is the unique pathogenic antiallergic effective treatment. **Aims:** to estimate the clinical efficacy of ASIT with Fostal-remedy in children suffering from pollinosis. **Methods:** 78 children with middle and severe seasonal bronchial asthma and pollinosis were divided into two groups. The first one (37 patients) was treated by symptomatic anti-inflammatory therapy. The second one (41 patient) was assigned ASIT with Fostal. **Results:** After 3 years of monitoring, the patients from the first group had deterioration of their condition in 37% of cases, addition by another form of allergy (bronchial asthma – 23%, atopic dermatitis – 8,1%), necessity in using the special exacerbation treatment – 72%, intensification of basic anti-inflammatory therapy – 28%, and regular visit to a physician. All patients from the second group showed a replacement of their clinical course severity to easier symptoms, decrease or absence of symptomatic supporting necessity, keeping of protracted remission. Also, after ASIT the spectrum of allergens that causes allergic symptoms were decreased in 61% of the patients. **Conclusions:**ASIT reduces sensitivity to allergens, that expressed by minimization or totally absence of clinical symptoms during naturally allergen exposition, that leads to the reduce or absence of needs in pharmacotherapy and physician visits.

P3.9 Immunologic and clinical efficacy of sublingual immunotherapy with house dust mite allergen vaccines in pediatric patients with persistent moderate allergic rhinitis.

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Background: Sublingual immunotherapy with allergens is not widely studied in pediatric patients with allergic rhinitis. **Objective:** To assess the immunologic and clinical efficacy of sublingual immunotherapy with allergenic extracts VALERGEN, in pediatric patients with persistent moderate allergic rhinitis. **Material and methods**: A controlled, randomized, clinical, diagnostic essay was carried out in 20 pediatric patients with persistent moderate allergic rhinitis. Sublingual immunotherapy was given through the deglutition technique with standardized allergenic extracts VALERGEN, manufactured in BIOCEN: Dermatophagoid siboney, Dermatophagoid pteronyssinus and Blomia tropicalis. Clinical, humoral immune and allergic response was assessed in vivo and in vitro, at the beginning and a year after treatment. **Results:** At the onset of the study, 100% of patients were sensitized to domestic dust mites and presented large amounts of eosinophils in the nasal cytogram, total Immunoglobulin E (ige)was elevated in 55% of the cases and it was determined that 30% of the patients had an associated Immunoglobulin A (iga) selective deficiency. After a year of treatment, eosinophil concentration in the nasal mucosa decreased. 80% of patients showed normal ige values. Clinical improvement was evident in all the patients and no collateral reactions were determined during treatment. **Conclusions:** Sublingual Immunotherapy with allergenic extracts VALERGEN is a safe and viable treatment that controls clinical symptoms and improves immunologic response in pediatric patients with persistent moderate allergic rhinitis.

P3.10 Allergy training and immunotherapy in Latin America: how survey-results lead to a regional overview

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Background: In April 2011 a group of Latin American (LA) allergy experts, leaders in their countries in the area of immunotherapy, met in Cordoba to discuss how allergy and immunotherapy can be improved in the region. The need for a situational sketch was expressed. **Aims:** a questionnaire on allergy training, allergen immunotherapy, extracts and legislation was sent out to 22 leaders in the field of nine Latin American countries to obtain an overview of the Latin American situation. Results are presented with descriptive statistics. **Results:** all 22questionnaires were returned (9 countries). Allergy training in 56% of the surveyed LA countries is at the third-level of medical care, after a core-training of 2-3y internal medicine or pediatrics; in 3 countries it is a second-level career and in one country there is no allergy training. Board certification with exam is only mandatory in a third of the countries; recertification being obtained without exam. Mostly, training is in general allergy; pediatric allergy training only exists in two countries. Both sublingual (SLIT, only in the form of drops) and subcutaneous (SCIT) immunotherapy are practiced in all countries, from the age of 3 years (mean, range 1-5yrs) onward. As no strict legislation exists IT can be managed by non-allergists in 7/9 countries. Mixed extracts are used with mostly 3-5 allergens/vial (range 2 to 6-10 allergens/vial) and all countries have bacterial vaccine. SCIT extracts come from US and European (89%) and 56% local providers. SLIT extracts are almost exclusively from Europe (Spain), but in Argentine, Brazil and Mexico also local SLIT extracts exist. There is rudimentary regulation concerning extract potency in two countries. IT is generally paid for by private patients. Insurance companies re-imburse IT in 56% of the countries, the social security system in 33% and in one country selected third level governmental hospitals supply IT. Publications on adverse events with IT are starting to appear (3 countries) and three countries have their own guidelines on IT (one only in pediatrics). **Conclusion:** There is an urgent need to have an LA consensus on how to improve allergy training and immunotherapy practice in the region.

P3.11 Cycloferon enhances the effectiveness of SCIT

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Background: It is known that the synthesis of ige under the control of cytokine network. Mediators of Th1 response (gamma interferon, IL2, TNF-alpha) inhibit the synthesis of ige. We prove the role of interferon inducers in allergic inflammation in bronchial asthma. SCIT is the gold standard in the treatment of allergic bronchial asthma. However, the unsatisfactory results of treatment ranged from 5 to 30%. The aim - finding ways to improve efficiency of SCIT through the use of interferon inducer – Cycloferon. Cycloferon - the first Russian low-molecular inducer of alpha- and gamma-interferon. At the heart of the drug is patented synthetic molecule - methylglucamine akridonatsetat. **Methods:** We examined 58 patients with bronchial asthma with poor rates of SCIT. Cycloferon in a 12.5% solution was administered at a dose of 2 ml by intramuscular injection 40 minutes before injection of the allergen. Opposed group, who received a standard course of SCIT, was 43. **Results:** In both groups after the SCIT observed positive dynamics of clinical severity index of day and night symptoms. Significant positive results achieved in lung function in patients of the first group. In this same group of patients had reductions in IL-4, ige, CIC, and increased rates IL-1b and IFN-gamma. In the second group there was a decrease of ige and IL-4. According catamnesis in patients of the first group poor effect of SCIT was observed in 6.9% of patients, and 25.9% in the second. **Conclusion** The inclusion of cycloferon to the classical SCIT improves its efficiency. Leveling reagin mechanism can explain the deviation of the cytokine balance under the cycloferon toward the Th1 response.

P3.12 Preliminary results of a national pharmacovigilance study of allergen injection and sublingual immunotherapy with house dust mite allergen extracts

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Introduction: Pharmacovigilance studies are helpful to follow the use of allergen vaccines in clinical practice. Industrially manufactured mite allergen vaccines (VALERGEN) have been recently introduced country-wide in Cuba for asthma treatment. **Objective:** To assess the safety and efficacy of allergen immunotherapy using VALERGEN vaccines in the routine clinical practice. **Methods:** A national prospective study was conducted using the cohort adverse event monitoring method. Patients have been followed at least for 6 months. The study has included 1719 asthmatic patients above 5 years old, attending 24 allergy services in 10 provinces. Efficacy variables were measured using a standard formulary reporting data on the last two weeks. **Results:** 53.2% of included patients are receiving SLIT and 46.8% SCIT, 48.4% are children. Only 92 patients (5.4%) have abandoned the study. Overall, 57 adverse reactions were reported. The frequency per administration of local reactions was 0.12% and 0.02%, respectively for SCIT and SLIT; whereas the frequency of systemic reactions was significantly greater for SCIT (0.191%) versus SLIT (0.027%). All systemic reactions by SLIT were classified as mild whereas 4 severe (Grade IV) reactions were reported by SCIT. The frequency of both local and systemic reactions was significantly greater in adults as compared to children. On the other hand, significant increase of Quality of Life, decrease of asthma severity and frequency of asthma manifestations and drug consumption was found, as compared to pretreatment values. **Conclusions:** The results safety profile of the sublingual route.

P3.13 Efficacy and safety of immunotherapy with valergens DP and BT extracts in asthmatic adults

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Introduction: Allergen-specific immunotherapy using House Dust Mite allergens is effective and advantageous for asthma, which has been widely acknowledged. However, the question of optimal dosing regimens is still controversial. **Objectives:** To evaluate the comparative efficacy and safety of two dosage schemes of subcutaneous immunotherapy, using mite allergen extracts in asthmatic adults. **Methods:** A randomized opened clinical trial was performed in 90 patients with mild to moderate asthma, sensitized to mites, as assessed by Skin Prick Test. Patients attended an Allergy service in Camaguey, a Cuban inland city. Patients were administered with SCIT with VALERGEN standardized allergen extracts of Dermatophagoides pteronyssinus, D. Siboney or Blomia tropicalis, and randomized to 2 dose-regimens: the first as indicated by the manufacturer (BIOCEN, Cuba), with an incremental phase of 13 weeks and monthly maintenance injection, and the second with a more gradual dosing-up (16 weeks) with biweekly injections. **Results:**Women from 18 to 27 years prevailed among the included patients. It was shown a reduction of clinical symptoms and medication at one year of treatment in both groups. This reduction was greater and significant in the first group ($p=0,020$) as compared to baseline. The skin reactivity to the administered allergen, as well as specific ige diminished significantly ($p<0.05$) in the first group. Most of adverse reactions were immediate and local, representing 1.37% and 0.33% of injections, respectively for the first and second group. **Conclusions:** A higher degree of efficacy, as well as satisfactory safety was demonstrated for the faster incremental schedule, as compared with previous conventional scheme, which can be important for treatment adherence.

P3.14 Sublingual immunotherapy and quality of life in asthmatic patients

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Introduction: The Quality of life index has been proposed as an objective validated end point for evaluating the clinical efficacy of allergen immunotherapy, as well as to assess a general impact on to patient's life. **Aim:** To assess change in the quality of life of asthmatic patients receiving allergen immunotherapy. **Methods:** A prospective open study was performed on mild to moderate asthmatic adult patients attending our allergy service receiving sublingual or subcutaneous immunotherapy with standardized allergen vaccines of House Dust mites (VALERGEN, biocen, Cuba). The study was conducted from January 2011 to February 2012; 115 patients were included with an age range from 15 to 60 years old. The Juniper AQLQ questionnaire was used at the beginning and at 6 months of treatment. **Results:** The Quality of life index increased significantly ($p<0.05$) in patients under immunotherapy from 3.57 (CI95%: 3.25-3.89) to 5.69 (5.22-6.16). All fields of the qol index increased significantly; namely, symptoms, limitation for activities, emotional function, environment stimuli. On the other hand, no significant difference ($p<0.05$) was noted between sublingual or subcutaneous administration routes. **Conclusion:** It with house dust mite allergens has a positive impact on quality of life of allergic asthmatic patients.

P3.15 Assessment of efficacy and safety of allergen immunotherapy with standardized mite allergen vaccines in the clinical practice

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Introduction: Allergen immunotherapy (IT) with House Dust Mite vaccines has been shown to be effective in clinical trials in asthmatic patients. The sublingual route is gaining in recognition and diffusion in Cuba. **Aim:** To assess the efficacy and safety of industrially manufactured Valergen® products in the clinical practice of it. **Methods:** A prospective longitudinal study was carried out in adult asthmatic patients sensitized to house dust mites Dermatophagoydes pteronyssinus (DP), Dermatophagoydes siboney (DS) o Blomia tropicales (BT), which received IT at our Allergy Service in Sancti Spiritus, an inland city in central Cuba, from January 2009 to February 2010. IT was administered by subcutaneous or sublingual routes following the manufacturer dosing regimen. 60 patients were included in this study, where women accounted for 64%, predominating the age group between 16 to 20 years. **Results:** Most patients accepted the sublingual route, while 78% followed the dose scheme without dose adjustments or any change. 88% showed clinical improvement after one year treatment, since symptom and medication score for asthma was below 60% of the initial value. Most patients (64%) showed also rhinitis manifestations, which were also generally improved. Adverse reactions were reported in less than 2% of administered doses, and were classified mostly as mild to moderate and of remote or possible causality. **Conclusion**: IT with the standardized mite VALERGEN vaccines has been well tolerated showing its efficacy and safety in clinical practice.

P3.16 A report of pharmacovigilance of adverse reactions to allergen immunotherapy by sublingual route in asthmatic patients

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Introduction: Sublingual allergen-specific immunotherapy (SLIT) is being increasingly used worldwide. In Cuba, three industrially manufactured allergen vaccines of dust mites (VALERGEN) have been approved for sublingual administration. **Objective:** To assess the safety of SLIT using VALERGEN vaccines in asthmatic patients, in the routine clinical practice in our allergy service, identifying and classifying the adverse events associated with their use. **Methods:** A prospective study was conducted using the cohort adverse event monitoring method. Patients have been followed at least for 6 months and the study is still ongoing. **Results:** 124 asthmatic patients were included, all from the Holguín Province in the eastern part of the country, 116 were children from 5 to 16 years old and 8 adults. 40 patients are receiving Dermatophagoides pteronyssinus, 44 D. Siboney, 39 Blomia tropicalis and only 1 a mix of D. Siboney and B. Tropicalis. Only 15 patients have abandoned the treatment, all by their own will, which indicates a 88% of adherence, a value much higher than the usual for subcutaneous IT. No adverse reactions have been reported at all. **Conclusions:** The results strongly support the safety profile and convenience to the patient of VALERGEN vaccines for SLIT in a routine clinical setting.

P3.17 Safety of sublingual immunotherapy with standardized vaccines of domestic mites

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Introduction: Allergen-specific immunotherapy, consists of administering gradually increasing doses of the allergen, to which the patient is sensitized, aiming at achieving tolerance to it and decreasing clinical symptoms. The sublingual immunotherapy (SLIT) was introduced as an alternative to subcutaneous route. Its use is being increased in the world and in Cuba, using standardized vaccines owing to greater safety. **Objective:** To assess the safety of sublingual standardized vaccines of three domestic mite species (VALERGEN) and its adverse events in the clinical practice at the Calixto García University Hospital, as well as the frequency of the vaccine prescription. **Methods:** A descriptive and cross sectional study design included 130 patients with treatment of SLIT with VALERGEN-DP (Dermatophagoides pteronyssinus), VALERGEN-DS (D. Siboney) and VALERGEN-BT (Blomia tropicalis) (BIOCEN, Cuba), who attended the Allergy Service in the period from January to September 2010. Age distribution: mean 19.6 years (range 1-75), 40.7 % was younger than 18 years. **Results:** The mixed multiallergen vaccine was the most used (63.8%). The most common allergen was *D. Pteronyssinus* followed by *B. Tropicalis*. 71,55% of administered allergens vaccines were in maintenance phase. We found 4 adverse events (3.1% of patients), all local, mild, and not requiring treatment or change of dosing schedule. **Conclusion:** VALERGEN vaccines by sublingual route were shown to be safe and well tolerated in Cuban allergic patients.

P3.18 Adverse reactions to subcutaneous allergen immunotherapy in patients with asthma, rhinitis and /or atopic dermatitis with an ultra rush buildup immunotherapy

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Background: Rush immunotherapy is prescribed infrequently because of the perception that accelerated immunotherapy buildup leads to a higher rate of systemic reactions. **Objective:** To evaluate the frequency of adverse reactions in patients with ige–mediated diseases receiving house dust mite (HDM) ultra rush immunotherapy. **Methods:** A retrospective study was conducted for patients with ige–mediated diseases receiving allergen-specific immunotherapy. Depigmented polymerized mites extracts were administered in two refracted doses of 0,2 and 0,3 ml at first injection, and in single 0,5 ml doses in subsequent monthly injections. A 30 min observation time was required after each injection. Systemic reactions were graded using the World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System. **Results:** Data from 577 patients were collected. 7141 injections were registered; 708 buildup and 6433 maintenance injections. The age range was 1 to 83 years. Most of patients had respiratory disease (546); asthma (314) or allergic rhinitis (507). 101 patients had atopic dermatitis. A total of 31 patients (5.37%) experienced 145 reactions (reactions / injections: 2.03%); 22 patients (3.8%) experienced 134 local reactions (local reactions / injections: 1.87%). Eleven patients (1.9%) experienced 11 systemic reactions (systemic reactions / injections: 0.15%). Seven systemic reactions were grade II and four grade I. No systemic reactions ocurred during buildup. Conclusion: Mites immunotherapy for patients with ige–mediated diseases using a depigmented polymerized mites extract with an ultra rush buildup, has similar frequency of systemic reactions than that seen in slow buildup immunotherapy. Accelerated buildup could improve patients' adherence to treatment and reduce dropout rates.

P3.19 Elevated tear fluid levels of mip-1 alfa in patients with atopic dermatitis

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Background: Atopic dermatitis (AD)one of the most chronic inflammatory skin diseases is characterized by dry and itchy skin and has been associated with a variety of ocular complications such conjunctivitis, keratoconus, iritis, retinal break, and retinal detachment. We present a patient with atopic dermatitis and ocular findings.The pathogenesis of ocular changes in CF is still unknown, but atopic dermatitis belongs to the large pathologic group of ocular surface epithelial diseases, termed keratoconjunctivitis sicca (KCS), that develop in dry eye syndrome. **Aims:** The aim of this study was to evaluate the levels of MIP-1 alfa (macrophage inflammatory protein-1 alfa) in the tear fluid of atopic dermatitis patients. We also investigated the correlation between the tear levels of this chemokine and clinical severity of ocular surface disease. We studied twelve patients with AD between 3 and16 years old. **Methods:** Chemokine levels were determined by ELISA. Complete ophthalmic examination, including dry eye tests, were used to study the ocular surface. **Results:** The tear levels of MIP-1alfa in the CF patients were significantly higher when compared with healthy controls. We found a positive correlation between the tear levels of MIP-1alfa and and the presence dry eye findings in AD patients. **Conclusion:** This current study indicates that chemokines play an important role in the ongoing inflammatory response. Our findings may help to explain one of the key factors contributing to the pathogenesis of ocular surface changes in AD patients.

P4.1 Allergic sensitization and ige binding profile to hen's egg allergens in cuban adults and children

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Introduction: Food allergy is increasing worldwide. Allergy to hen's egg can be important, particularly among children, and is relatively common in Cuba, although the exact sensitization prevalence is not well known. **Objective:** To perform a preliminary assessment of allergic sensitization and ige specificity profile to hen's egg white allergens in Cuban allergic patients. **Methods:** The Skin Prick Test was performed to each patient/subject, using a glycerinated allergenic extract, prepared in BIOCEN, Cuba, at 5 mg/ml protein content. Two groups of subjects were studied: a cohort of general adult population (N=292), and patients attending allergy services at 4 hospitals in Havana, comprising 100 adults (above 16 years old) and 157 children (5-16 years). The ige and igg4 binding profile of 20 selected SPT positive patients, was further analyzed by Western Blotting. **Results**: In the general adult cohort the prevalence of sensitization was 3.8%. Whereas, among patients suspecting food allergy the positivity rate was much higher: 34%, including 61% for adults and 38% for children. The most frequent clinical manifestation among positive patients was Urticaria or Dermatitis (64.2%), although respiratory symptoms were also common (44.2%). Ige binding was shown mostly by the 45kda band, tentatively identified as Gal d 2, a known major allergen of clinical importance. Also, the 77kda band (probably Gal d 3) showed binding in 2 patients. The igg4 binding profile was similar to ige, although with some additional bands. **Conclusions:** Allergic sensitization to egg can be important in Cuba, with prevalence values similar to other countries. Therefore, it should be taken into account for improving the specific diagnosis of allergy.

P4.2 Sensitization to storage mites, wheat and yeast allergens in cuban bakers

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Introduction: Bakers are considered a professional group at risk of developing allergic diseases by factors related to the work environment and the raw materials used in their work. **Objective:** This study aimed at assessing the frequency of work-related symptoms and sensitivity to mites and other occupational allergens among bakery workers through skin prick test (SPT). **Methods:** A no-matched case-controlled study was performed The study group included 17 bakery workers in Havana, Cuba, median age 33 (range 18-55 years old), and the control group comprised 14 non-exposed subjects, median age: 28 (range 22-47 years old). Both groups had male predominance. A medical resume and SPT were performed to all subjects. **Results:** Of bakery workers, 47,05 % had one or more work-related symptoms and the respiratory symptoms were the most reported. The higher sensitization frequency in bakers corresponded to yeast (88.2%), followed by storage and house dust mites, A. Siro and Dermatophagoides siboney (a Caribbean species similar to D. Farinae) and wheat with 82.3% each one. The least sensitization value was found to D. Pteronyssinus (only 47%), in contrast to the response pattern found in the control group where this mite was the predominant sensitizing allergen. The largest wheal diameters were found to A. Siro and T. Putrescientae in the bakers group and to D. Farinae and B. Tropicalis in the control group. **Conclusions:** There is a high sensitivity to storage mites and wheat flour and yeast allergens among bakery workers, which can be an important risk factor to consider for their occupational safety.

P4.3 Cross-reactivity between olea europaea and *betula verrucosa* extracts

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Background: Sensitization to Betula verrucosa pollen is common in the North of Europe. In Spain, it is also frequent in patients from Galicia, a region located in the Northwest of the country. Sensitization to olive (Olea europaea) pollen is very common in the Mediterranean countries and the presenceof sharedallergens in olive and birch pollens, such as profilins and polcalcins, has been described. In spite of this, there are few studies analyzing the in vitro cross-reactivity of theseimportant allergenic species. **Objectives**:The main objective of this study was to evaluate the cross-reactivity between of*B. Verrucosa* and *O. Europaea*, as well as the contribution of the major allergen of B. Verrucosa, Bet v 1, to this potential reactivity in patients with birch pollen sensitization. **Methods**:The sera from 39 birch pollen allergic patients from Galicia, Spain were used to study the cross reactivity between proteins from olive and birch pollen. All patients suffered from allergic rhinitis during the birch pollen season. Ige reactivity to *B. Verrucosa*, rbet v 1 and *O.Europaea* was evaluated by ELISA and Western Blot. Cross-reactivity between O. Europaea, B. Verrucosa, as well as rbet v 1 contribution was determined by inhibition ELISA using apool of sera. Mass spectrometry analyses were performed to identify common proteins in both extracts which could be responsible of cross-reactivity. **Results**: Specific ige determinations by ELISA revealed that 17(44.73%)ofthesepatients were sensitized to *O.europaea* pollen and 31 (81.57%) to Birch pollen. Immunoblot assays confirmed specific ige binding to proteins of birch and olive extracts. Proteomic analysis revealed the existence of mayor allergens as well as common allergens in both species. Inhibition ELISA performed with native extracts of *O. Europaea, B. Verrucosa* and rbet v 1 demonstrated the existence of cross-reactive allergens. Bet v 1 does not contribute to thecross-reactivity observed in this population. **Conclusion**: In spite of the lack of contribution of major allergens,there is a relatively high degree of cross-reactivity between olive and birch pollens, probably due topanallergens such asprofilins and polcalcins, or Ole e 10 homologous. The clinical implications of these findings may be that patients sensitized to olive pollen may have symptoms in Northern Europe during the birch pollen season, and viceversa.

P4.4 Allergic sensitization and IgE/IgG4 profile to soy food and respiratory allergens in Cuban adults and children

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Introduction: Food allergy is increasing worldwide. Soy is widely used as a hidden ingredient in many food products. Sensitization to Soy allergens can be important in Cuba, although the exact sensitization prevalence is not well known. **Objective:** To perform a preliminary assessment of allergic sensitization and ige specificity profile to Soy allergens in Cuban allergic patients. **Methods:** The Skin Prick Test was performed using a glycerinated allergenic extract of soy beans (BIOCEN, Cuba) at 5 mg/ml protein content. Two groups were studied: a cohort of general adult population (N=292), and patients suspected of food allergy, attending allergy services at 4 hospitals in Havana, comprising 90 adults (above 16 years old) and 128 children (2-16 years). A group of 40 bakers exposed to soy flour was also studied. The ige and igg4 binding profile of 32 selected SPT-positive patients, was further analyzed by Western Blotting. **Results:** In the general adult cohort the prevalence of sensitization was 4.5%. Whereas, among patients suspecting food allergy the positivity rate was much higher: 78% for adults and 41% for children. The most frequent clinical manifestation among positive patients was Urticaria or Dermatitis (64%), although respiratory symptoms were also common (42%). 56% of bakers were SPT-positive, showing mostly respiratory symptoms. Ige binding was mostly shown by a 30kda band in bakers (possibly Gly m Bd30K, a cysteine-protease). The igg4 binding profile was similar to ige. Conclusions: Allergic sensitization to soy as food can be important in Cuba, with prevalence values similar to other countries. Therefore, it should be taken into account for improving the specific diagnosis of allergy.

P4.5 Sensitization to mite allergens and its role in atopic dermatitis in children

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Background: Some studies suggest that inhalation or contact with aeroallergens may be involved in the pathogenesis of AD; the finding of positive skin prick test (SPT) to this allergens in this group of patients is one of the evidences supporting its role in AD. **Aims:** the purpose of this study was to determine the presence of AD and its relation with the sensitization to dust mites in children. **Methods:** Randomized and analytical study in 100 school children aged 6-7 years. The ISAAC questionnaire was applied to determine the presence of atopic dermatitis (AD). The sensitization to dust mites was explore by skin prick tests (SPT). The statistical associations were determine by chi-square tests. **Results:** the 26% of the selected sample suffered from atopic dermatitis (AD). The 58% of the children with dermatitis had at least one positive SPT to dust mites. Blomia tropicalis was the most common sensitization in these children (46%). The 69% of the school children with dermatitis had allergic rhinitis and the 31% had asthma. From the children with dermatitis, rhinitis and asthma, the 62% had positive SPT to dust mites. There was a significant statistical association between positive SPT to Dermatophagoides siboney and the presence of AD. **Conclusions:** there was a high prevalence of AD in this study, it was relevant the sensitization to dust mites, mainly to Blomia tropicalis in the children with dermatitis and there was a statistical association between positive SPT to Dermatophagoides siboney and the presence of AD.

P4.6 Serodiagnosis of allergy to *blomia tropicalis* using recombinant antigens instead of whole mite extract increases the assay specificity.

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Introduction:*Blomia tropicalis* is the more frequent sensitizer agent in tropical regions. Ige raised by B. Tropicalis cross-react with antigens of Ascaris lumbricoides, so the serodiagnosis of hypersensitivity to this mite using as antigen the mite extract have low specificity. **Aims:** To obtain recombinant antigens of B. Tropicalis with the purpose of a developing diagnostic tests for allergy to B. Tropicalis more specific than those available nowadays. **Methods:** The sequences encoding the antigens of B. Tropicalis (Blo t 5 and 21) were obtained from their deposits in the database genbank / NCBI. The inserts obtained by RT-PCR were cloned into the expression vector prseta. The proteins were expressed in Escherichia coli and purified by affinity chromatography. Recombinant antigens and B. Tropicalis extract were used to indirect ELISA assay with sera from 62 individuals with history of allergy to the mites and moderate to severe asthma. Inhibition tests with 20 sera absorbed with A. Lumbricoides antigen were performed with the extract of B. Tropicalis, rblo t 5 and rblo t 21. **Results:** The recombinant antigens rblo t 5 and rblo t 21 showed ige reactivity of 87.1% and 88.7%, respectively. Moreover, recombinant antigens showed less inhibition of the binding of specific ige when compared to crude extract of B. Tropicalis using sera absorbed with A. Lumbricoides. Conclusion: The recombinant antigensrblo t 5 and rblo t 21, produced in E. Coli, retained important epitopes recognized by ige antibodies of patients allergic to B. Tropicalis. Moreover, they had lower cross-reactivity with A. Lumbricoides antigen, demonstrating higher specificity for immunodiagnostic purpose.

P4.7 IgYpoliclonal antibodies conjugated to colloidal gold nanoparticles as a novel tool for the detection of of dust mite allergens

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Introduction: The use of nanotechnology is a useful tool in biomedicine. Aviar igy has a comparatives advantages because of its low and simplicity cost in its production and its ionic stability. The combination of these antibodies with colloidal gold nanoparticles is an innovation that has been used in many immunoassays. **Objective:** The purpose of this study was to produce and evaluate a novel immune-conjugates igy antibody anti dust mite allergens with colloidal gold nanoparticles (gnps) obtained by immunization of Hi Line Brown hens with synthetic oligopeptides from the dust mite group 1 allergens. **Methods:**The synthesis of gnps was performed by reducing haucl4 by boiling trisodium citrate at 1%. And were conjugated with the igy-anti cisteinprotease at room temperature incubation. The GNP immune-conjugate was characterized by UV-visible spectroscopy and by EM. The capacity of the gnps immune-conjugates to detect and bind to the allergens of Dermatophagoides farinae was demonstrated by Dot-Blot using a somatic proteins extract from whole body of the mites. **Results**:These synthesized gnps showed a maximum absorption peak at 520 nm.Dot-blot experiments shows evidence of this immune-conjugate bind to allergens present in the D. Farinae mite whole body extract (detection limit: 1.5 µg total protein). **Conclusion**: A novel conjugate hen igy- colloidal gold nanoparticles was produced and this gold labeled igy antibody is able to recognize allergens from Dermatophagoides farinae, representing a new tool for detection and control of mites allergens that could be used in the control of these allergens and could benefit sensitized atopic patients to mites, who daily are exposed to pollution from indoor spaces.

P4.9 House dust mite fauna and its relationship to allergen skin tests in six mexican states.

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Background: House Dust Mites (HDM) are important respiratory allergens all over the world. In Mexico, there have been only few studies describing the HDM fauna, and mostly limited only to Mexico City. This study aimed to asses the HDM fauna and its relationship to allergen sensitization in different cities with climatic variations in Mexico. **Methods:** A total of 60 dust samples were collected from mattresses in 6 Mexican states: Oaxaca, Tamaulipas, Veracruz, Puebla, Chiapas and Campeche; during a period from February to August, 2010 and in May, 2011. Mites were isolated under a stereomicroscope using lactic acid - 0.9% nacl solution (1:1). Identification was performed on fixed slides prepared with Hoyer solution. Skin Tests were performed with allergen extracts of different HDM species in the 60 mattress' owners, which had previously been diagnosed with respiratory allergy. **Results:** The Pyroglyphidae family was predominant, being found in 100% of dust samples. Dermatophagoides pteronyssinus (Dp) and Dermatophagoides farinae (Df) were the species most frequently found (in over 90% of samples). These findings were in agreement with the Skin Tests results, where 100% of patients were positive to Dp whereas 70% was positive to Df. It was evidenced for the first time the presence of Blomia tropicalis (in Tamaulipas, Veracruz and Campeche) and Dermatophagoides siboney (in Campeche). Both species are important allergenic sources in tropical/subtropical climates, and the last one had been previously reported only in Cuba. Other species found were Acarus siro, Cheyletus sp., Suidasia pontificia, and Gamasidae and Oribatidae families. **Conclusions:** These results confirm the importance of pyroglyphid HDM, as indoor sensitizers in different climatic and geographical regions in Mexico, as well as, the relevance of tropical species, particularly Blomia tropicalis and D. Siboney, in certain areas. They support the need of using allergen extracts of these mite species for improving allergen-specific diagnosis and immunotherapy.

P4.10 Sensitization to Dermatophagoides *pteronyssinus*, *Dermatophagoides siboney* and *Blomia* tropicalis in children from three family physician's offices.

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Introduction: Morbidity of allergic diseases mediated by ige antibodies has increased in the last 40 years. House dust mites have been identified as a major ethiological agent. In Cuba, the most common mites are Dermatophagoides pteronyssinus (Dp), Dermatophagoides siboney (Ds) and Blomia tropicalis (Bt). **Objective:** To assess the allergic sensitization to these mites in a child population and its relationship to house dust exposure. **Methods:** One hundred and three allergic children from the Guatao village, a suburban area of Havana city, were selected and classified in two groups according to the degree of exposure to domestic dust, as roughly assessed by a formulary administered by clinical investigators. All of them underwent the skin puncture test in both forearms with standardized allergen extracts: VALERGEN-BT (B. Tropicalis), VALERGEN-DS (D. Siboney) and VALERGEN-DP (D. Pteronyssinus) at 20 000 BU/ml (BIOCEN, Cuba). **Results:** Sensitization to three mites was significantly higher ($p<0,01$) in the group with greater exposure. In addition, the sensitization frequency was significantly higher ($p<0,01$) for Dermatophagoides species, specially for Dp (87,9 %), as compared to Bt (33,3 %) The Dp extract provoked a mean size of the wheal higher than the other products among the positive patients of both exposure groups ($p<0,04$). **Conclusions:** We concluded that a greater exposure of patients to domestic dust is related to a higher sensitization intensity and frequency to house dust mites, including Blomia tropicalis.

P4.11 Allergic sensitization and IgeE/IgG4 profile to wheat food allergens in Cuban adults and children.

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Introduction: Food allergy is increasing in developed countries. Wheat is widely used as an ingredient in many food products. Sensitization to wheat has been anecdotically described in Cuba, although its prevalence is not well known. **Objective:** To perform a preliminary assessment of allergic sensitization and ige specificity profile to wheat allergens in Cuban allergic patients. **Methods:** The Skin Prick Test was performed using a glycerinated allergenic extract of wheat flour (BIOCEN, Cuba) at 1mg/ml protein content. Two groups were studied: a cohort of general adult population (N=292), and patients suspected of allergy to wheat containing foods, attending allergy services at 4 hospitals in Havana, comprising 77 adults (above 16 years old) and 115 children (2-16 years). A group of 41 bakers exposed to wheat flour was also studied. The ige/igg4 binding profile of 10 selected SPT-positive patients was further tested by Western Blotting. **Results:** In the general adult cohort the prevalence of sensitization was 2.6%. Whereas, among patients suspecting food allergy the value was much higher: 84% for adults and 68% for children. The most frequent clinical manifestation among positive patients was Urticaria or Dermatitis (50.6%). Respiratory symptoms were also common (44.6%). 41% of bakers were SPT-positive, showing only respiratory symptoms. Ige binding was shown mostly by the 16kda band, tentatively identified as Tri a 30 a-amilasa inhibitor, a known important allergen. The igg4 binding profile was similar to ige. **Conclusions:** Allergic sensitization to ingested wheat can be important in Cuba, with prevalence values similar to other countries. Therefore, it should be taken into account for improving the specific diagnosis of allergy.

P4.12 In silico dimerization propensity study of house dust mite allergens from families 5 and 21

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House dust mites are an important source of allergens that cause asthma, eczema and rhinitis. The purpose of my research is to model the structure of some family 5 and 21 major house dust mite allergens. Their biological function remains unknown and their tridimensional structures have not been resolved experimentally except for Blo t 5 from Blomia tropicalis and and Der p 5 from Dermatophagoides pteronyssinus. These two allergens are formed of three similarly sized ±helices, but, in contrast to Blot5, which is reported to be monomer, Der p 5 is a homodimer forming a large hydrophobic cavity in which hydrophobic ligands (eg fatty acids/LPS) can bind and could provoke Th2-type immune response. We have studied the propensity if the allergens (Der f 5 and Der f 21 from Dermatophagoides farinae, Dermatophagoides pteronyssinus Der p 21, Sui m 5 from Suidasia medanensis and Lepd5 from Lepidoglyphus destructor) could dimerize and form a large cavity in the same way as Der p 5, using molecular modelling approaches. Monomeric models were performed for each allergen by MODELLER using the experimental structures of Der p 5 and Blo t 5 as templates. Then, the cluspro server processed the selected monomers in order to assess their capacity to form dimeric structures with a positive result for Derf5 only. The other allergens (Blo t 5, Sui m 5, Lep d 5, Der p 21 and Der f 21) did not present such a propensity. Furthermore, we identified mutations that should destabilize and/or prevent the formation of the Der p 5 dimeric structure which could help us to understand the role of the dimerization process in the allergic response these allergens.

P4.13Allergen sensitization patterns in mexican patient with rhinitis symptoms: a prospective nation-wide study

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Background: Allergic rhinitis (AR) is a disease with changeable expression depending on climate and allergen exposure. These variables differ between Europe and Latin America. We investigate the characteristics of allergic sensitization in skin prick testing of AR patients in Mexico. **Method:** In a carefully designed trial patients of either sex, 2-12 and 13-65 years of age, with rhinitis symptoms and a positive screening allergen skin test, seen by allergists in 26 centers spread over the 6 climatologic zones in Mexico were studied. The subjects filled in a validated questionnaire on allergic rhinitis and underwent skin prick testing with a standardized panel of 18 allergens using a uniform method. We report here on the skin prick test results using descriptional statistics. **Result:** A total of 529 skin prick tests were analyzed. The nationwide results are as follows (% positivity of all tests done per allergen): HDM (house dust mite Dermatophagoides pteronyssinus and farinae mix) 56, Blomia 25, Cat 22, Cockroach 21; Ash 24, Oak 23, mesquite 22 and cypress 11 pollen; Bermuda grass 26, Kentucky Bluegrass 21 and Timothy 20 pollen; lamb's quarter 14, ragweed 13 and sunflower 12 pollen; aspergillus 6, alternaria 7. We also tested 2 pan allergens: peach 8.5 and profilin 10%. Ash and oak sensitivity is less frequent in the dry North, where sensitivity to Mesquite - which grow better in dry zones- predominates among trees. Bermuda grass, as a tropical grass, causes most frequently grass pollen sensitivity in Mexico. Ragweed pollen sensitivity is mostly found in the dry (North) and semi-dry (Queretaro) zones (18-26%), but is not of importance in the area around Mexicocity or in the humid South-East (6-9%). Remarkable regional differences were found. The very dry Northern zone (Sonora): low HDM 18.5% and cat 7.4%, high aspergillus 26% and ragweed 26%. The semi-dry center (Queretaro) very high positivity for tree 63%, grass 55% and weed 37% pollen. Rhinitis patients from the very humid, semi-hot South-East with rainfall all year long (Tampico) have almost only skin prick sensitivity to mites 87% (both HDM and Blomia) and cockroach 30%. Hardly any to pollens nor cat (7%). **Conclusion:** Allergic sensitization pattern in Mexico varies from that reported in US and Europe. Striking regional differences exist in allergic sensitization of patients with rhinitis symptoms, within one country in which several different climatologic zones can be detected, as in Mexico.

P4.14 Sensitization to three domestic mites in allergic children in a suburban area in Cuba

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Introduction: Allergic diseases are a global health problem, being house dust mites among its principal etiological agents.**Objectives:** To characterize the sensitization to three mites: Dermatophagoides pteronyssinus, D. Siboney and Blomia tropicalis, in allergic children in Bejucal, a suburban area of Havana city. **Methods:** Skin prick testing with standardized allergen extracts at 20 000 BU/ml (VALERGEN, BIOCEN) were performed to 103 children, aged 3 to 15 years, with symptoms of respiratory allergy. Wheal diameters were measured. For positive patients, ige reactivity was assessed by Western Blotting. **Results:** The highest sensitization frequency was to D. Pteronyssinus (36.9%), followed by D. Siboney (35%) and B. Tropicalis (33%). The groups of 9-11 and 12-15 years old showed the highest sensitization frequency to each mite. Age was directly related to sensitization frequency for each mite separately ($p<0.05$) and, particularly to the number of sensitizations ($p<0.001$). The bands corresponding to major allergens were recognized by ige in Western Blotting, at all ages, however there was noted a trend towards spectrum widening at older ages. There was a significantly higher percentage of sensitized patients with both rhinitis and asthma, as compared to rhinitis alone ($p<0.001$), but the values were similar between mite species. However, for Dermatophagoides, but not for Blomia, the skin reaction size was significantly greater ($p<0.05$) in patients with asthma and rhinitis, as compared to rhinitis alone. **Conclusions:** Intensity and frequency of sensitization to tropical mites, including Blomia tropicalis, was associated with clinical manifestation of asthma and rhinitis. These data highlight the need to extend the use of specific immunotherapy in allergic children.

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P4.15 Is sensitization to Cupressus spp. (Cypress) and Platanus spp. due to allergen exposure or cross-reactivity?

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Background: The inhalation of Cupressus spp. (Cypress) and Platanus spp. (London plane tree) pollen is an important cause of allergic sensitisation in Southern Europe. However, cross-reactivity betweencupressus and Olea and between Platanus and grass species has been reported in a limited number of studies. Therefore, it has been suggested that the high prevalence of sensitisation to Cupressus andplatanus could be due to cross-reactivity instead of a primary sensitisation caused by exposure to pollen of Cypress and Platanus in the environment. The aim of this study was to investigate if there is a significant correlation between Cypress and Platanus atmospheric pollen counts and sensitisations in different cities of Spain. **Method:** We performed pollen counts for 1 year in 10 Spanish cities using a Burkard sampler, and conducted skin prick tests with several standardized pollen extracts in 1,112 allergic patients in the same geographical locations where the samplers were located. Spearman´s rank correlation was used to correlate the quantity of Cypress and Platanus pollen grains collected at each site with the prevalence of positive skin tests to these pollen extracts in each region. **Result:** A significant correlation between total annual atmospheric pollen counts for Platanus and the prevalence of positive skin prick tests for Platanus extracts was obtained (rs = 0.66, p=0.03). A non significant, but close correlation was obtained for Cupressus (rs = 0.57, p=0.08). **Conclusion:** The high rate of sensitization detected in Spain to Cupressus and Platanus seems to be due to a natural exposure to pollen and not to cross-reactivity. The approach followed in this study is helpful to evaluate potential clinical cross-reactivity between tree species. The large amount of trees planted during the last years and Global Climate Change in Spain, may account for these findings.

P4.16 Is allergy to peanut important in Cuba?

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Introduction: Peanut allergy is alarmingly increasing in developed countries. Roasted peanut (Arachis hypogaea) is a common food in Cuba. Nevertheless, reported values of sensitization to peanut are usually low. **Objective:** To perform a preliminary assessment of allergic sensitization and ige specificity profile to peanut allergens in Cuban allergic patients. **Methods:** The Skin Prick Test was performed to each patient/subject, using two glycerinated allergenic extracts, prepared from raw or roasted peanuts, in BIOCEN, Cuba, at 1 mg/ml protein content. Two groups of subjects were studied: a cohort of general adult population (N=292), and patients attending allergy services at 4 hospitals in Havana, comprising 16 adults (above 16 years old) and 66 children (2-16 years). The ige binding profile of 10 selected SPT positive patients was further analyzed by Western Blotting. **Results:** In the general adult cohort the prevalence of sensitization was 2.7% and 4.7%, to raw and roasted peanut, respectively ($p<0.05$, mcnemars). Unexpectedly, among patients suspecting food allergy, the positivity rate was higher to raw peanut (62%) versus roasted (51%). Interestingly, sensitization to roasted, but not to raw peanut, increased from 45% in children, to 75% in food allergic adults. Ige binding was shown mostly by the 60-64 kda bands, tentatively identified as (Ara h1 and Ara h3), known as clinically relevant allergens. **Conclusions:** Allergic sensitization to peanut seems not to be as important in Cuba as in developed countries, although it should be taken into account for improving the specific diagnosis of food allergy. Differences between sensitization to raw or roasted peanut deserve further investigation.

P4.17 Blo t 12 as a species-specific marker of *Blomia tropicalis*

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Background: Blomia tropicalis is an important allergen source in the tropics. Cross-reactivity with other mites, such as Dermatophagoides pteronyssinus, has been documented. Species-specific markers of exposition and sensitization help to improve diagnosis and treatment of allergy. Objective: To evaluate whether Blo t 12 is a species-specific marker of B. Tropicalis and the usefulness of P. Pastoris- and Origami BL21(DE3)- produced-Blo t 12.0101 (pblo t 12 and orblo t 12, respectively) for this purpose. Results: ELISA and WB showed that anti-pblo t12 rabbit polyclonal antibodies (pabs) reacted to Blo t 12 recombinant isoforms and B. Tropicalis extracts (body and faeces), but not to D. Pteronyssinus, several storage mite species' (such as T. Putrescienceae and L. Destructor) and cockroach extracts. Glycosylation was detected in pblo t12 and affinity-purified native Blo t 12. Human-ige binding to pblo t 12 was more intense after chemical deglicosylation (1.59 vs. 2.17 OD units, p=0.03). Metaperiodate-oxidation of pblo t 12 increased its recognition by pabs. Since human ige recognition of pab affinity-purified native Blo t 12 (nblo t 12) was not modified by metaperiodate-treatment this allergen was subcloned and expressed as a non-glycosylated protein in E. Coli. Although there was correlation between ige levels to nblo t 12 and those to both recombinants, it was higher with orblo t 12. **Conclusion:** Blo t 12.0101 could be used as a species-specific marker of B. Tropicalis. Hyperglycosilation of pblo t 12 affects its immunological reactivity, then, orblo t 12 seems to be more representative of this allergen.

P5.1 Food and nutrition insecurity: a marker of vulnerability to asthma symptoms

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Introduction: The mite Blomia tropicalis is the more frequent sensitizer agent in tropical regions, including in cities of Northeastern Brazil. Immunotherapy for the treatment of allergy to mites is indicated, but the use of total extract of the allergen agents has been contraindicated due to possible anaphylactic reactions. **Aims:** We obtained recombinant antigens of B. Tropicalis with the purpose of developing a useful experimental model for investigating the use of hypoallergenic recombinant antigens in immunotherapy of allergy to this mite. **Methods:** The sequences encoding the antigens of *B. Tropicalis* (Blo t 5 and 21) were obtained from their deposits in the database genbank. The proteins were expressed in Escherichia coli and purified by affinity chromatography. Groups of A/J male mice were sensitized with B. Tropicalis extract, rblo t 5, rblo t 21 or both intraperitoneally. The challenge was carried out by intranasal instillation. In 22 days, animals were anesthetized to obtain the samples and analysis of the following parameters: total white cell count in the BALF, measurement of the eosinophil peroxidase activity in BALF and macerated lungs, serum levels of specific ige antibodies. **Results:** Animals sensitized with *B. Tropicalis*, rblo t 5 , rblo t 21 or both, showed an infiltrate of inflammatory cells in BALF and increased levels of specific ige antibodies. The groups sensitized with B. Tropicalis, rblo t 5 and rblo t 5 plus rblo t 21 showed an increase of peroxidase activity in eosinophilic lung macerate. **Conclusion:** The rblo t 5 and rblo t 21 sensitization induced an airway inflammatory profile in A/J mice strain similar to those caused by the total mite extract. However, further studies are needed to evaluate and to standardize the experimental model of allergy to B. Tropicalis recombinant antigens.

P5.2 Prevalence of asthma and its association with body mass index and abdominal obesity in children from a small brazilian town

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Introduction: Some studies reported the impact of obesity on asthma. Decreased pulmonary functional residual capacity, tidal volume and lung inflammation described in obese individuals have been cited as predictors of asthma. This work aimed at evaluating a possible association between asthma and obesity, and the relative importance of BMI and abdominal obesity in the occurrence of asthma in Brazilian children. **Material and methods:** This is a cross-sectional study of children, 6 to 12 years old, from the public elementary schools of São Francisco do Conde, Bahia, northeastern Brazil. Asthma symptoms were collected using a questionnaire from the International Study of Asthma and Allergies in Childhood Program (ISAAC) phase III, adapted to Portuguese. Anthropometric, demographic and socioeconomic information were collected. Multivariate logistic regression analyses were used to assess the associations of interest. **Results:** 10.6% had histories of asthma. Excess weight was observed in 16.2%, 10.5% and 7.9% of the sample, measured by body mass index (BMI), waist circumference (WC) and the waist-to-height ratio (whtr), respectively. The percentage of patients with asthma attributable to BMI (8.2%) slightly exceeded those identified with abdominal obesity, measured by the WC (7.3%) and whtr (7.1%). **Conclusion:** We confirmed the predictive power of obesity in the occurrence of asthma. The results suggest that excessive fat deposits, either in the abdominal region or elsewhere in the body, increased the risk of asthma. Considering that obesity is an important public health problem worldwide, the control and eradication of this disease may partially reduce the occurrence of asthma.

P5.3 Atopy as a risk factor for recurrent upper respiratory tract infections in children under 5 years

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Introduction: Upper Respiratory Tract Infections (URTI) constitute an important morbility cause in children in preschool age. URTI are usually associated to atopy and allergic manifestations. **Aim:** to identify risk factors of URTI in children in preschool age. **Methods:** A longitudinal study of cases and controls cohort was carried out on a population sample of 320 children, half of them with URTI and the other half, healthy controls. A questionnaire was applied to each group, as well as, microbiologic examination of the upper airways, Skin Prick Test with House Dust Mite allergens (VALERGEN, 20 000 BU/ml) and tests for humoral immunity. **Results:** Allergic sensitization was the strongest risk factor for URTI, specifically, positivity to D. Pteronyssinus (OR=106, CI95%: 37-303), followed by positivity to D. Siboney (OR=56, CI95%: 17-184) and to Blomia tropicalis (OR=20, CI95%: 8.5- 49). Significant values ($p<0.01$) were shown also by existence of allergic parents, elevated total ige, decreased total igg and iga, and exposure to tobacco smoke. The strongest protecting factor was exclusive maternal breastfeeding (OR=0.12, CI95%: 0.05-0.26). Interestingly, non-significant difference was found regarding microbial isolates. Conclusions: Atopic status was the most prominent risk factor for URTI in preschool children. More effective strategies should be designed and enforced to diagnose and manage these risk factors in our child population.

P5.4 The specific ige anti *ascaris lumbricoides* seems to influence the serologic IgE response to indoor allergens in colombian pediatric asthmatic patients

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Introduction: Dust mite Df, Dpt, Bt and cockroach _Pa are important sources of allergens in the tropic. Host immune response against Ascaris lumbricoides (Al) could contribute to understand the influence of helminth antigenic proteins to modulate the allergic response to mites in allergic patients. The objective of this study was to establish the serological ige Immune response to Bt, Df and Pa, and to analyze its possible association with the ige specific response. **Method:** This was an open study conducted between January of 2010 and October 2011. 60 asthmatic patients were enrolled. Level of total ige were determinated by a commercial ELISA test. Specific ige was determinated by a in house indirect ELISA assay. Native extracts produced by us were characterized previously by SDS-PAGE and enzymatic activity test (APY ZYM). The ige antibody reaction was identified by Western blotting. **Result:** SDS-PAGE showed the presence of 21 major bands in the extract of Al, 12 in Df and 10 in Bt. All native extracts were positive for enzymatic activity. 4/60 (6,66%) patients showed low levels of total ige (< 40 IU/ml) (average = 23, 025 SD = 8, 60634); 20of 60 (33.33%) had normal serum ige level(40-150 IU/ml) average = 37, 90 ; SD = 34, 19); and 36/60 (60%) showed high ige levels (> 150 IU/ml) SD = 814,8 SD = 1532, 9). ELISA assay show the following results: 34/60 (56.66%) had a positive ige response to the Ascaris sp. Average = 0.325357;SD = 0. 219055. 46/60 (76.66%) were positive to Bt and Df. Of those 13 had a positive response for both mite species. (21,66%), 30/60 were ige positive to Bt. 17 patients (28.33%) were ige positive for Pa ;average = 0. 149824;SD = 0. 0648327. The statistical analysis comparing total ige, specific ige to mites and ascaris sp displays proportional significance. Bt : p = 0, 007.Df : p = 0, 027. Western Blot results identified proteins fractions in extracts from Bt, Df, Ascaris sp and Pa with nearby similar molecular weights between 200 - 45 kda. **Conclusion:** The specific ige response against Ascaris sp appear to influence the ige serological response to Bt, Df, and Pa (CR) allergens in patients from the tropic. Total ige response seems to be inversely to a specific ige response against mite allergens but those serological patter is not the same when its compare total ige and anti Ascaris specific ige in this group of patients.

P5.5 Sensitisation to Der p1, Der p2, Der p10, ascaris suum and shrimp in a population of allergic patients living on the tropical caribbean island of martinique

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Background: Martinique is a French Island located in the Caribbean Basin. It belongs to the Lesser Antilles, and has a tropical climate, which is very conducive for the development of house dust mites. Several species have been identified. Regular parasite eradication campaigns are conducted to reduce the number of parasite infested children and young adults. The allergic population is frequently sensitised to several mite and parasite species, shrimps and to different individual mite allergens. To establish the rate of sensitisation to Der p1, Der p2, Der p10, *Ascarissuum* and shrimp in a population of allergic patients living on the tropical Caribbean Island of Martinique.**Method:** 260 consecutive patients (mean age: 12.46 years; range: 1,17 to 61,87) were evaluated at an outpatient Allergy Clinic in Fort de France, Martinique. All patients had allergic rhinitis with or without asthma and atopic dermatitis. Specific ige was determined using the Immuno Cap (Phadia). Besides D*ermatophagoide spteronyssinus* and *Blomia tropicalis*, the following allergens were also tested: Der p 1, Der p 2, Der p 10, *Ascaris* and shrimp. **Result:** The following sensitisation results were obtained: Der p1: 83.08%; Der p2: 66.15%; Der p10: 32.30%; *Ascaris*: 69.88% and shrimp: 79.3%. Sensitisation to Der p 10 was 10% in the *Ascaris* (—) group versus 40% in the *Ascaris* (+) group ($p<0.001$). Other significant differences were obtained in the group of patients sensitised, or not to shrimp. Sensitisation to Der p 10 was 0% in the shrimp (—) group versus 40% in the shrimp (+) group; sensitisation to *Ascaris*was 40% in the shrimp (-) group versus 80% in the shrimp (+) group ($p < 0.001$). Increased sensitization rates to Der p 2 were found in the *Ascaris* (+) group. No significant differences were obtained regarding sensitisation to Der p 1 in these groups. **Conclusion:** The mite allergic population of Martinique is sensitised to a variety of allergens, especially Der p 1, Der p 2, tropomyosin, shrimp and *Ascaris*. There seems to be an intriguing mechanism in which these allergens modulate the immune response of these patients.

P5.6 Cross-reactivity between *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Ascaris suum*, *Anisakis simplex*, Shrimp, and 2 Tropomyosins of different shrimp species in martinique.

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Background: Martinique is a French tropical island located in the Caribbean. It belongs to the group of the Lesser Antilles. Mites are the main source of allergens, although sensitisation to parasites and shrimps is also frequent among allergic individuals. Infection with intestinal parasites is also common in young children, in spite of preventive parasite eradication campaigns. Objectives: The main objective of this study was to evaluate cross-reactivity among *D. Pteronyssinus*, *B. Tropicalis*, *Ascarissuum*, *Anisakis simplex*, shrimp and shrimp tropomyosin. **Method:** The sera of 372 randomly selected patients (200 women and 172 men, mean age 24.5 years) were evaluated. All patients had been evaluated at an allergic clinic for potential allergic respiratory complaints. Specific ige to the different allergens was measured by direct ELISA using 50 micro liters of serum. Tropomyosin from 2 different shrimp species were used, i.e. Pen i 1 and Pen m 1. ELISA Inhibition assays were conducted to evaluate cross-reactivity. For this purpose, two different serum pools were used. One pool contained high titers of specific ige to both mites (10 individuals), and a second pool containing high titers of specific ige to all the allergens tested (11 individuals). SDS-PAGE followed an igeimmunoblot were carried out to determine the protein and allergenic profiles. **Result:** The main allergen sensitivity was detected to *D. Pteronyssinus* followed by *B. Tropicalis*. Variable correlation coefficients (Pearson) were obtained between specific ige levels, ranging from 0.98 between both tropomyosin and among shrimp and both tropomyosins (0.8). Correlation coefficients of specific ige to Ascaris ranged from 0.35 to *B. Tropicalis* to 0.66 to *A. Simplex*. ELISA Inhibition assays using the serum pool which contained specific ige to all allergen extracts tested showed several degrees of cross-reactivity among the different allergens. When shrimp was used on the solid phase, cross-reactivity was obtained with tropomyosin, and both mite species. No cross-reactivity was detected between *B. Tropicalis* and *D. Pteronyssinus*. **Conclusion:** This study shows variable degrees of cross-reactivity between mites, parasites, shrimp and shrimp tropomyosins. Tropomyosin seems to play an important role in allergic sensitization in Martinique, especially since it is present all evaluated allergens. More data are needed to confirm the clinical implication of tropomyosin in the Caribbean area.

P5.7 Association between asthma and ige levels

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Introduction: Asthma is a multifactorial chronic inflammatory disease that presents with varying degrees of bronchospasm which can be triggered by multiple causes. One of the best known triggers is allergies, or contact with allergens that have different immunological mechanisms leading to activation of the inflammatory process characteristic of asthma. Immunoglobulin E is a protein that normally rises in the allergic process and used as a marker. **Objectives:** To assess the prevalence of ige as a key factor in asthma and to estimate the incidence of non ige-mediated asthma. **Methods:** 50 Patients (72% female and 28% male) aged 12 and 50 years old, diagnosed with asthma who met the inclusion and exclusion criteria, were taken from the Allergy and Immunology department. A complete medical history was performed, and the diagnosis was confirmed through a clinical history and spirometric criteria, according to GINA 2007. Subsequently, total ige in serum were measured by electrochemiluminescence using the Cobascore equipment. **Results:** 72% of the patients in the study, had an elevated total ige which suggests that this group has an allergic cause asa trigger for their disease, meanwhile the cause for the remaining patientswere due to other causes. **Conclusion:** Evaluation of serum ige levelsshould be considered in asthmatic patients in order to determine the specific etiologic treatment. Additionally, these results enhance the importance of a multidisciplinary working diagnosis, and management of this condition,considering that up to a quarter of asthma patients may have Non-igemediated etiology.

P5.8 Cross-reactivity between anti-mite and anti-helminth IgE antibodies and their effect in the diagnosis of atopy by immunocap assay

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Introduction: Conventional indirect ELISA and Western blot techniques are usually performed to verify cross-reactive ige antibodies between nematodes and arthropods. **Aims:** In this study we used the Pharmacia immunocap system, a highly sensitive in vitro test, to measure the cross-reactive between mites (Blomia tropicalis and Dermatophagoides pteronyssinus) and helminthes (Ascaris lumbricoides). **Methods:** Sera from 25 atopic children, serum ige positive for B. Tropicalis and D. Pteronyssinus, were pre-incubated with two different concentrations of A. Lumbricoides extract (0.3 and 3 □g/ml) and bovine serum albumin (BSA; 3 □g/ml) , was used as control. All sera were tested for specific ige to B. Tropicalis and D. Pteronyssinus allergen before and after their incubation with the mentioned antigens. **Results:** When the tested antigen was B. Tropicalis, a statistically significant difference was found between serum inhibition with BSA and A. Lumbricoides extract at 3 m□/ml. However, when the tested antigen was D. Pteronyssinus, some degree of inhibition was found, although no statistically significant difference where found between the inhibitions by the different antigens used in the serum absorptions. Conclusion: *A. Lumbricoides* and *B. Tropicalis* antigens produce cross-reactive ige in a highly used and sensitive assay, the PHADIA immunocap system. The influence of this cross-reactivity in the pathogenesis of allergic diseases is still unknown, although it may have impact on serodiagnosis of allergy and helminth infection when anti-Ascarid specific ige are used as marker of these parasite infections.

P5.9 Identification of protein fractions obtained from somatic extract of toxocara canis with immunoregulatory effects on peripheral blood mononuclear cells of allergic and non-allergic donors

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Introduction: Some studies reported the impact of obesity on asthma. Decreased pulmonary functional residual capacity, tidal volume and lung inflammation described in obese individuals have been cited as predictors of asthma. This work aimed at evaluating a possible association between asthma and obesity, and the relative importance of BMI and abdominal obesity in the occurrence of asthma in Brazilian children. **Material and methods**: This is a cross-sectional study of children, 6 to 12 years old, from the public elementary schools of São Francisco do Conde, Bahia, northeastern Brazil. Asthma symptoms were collected using a questionnaire from the International Study of Asthma and Allergies in Childhood Program (ISAAC) phase III, adapted to Portuguese. Anthropometric, demographic and socioeconomic information were collected. Multivariate logistic regression analyses were used to assess the associations of interest. **Results:** 10.6% had histories of asthma. Excess weight was observed in 16.2%, 10.5% and 7.9% of the sample, measured by body mass index (BMI), waist circumference (WC) and the waist-to-height ratio (whtr), respectively. The percentage of patients with asthma attributable to BMI (8.2%) slightly exceeded those identified with abdominal obesity, measured by the WC (7.3%) and whtr (7.1%). **Conclusion:** We confirmed the predictive power of obesity in the occurrence of asthma. The results suggest that excessive fat deposits, either in the abdominal region or elsewhere in the body, increased the risk of asthma. Considering that obesity is an important public health problem worldwide, the control and eradication of this disease may partially reduce the occurrence of asthma.

P5.10 Association between feno and ige levels in patients with allergic asthma

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Introduction: Asthma is a chronic multifactorial disease characterized by inflammation with multiple triggers. Inflamation of the airways is the main pathologic pathway in asthma, and not only determines the diagnosis and severity of symptoms, but is also useful to establish treatment and predict potential complications. By measuring feno levels, an indirect level of pulmonary inflammation can be obtained since it is produced by alveolar macrophages in response to an inflammatory stimulus induced by cytokines. One of the main known causes of this inflammatory response is an allergic reaction. This allergic reaction sets off a cascade of biochemical events that leads to the expression of inflamatory mediators, preformed or de novo, and ige being the principal of such mediators. **Objectives:** To evaluate the relationship between levels of feno and ige to inflammation and allegy severity. **Results:** 21% of the participants showed normal ige values (<100 UI/Ml) and 33% of the participants had normal feno measurements (<20 ppm). A 0.29 coefficient was measured using a Pearson's correlation test, which suggests a low positive correlation between the two observed variables. **Conclusion:** The results showed a low correlation between the ige and feno levels. This result does not allow for a correlation between both parameters; which leads to a conclusion that high levels of ige from an allergic reaction is not necessarily going to lead to a high feno, thus a pulmonary inflammation. This also strengthens the use of the steroid treatment since it has been shown to lower ige levels but has no effect on feno post treatment.

P5.11 Longitudinal study "natural history of asthma in havana, cuba": results from the first year.

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Introduction: Factors behind the growing prevalence of asthma in modern societies, and particularly in Cuba, are not clear. **Aim:** To determine prevalence of wheezing and association factors in infants of Havana, Cuba. **Method:** A cross sectional study was performed, from March 2010 to March 2011 in 1956 healthy infants belonging to the "Natural history of asthma in Cuba (HINASIC)" cohort. A modified ISAAC questionnaire was applied adding a group of questions and laboratory tests (differential blood count, eosinophil count and total ige). To identify association factors, bivariate analysis and dichotomous logistic regression were used rendering prevalence ratios (PR) and 95% confidence intervals. **Results:** 44.6% of infants had wheezing and 19.9% recurrent wheezing (RW). Of them, 71.1% had their first wheezing episode at 6 months of age. Persistent episodes occurred in 7.2% of infants with wheezing and 13.5% in recurrent wheezing. 9.0% of infants used emergency services. Bronchiolitis, asthma and pneumonia were diagnosed as causes of wheezing. Major risk factors were male gender (PR=1.48), low birth weight (PR=1.59), having a family history of asthma (PR=1.93), personal history of eczema (PR=1.82) and inadequate lifestyle. 36.5% of cases showed elevated total ige, of which 36.0% had RW. Eosinophilia was in found in 8.6% (9.1% in RW). Eosinophilia plus high ige was found in 3.7% of the RW. **Conclusions:** A high magnitude of wheezing in Cuba was found. Male gender, family history of asthma, personal history of eczema and inappropriate lifestyles were associated with wheezing. Inflammatory cell patterns and elevated total ige dazzle the immunoepidemiological pattern of present and future allergic population.

P 6.1 Allergenic potency of parietaria judaica *and* olea europaea *using different serum pools containing, or not, specific IgE to the major allergens*

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Background: *Parietaria judaica* and *Olea europaea* are two important sources of outdoor allergens. Although their distribution is mainly Mediterranean, these species are distributed in many countries of the world. The main allergens are *Par j 1* and *Par j 2* in *P. Judaica* and *Ole e 1*in *O. Europaea*. Allergen standardization relies on the in vitro characterization of allergen extracts using specific ige antibodies. Supposedly, these pools should have antibodies against the major allergens.The aim of the present study was to determine the allergenic potency of *Parietaria* and *Olea extracts* using different serum pools. **Methods:** Total potencies of 2 *P. Judaica* and 2 *O. Europaea* extracts were determined by ELISA inhibition assays. The main characteristic of the 4 serum pools used was: positive specific ige to the total extract (*Parietaria* or *Olea*) but negative to the major allergens (PTNMA), or positive to the total extracts and positive to the major allergens (PTPMA). PTNMA pools were obtained from Plasmalab (USA) and PTPMA from Valencia and Sevilla, Spain. The analyses of the extracts consisted of total protein and major allergen content, ELISA and ELISA inhibitions, and SDS-PAGE and immunoblots. **Results:** ELISA inhibition data of the extracts showed significant differences in total potency using both types of serum pools. 50% inhibition values for *Parietaria* extracts using PTNMA were 0.21 ìg/ml and 0.17 ìg/ml, and 0.03 and 0.015 ìg/ml using PTPMA (a mean difference in potency determination of 8.8 times lower using PTNMA). In *Olea* extracts, these values were 0.17 and 1.14 using PTNMA and 0.085 and 0.583 using PTPMA (a mean difference of 2). Immunoblots revealed an absence of signal for the major allergens *Par j 1*, *Par j 2* and *Ole e 1* using PTNMA, but not with PTPMA.

P6.2 Quality risk management application in the manufacturing process of raw allergenic material of house dust mite vaccines

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Introduction: Quality Risk Management (QRM) is important for manufactures of biological and biotechnological products, according to current Good Manufacturing Practice regulations, allowing an appropriate process quality improvement. **Aim:** The objective of this work is to show the use of QRM as an effective tool in the production of raw allergenic material of house dust mite vaccines. **Methods:** A multidisciplinary specialist's team was created including personal from quality unit, development, engineering, regulatory affairs, production operations and statistics. This group studied the QRM methodology according to ICH Q9 and applied it to this particular process. A flow diagram, process mapping, Cause and Effect Diagrams, and other quality improvement tools were used. Failure Mode Effects Analysis (FMEA) was employed and the Risk Priority Number, RPN was determined. Risks were evaluated by a subjective measure of the severity of the effect (S), estimate of the expected probability of its occurrence (O) and means of detection (D). **Results:** Quality risk management methodology was applied in BIOCEN, as effective quality tool according to the current GMP and quality continuous improvement, for the first time into the process of manufacturing of allergen products, particularly at the mite culturing stage (raw allergenic material). Risk Priority Numbers were calculated and corrective and preventive actions were taken for risks mitigation. **Conclusion:** Application of QRM is a useful methodology for defining strategies of Quality Improvement for allergen products.

P6.3 Grass and mite mixtures: how does the proteolytic activity of dermatophagoides pteronyssinus affect phleum pratense extracts?

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Background: Some recommendations have been issued indicating the extracts that can, or cannot be mixed for allergen immunotherapy. Mixtures containing mite species and pollen allergen extracts are not recommended since some of the house dust mite allergens are potent enzymes, including Group 1, Group 3, Group 6 and Group 9. We have shown that polymerized mite allergen extracts have a significantly reduced proteolytic activity. **Objectives:** To investigate the stability of a *Phleum pratense* extract in mixtures containing native or allergoids of *D. Pteronyssinus*. **Methods:** Native or allergoid extracts of *D. Pteronyssinus* were mixed with an unmodified extract *P. Pretense.* The samples were stored at 4ºc and analyzed at 3 different time points: t_0= 30 minutes, t_1=8 days and t_2=20 days. SDS-PAGE followed an ige immunoblots were carried out to determine the protein and allergenic profiles. An ELISA competition test was developed to determine the allergenic activity of the extracts. The mayor allergens Phl p 1, Phl p 5, Der p 1 and Der p 2 were measured. **Results:** SDS-PAGE and ige immunoblots results showed that the *D. Pteronyssinus* native extract was able to degrade the *P. Pratense* proteins faster than allergoids.*P. Pratense* bands begin to disappear after 30 minutes of incubation (t_0), which was not the case with mite allergoids. Competitive ELISA assays showed that the allergenicity of *P. Pratense* decreases significantly in mixtures containing the mite native extract. The Inh_{50} points changed from 0.131 µg at 30 minutes to out of range at day 20. When incubating the *P. Pratense* extract with the mite allergoids the reduction of the allergenicity was less pronounced changing from 0.079 µg at 30 minutes to 0.27 µg at day 20. Phl p 1 was significantly more affected than Phl p 5 after mixing the grass extract with the mite extracts. Der p 1 and Der p 2 levels were not altered. **Conclusions:** *P. Pratense* native allergen extracts suffer a significant degradation of their allergens when mixed with unmodified mite allergen extracts, but not with allergoids. Some allergens seem to be more affected than others.

P6.4 Process consistency of *dermatophagoides siboney* purified allergen fraction as the active pharmaceutical ingredient for a novel adjuvanted allergen vaccine

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Introduction: Novel allergen vaccines intended for allergy immunotherapy are based on recombinant or purified natural allergen proteins and adjuvants. The allergens are regarded the active ingredients since they induce an adaptive immune response. Objective: To asses the process consistency and effectiveness of the purification steps of a Dermatophagoides siboney major allergen fraction. **Materials and methods:** A purification process in GMP conditions was followed starting from a freeze-dried allergen extract of D. Siboney (VALERGEN-DS, BIOCEN) comprising salting-out steps and chromatography in SUPERDEX-200. The major allergen content (Der s 1) was assessed by mab-ELISA and the allergenic activity was measured using ige competition ELISA with human sera. Purity was determined by SDS-PAGE. **Results:** The purification process showed consistent results after 20 consecutive batches. The average concentration of Der s 1 was 154µg/ml, with a CV of 8.4%. Main total protein content was 254µg with a CV of 15%. Purity of Der s 1 (25kda) and Der s 2 (15Kda) components was higher than 90 % for all the batches. Overall Der s 1 recovery was above 60% during the process and batch yield was 4.3 ± 0,6 mg. No batches were found to be out of the specification or ±3σ limits. The maximum failure probability according to Process capability Analysis was 3 % for the Der s 1 content and allergenic activity. These values are regarded as satisfactory considering the high variability of the analytical methods, which is estimated to be much greater that the intrinsic process variability. **Conclusion:** It was demonstrated the consistency of the main quality parameters of the active ingredient for a novel allergen vaccine, which is relevant to clinical development of the product.

P6.5 Stability study of food allergen extracts in glycerin and aqueous formulation

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Introduction: The stability of allergen extract is an important factor that ensures its quality. Glycerin is well known as a stabilizer for allergen extracts. Real time evaluation of shelf-life stability of a biopharmaceutical product is required during the pharmaceutical development phase, prior to advancing to clinical trials. **Objective:** To determine the stability of food allergen extracts of wheat and egg white in glycerin and aqueous formulation. **Methods:** Three pilot batches of each allergen extract, stored at 4°C, in aqueous or glycerin formulation, were sampled at 0, 15, 30, 60 and 90 days and 0, 3, 6, 9 and 12 months, respectively. The possible degradation was monitored by total protein content (Lowry) and SDS-PAGE with densitometric analysis for protein quantitative profiling. Content of glycerin was also monitored, as well as other general parameters. Vials were kept at normal and inverted positions. Preservation of integrity of relevant allergens was checked by ige Western blotting with patient's sera. Sterility was determined at the beginning and end of the study. Acceptance limits matched those used for product release. **Results:** No deviations of quality specifications were detected in any parameter, after 12 months of storage (for glycerinated products) or after 3 months for aqueous products. Physico-chemical features and allergenic activity of proteins were preserved. Batch to batch regression analysis of key quality parameters, indicated no significant trend towards degradation of any parameter ($p=0.05$). **Conclusions:** This study proved the stability of allergenic extracts in glycerin and aqueous formulation during 12 months and 3 months, respectively, as a basis for approval of a reliable expiration period.

P6.6 Stability study of bulk allergen extracts of house dust mites in freezing state

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Introduction: Stability of biopharmaceutical products at intermediate process stages is a requirement for assuring the quality of the finished product. Allergen extracts, as protein mixtures, are inherently unstable and subject to degradation. Storing intermediate products in freezing conditions could be useful for preserving in-process product stability. **Aim:** To assess the stability of bulk allergen extracts of *Dermatophagoides siboney* and *Blomia tropicalis* during storage at -20°C. **Methods:** A real time stability study was performed using three batches of bulk allergen extracts (active pharmaceutical ingredient) of *Dermatophagoides siboney* and *Blomiatropicalis*, stored in plastic ethylvinyl acetate bags at -20±5 °C. Major allergen allergen content (Der s1) was measured by Mab-ELISA and allergenic activity by ige Inhibition ELISA. Protein composition was monitored by SDS-PAGE. Samples were analyzed at 1, 3, 6, 9 and 12 months. Sterility was assayed at the beginning and end of the study. **Results:** All quality parameters were kept within the specification limits at all dates. The regression analysis of the Der s1 content and ige activity did not show a significant trend towards degradation (P>0.05) of these parameters. Taking the upper value of the slope 95%CI, a mathematical prediction was calculated, which indicated a period of more than 6 months for reaching the lower 90% limit of the nominal specification value. Thus this time period is proposed as a shelf-life expiration period for the active ingredients in frozen state. **Conclusions:** Bulk allergen extracts of *Dermatophagoides siboney* and *Blomia tropicalis* can be stored in freezing conditions for at least 6 months, maintaining its quality.

P6.7 A simple method for assessing ultrafiltration efficiency for allergen extracts.

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Introduction: Ulrafiltration or diafiltration is extensively used as a purification process for removing low molecular weight (LMW) components from allergen extracts. An important aspect for developing a validated diafiltration process is to establish optimal parameters regarding efficient removal of unwanted components and retention of active allergen proteins. **Aim:** To establish an analytical method for assessment of the ultrafiltration process efficiency for diafiltered allergen extracts. Methods: Samples of three pilot batches of Soy allergen extracts were analyzed. Samples were taken before and after the diafiltration step, with a cutoff value of 10 Kda. A gel filtration chromatographic analysis was performed using a column of Sephadex G-25 superfine and a bioline Chromatographic system (KNAUER), measuring adsorption at 280 nm. Chromatograms were further processed using the claritychromprep software (KNAUER). **Results:** Chromatograms clearly discriminated the low molecular fraction (below 10 Kda) and the retained fraction (>10Kda). The content of each fraction was estimated as the area under each curve (AUC). Then, diafiltration process efficiency showed a mean value of 83% for removing of LMW fraction with a CV between batches of 3.0%; and a mean recovery value of the active fraction of 92% (CV=1.83%). Thus process efficiency was measured and batch to batch consistency evidenced. **Conclusions:** The established method is fast, simple and accurate. Then, it can be used for routine batch control and as a tool for validating the ultrafiltration process.

P6.8 Validation of sterile filtration processes of house dust mite allergen vaccines

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Introduction: Sterility is a critical safety requirement for injectable pharmaceutical products, including allergen vaccines. Sterile filtration is the preferred sterilization process for biological products. The validation of this process is the experimental demonstration of its capability for removing microorganisms from the product. **Aim:** To validate the sterile filtration process for allergen extracts. **Methods:** Sartrobran P capsules (Sartorius Stedim) were used for 0.2 µm sterile filtration process. Microbiological challenge was employed for testing the process performance. Microbial burden was measured prior and after the filtration process according to US Pharmacopeia. A non destructive integrity test was applied to filtration capsules. **Results:** The bacterial retention test, performed at process scale, showed the actual capability of filtration capsules of retaining a high microbial burden of Brevundimonas diminuta ATCC 19146 at a concentration of 107 UFC/cm2 per effective filtrating area. Viability assessment of Brevundimonas diminuta showed its impaired growth in the buffer solution used for allergen extract process. Bioburden in the non-aseptic stages prior to sterile filtration was below 103 UFC/ml, complying with regulatory requirements. The filter integrity, after the filtration process, was also confirmed showing a bubble point below 3.2 bar and a diffusion value below 5ml/min. **Conclusion:** The effectiveness of the sterilizing filtration step was proven, assuring the sterility of allergen extracts, a critical quality feature for its clinical safety.

P6.9 Use of gamma-sterilized rubber stoppers for assuring a lower moisture content in lyophilized house dust mite allergenic extracts

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Background: Long-term storage stability is a requisite for pharmaceutical products. Lyophilization is commonly used for biological products, and particularly, for allergen extracts for assuring stability. However, lyophilized products can be affected by the moisture released by stoppers, among other factors. **Objective:** To monitor the residual moisture content in different batches of lyophilized allergenic dust mite extracts and during real time stability studies. **Methods:** Results of 20 batches of freeze-dried allergen extracts produced in biocen between 2006 and 2011 were analyzed. Moisture content was measured by the Karl Fisher potentiometric method, according to USP. Batches used either autoclaved or gamma radio-sterilized bromobutyl stoppers. Later on, two batches were put into stability studies at 4°C for 3 years following the ICH methodology. **Results:** It was noticed that even when all batches produced in biocen between 2006 and 2011 were in compliance with the specification limit regarding moisture content (below 5%), the mean+3Ãvalue was above the specification limit. The mean value was higher for D. Pteronyssinus extracts. The autoclaving of stoppers results in a significant increase of the product moisture, as compared to radiosterile stoppers. Moisture content during the stability study using autoclaved stoppers showed a significant increasing trend ($p<0.05$). Nevertheless, the allergenic activity and other specification requisites stayed in compliance with the specification limits. **Conclusions:** The most probable cause for the increase of moisture content in time was the transference of the residual humidity from stoppers to the lyophilized product .The use of radio-sterile stoppers proved to be useful to decrease this moisture increment in lyophilized extracts.

P6.10 Microbial bioburden during the culture process of *Dermatophagoides pteronyssinus*

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Introduction: House Dust Mites coexist in their natural habitat with different microorganisms, possibly with symbiotic interactions. From the pharmaceutical point of view, GMP require the establishment of limits for microbial contamination in Raw Materials and intermediate drug products, including the Allergenic Raw Material for manufacturing allergen vaccines. **Objective:** To perform an assessment of the microbial burden during the culture process of the house dust mite *Dermatophagoides pteronyssinus*. **Methods:** Samples of different culture flasks were collected and subjected to microbiological analysis. Total count of Colony Forming Units (CFU) was accomplished by spread plate method. The identification of enterococcus and Gram-negative bacteria, was accomplished using selective chromogenic media (cromocen, BIOCEN). **Results:** Bacterial and fungi CFU counts showed approximately the same pattern decreasing inversely to mite growth density, although peaks were noted at the beginning of the culture process and after addition of fresh media at the 10-11th weeks, with a later decrease at the end of the culture period. CFU values ranged from 0.12 to 1.66x10² CFU/g for bacteria, and from 0.201 to 4,09x10 t†CFU/g for fungi. The most frequent bacteria were Enterococcus, *Bacillus spp*, enteric bacteria, Citrobacter and in lesser degree *Staphylococcus spp*. The most frequent fungi were Mucor, *Penicillium spp* and scarcely *Rhizopus sp*. No clear relationship was found between microbial levels and the mite life cycle. The source of these contaminant microorganisms was tracked to mite inoculums. **Conclusion:** These data is valuable for the establishment of permissible microbial limits for the mite culture process, assuring the quality of the source allergenic material.

P6.11 Risk analysis applied to quality improvement of the manufacturing process of the active pharmaceutical ingredient of allergen vaccines

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Introduction: As stated in ICH Q9, the Quality Risk Management (QRM) is a systematic process for the evaluation, control and revision of manufacturing process in pharmaceutical industry throughout the products lifecycle. **Aim:** To design and to apply a methodology for QRM for the manufacturing process of allergen vaccines of House Dust Mites at the Active Pharmaceutical Ingredient (API) stage. **Methods:** The Failure Mode and Effect Analysis (FMEA) was employed as a procedure. Potential failure of product's quality parameters, equipment and operation were analyzed. The Risk Priority Number, was determined. Risks were evaluated by a subjective measure of the severity of the effect (S), estimate of the expected probability of its occurrence (O) and means of detection (D). **Results:** The methodology was applied to the manufacturing of API of three VALERGEN products, starting from 2009 to the present day. The risks with major impact and recurrence were identified. Corrective and Preventive actions were taken in order to mitigate risks, according to the procedures established in the Quality System. In addition, the efficacy of QRM and improvement measures was evidenced during the 2011 campaign. **Conclusion:** The aapplication of QRM is a useful methodology for defining strategies of Quality Improvement for allergen products, and to assure the effective application of Good Manufacturing Practices.

P6.12 Establishing a validated pharmacopeia sterility test for glycerinated allergen extracts

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Introduction: A validated sterility test is required for biopharmaceuticals intended for injection, including allergen extracts and vaccines. Particularly, this validation should deal with the evaluation of inhibitory activity to microbial growth. Glycerine 50% is commonly used as stabilizer and preservative for formulating allergen extracts. **Aim:** To establish a suitable sterility testing procedure, fulfilling the validation requirement regarding fungistasis and bacteriostasis, for glycerinated food allergen extracts. **Methods:** Glicerynated final products and bulk allergen extracts (active ingredient) of wheat, egg white, peanut and soy at 1 mg/ml protein content, were tested. The USP membrane filtration method and direct inoculation method were performed for each product. The bacteriostasis/fungistasis effect was assessed testing the growth of *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231 y *Aspergillus niger* ATCC 16104, representing different microbial groups. **Results:** In spite of the high viscosity of finished glycerinated products, the USP filtration method was fully functional for peanut, egg white and wheat allergen extracts, although for egg white and wheat a prior 1:10 dilution of samples was required. However for soy glycerinated product it was not possible to perform the USP filtration method. No inhibitory effect was detected for filtered extracts, indicating no interference of the Glycerol preservative activity to the sterility test. **Conclusion:** Sterility testing of glycerinated allergen extracts of wheat, egg white and peanut were validated, assuring the quality and clinical safety of these products.

P6.13 Cuban national regulatory authority: legal basis for allergenic products

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Introduction: Until the decade of the 80 allergenic extracts were not considered pharmaceuticals products. The first effort to develop a standard for the Registration of allergen products as pharmaceuticals was conducted by the Nordic Council of Medicines in 1982. In Cuba, starting from 2002, it is mandatory the marketing authorization of allergenic extracts as biological products, with the approval of the CECMED´s Regulation 30-2002 on the Registration of allergen products. **Objective:** To review the regulatory framework for clinical evaluation of allergen vaccines. **Methods:** It was conducted a literature review of guidelines, guides, international and national regulations related to the evaluation of allergen products and a quantitative analysis of the procedures for approval and modification of clinical trials of allergen vaccines, as well as a quantitative analysis of the registered vaccines. **Results:** Documents issued by international and foreign regulatory agencies (NLN, WHO, FDA, EMEA) related to the regulatory aspects and guidelines for the evaluation of allergen vaccines, were revised. In terms of quantitative activity in Cuba, it was found that 29,8 % (17) of all authorization applications of clinical trials for vaccines, in general, corresponded to allergen products. Three allergen vaccines are registered for both subcutaneous and sublingual routes, out of a total 15 registered vaccines. We identified the key points (safety, immunogenicity and efficacy) to be considered for clinical evaluation of allergens. **Conclusions:** It is recommended to promote the implementation and application by Allergy Services of recording of adverse events during immunotherapy, as well as more stringent measures of treatment effectiveness. Also, to follow carefully the international regulatory developments regarding new allergy vaccines.