Friday, January 25

Oral Abstract Presentation I - Lymphocytes
09:20 - 11:00

O01 Activated Th2 Cells Are Characterized By Fatty Acid Metabolism In Vivo

Graham Heieis, Stephan Loeser, Holly Webster, Nicola Britton, Rick Maizels, Georgia Perona-Wright

University of Glasgow, Glasgow, United Kingdom

Introduction
Cellular metabolism is a potent regulator of immune cell function, but relatively little is known about its impact on Th2 cells. Current dogma is that T cell activation and differentiation is driven by rapid increases in both glycolysis and oxidative phosphorylation. Th2 cells in particular show a dramatically increased glycolytic rate and high sensitivity to glycolytic inhibition in vitro, but we have yet to dissect the importance of metabolic pathways in vivo.
Method
We assessed Th2 cells generated in vivo in two mouse models: a house dust-mite (HDM) model of allergic asthma and a hookworm infection with Nippostrongylus brasiliensis.
Results
Th2 cells in both house dust-mite challenge and helminth infection showed very little glycolytic gene expression. Instead, we observed striking up-regulation in genes related to the uptake and breakdown of fatty acids (FA). We found that lung Th2 cells had enhanced FA uptake compared to Th2 cells in the lymph node, and this increase was restricted to cells expressing the IL-33 (ST2) receptor. Up to 75% of ST2+ Th2 cells in the lung co-expressed programmed death receptor-1 (PD1), a known promoter of fatty acid-fueled mitochondrial metabolism, and, indeed, PD1+ST2+ Th2 cells in the lung possessed the greatest mitochondrial mass and highest mitochondrial membrane potential. We therefore propose that tissue-restricted signals, mediated by PD-1, facilitate terminal Th2 differentiation by promoting FA oxidation.
Conclusion
Together, our data highlight a new role for PD1 in controlling terminal Th2 differentiation through metabolic regulation. PD1 therapy has shared enormous success as a therapy in other immunological fields, while metabolic targeting is also showing significant therapeutic promise. Our research could therefore support the translation of effective, validated and efficacious treatments to Th2-driven disorders such atopy, allergy and asthma.

O02 Experimental Rhinovirus Infection Induces Extensive Antiviral Response In Circulating B Cells From Asthmatic Patients

Oliver Wirz[1], Kirstin Jansen[1], Willem Van De Veen[2], Milena Sokolowska[1], Ge Tan[3], David Mirer[1], Simon Message[4], Tatiana Kebadze[4], Nicholas Glanville[4], Patrick Mallia[4], Nikolaos Papadopoulos[5], Cezmi Akdis[2], Sebastian Johnston[4], Kari Nadeau[6], Mübeccel Akdis[1]

1. Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland
2. Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich; Christine Kühne – Center for Allergy Research and Education (CK-CARE), Davos, Switzerland
3. Functional Genomics Center Zürich, ETH Zürich/University of Zürich, Zurich, Switzerland

4. Airway Disease Infection Section, National Heart and Lung Institute, Imperial College London, London, United Kingdom
5. Division of Infection, Immunity & Respiratory Medicine, The University of Manchester, Manchester, United Kingdom
6. Sean N. Parker Center for Allergy and Asthma Research, Department of Medicine, Stanford University, Palo Alto, United States

Keywords: B Cells, Asthma, Rhinovirus

Introduction
Rhinoviruses (RV) are the most common cause for viral induced respiratory diseases and these infections strongly associate with asthma exacerbations. While B cells exert a crucial antiviral function secreting protective antiviral antibodies, their cellular responses to rhinovirus infection remain largely unknown. The current study aimed to characterize in-vivo B cell responses in asthmatic and healthy subjects, before and after experimental rhinovirus infection.
Method
Asthmatic and healthy volunteers were experimentally infected with RV-16. Peripheral blood mononuclear cells (PBMCs) were isolated prior and three days after infection. CD19+ B cells were purified using fluorescence-activated cell sorting and next generation RNA-sequencing (NGS) was performed. In a functional experiment, we stimulated purified B cells in-vitro and performed NGS to assess, whether systemic cytokines or direct virus contact leads to the antiviral gene program observed in ex-vivo-isolated B cells. Lastly, PBMC were stimulated with RV-16 in-vitro and cytokine-positive B cells were analyzed using flow cytometry.
Results
At baseline, prior to infection, most genes that were differentially expressed between asthmatic and healthy subjects were involved in immune system processes. This included antiviral, cytokine and B cell receptor signaling responses. Expression of genes encoding IgE and IgG4 as well as IgG1, IgG2, IgG3 and IgA2 and several proinflammatory cytokines was higher in B cells of asthmatic patients. Unexpectedly, in-vivo infection induced a broad antiviral transcriptional program in B cells. Asthmatic patients showed elevated expression of several interferon-induced genes including IFI44L, IFIT3, IFI6, MX2 and STAT1 compared to healthy subjects. Here, we demonstrated that virus-induced cytokines rather than virus itself elicit the transcriptional gene program found in B cells of experimentally infected subjects. Also, we showed increased expression of pro-inflammatory cytokines in response to infection.
Conclusion
In this study, we provide novel evidence that peripheral B cells from asthmatic patients have aberrant gene expression at baseline and also respond differently upon experimental RV-infection. This study suggests, that future therapies for rhinovirus infection should not only focus on local tissue responses but also address peripheral immune system.

O03 MicroRNA Expression Patterns In IL-33 Challenged Bone Marrow And Lung – Role Of MTOR And MAPK Signaling Pathways

Emma Winberg, Kristina Johansson, Carina Malmhäll, Cecilia Lässer, Madeleine Rådinger

University of Gothenburg, Krefting Research Centre, Gothenburg, Sweden

Keywords: ILC2, IL-33, MicroRNAs, MTOR

Introduction
Bone marrow (BM) type 2 innate lymphoid cells (ILC2s) have previously been identified to play key roles in IL-33-induced eosinophilic inflammation. However, the mechanisms regulating the properties of ILC2s in this model are unclear. MicroRNAs (miRNAs) are regulators of mRNA translation and have been involved in immune regulation of several

diseases, such as allergy and asthma. In this study, we determined the miRNA expression patterns in BM and lung tissue samples from IL-33 challenged mice. We also focused on the regulation of BM derived ILC2s in response to IL-33 challenge.

Method

Wild type mice were challenged to recombinant IL-33 (rIL-33) or PBS intranasally every other day for five days. BM and lung tissue were collected 24 h after the final challenge. RNA was isolated and microarray analysis was performed. A miRNA was determined to be differently expressed if the fold change was > 2 and p-value < 0.05. KEGG pathway analysis was performed using miRSystem. Naïve BM cells were stimulated with rIL-33 or culture medium as a control. The activity of the mTOR target ribosomal protein S6 (rps6) in ILC2s was measured by flow cytometry.

Results

In total 62 and 34 miRNAs were up-regulated in the lung and BM, respectively, in the IL-33 challenged group. mTOR and MAPK signaling pathways were identified as top candidates for differentially expressed miRNAs in both BM and lung. In the BM, the mTOR pathway was identified on placement 2 out of 124 for the upregulated miRNAs in the IL-33 challenged group. In vitro studies revealed that the activity of the mTOR target; ribosomal protein S6 (rps6) was increased in IL-33 stimulated BM ILC2s compared to the unstimulated control group.

Conclusion

Our data suggest that miRNAs may have a regulatory role in IL-33-induced inflammation involving both mTOR- and MAPK-signaling pathways. Furthermore, mTOR signaling may be involved in IL-33-induced ILC2 driven eosinophilia in the BM. However further studies need to be performed in order to rule out the exact role of miRNA regulation in these processes.

O04 In Vivo T Regulatory Cell Regulation During Human Rhinovirus Infection

Kirstin Jansen[1], Oliver Wirz[1], Willem Van De Veen[1], David Mirer[1], Sebastian Johnston[2], Cezmi A. Akdis[1], Nikolaos G. Papadopoulos[3], Kari Nadeau[4], Mübeccel Akdis[1]

1. Swiss Institute of Allergy and Asthma Research, Davos Platz, Switzerland
2. Imperial College London, London, United Kingdom
3. University of Manchester, Manchester, United Kingdom
4. Sean N. Parker Center for Allergy and Asthma Research - Stanford University, Palo Alto, United States

Keywords: T Regulatory Cells, Rhinovirus, Tolerance, Asthma

Introduction

Human rhinovirus (HRV) infections are strongly associated with asthma exacerbations and pose a severe health risk for allergic individuals. How chronic allergic diseases and HRV are linked, and which role HRV plays in the breaking of allergen-specific tolerance is unknown. T regulatory cells (Tregs) play an important role in the induction and maintenance of immune tolerance. Therefore, the aim of this study is to investigate the effects of HRV on Tregs.

Method

Healthy and asthmatic individuals were experimentally infected with HRV16 in vivo. Peripheral blood mononuclear cells (PBMCs) were obtained before infection and three after infection and seven days after infection (only for healthy individuals). Tregs were sorted from the PBMCs according to the flow cytometric profile CD4+CD3+CD25+ CD127- and were analyzed with next generation sequencing.

Results

We have found that on baseline there are clear differences in Tregs from asthmatics compared to healthy individuals. Tregs from asthmatics show a more Th2 type profile with increased expression of IL13, IL4, IL5, PTGDR2 and reduced FOXP3, and show upregulated histone related genes, which suggest epigenetic changes.

After infection with HRV a strong antiviral response is induced in Tregs from healthy and asthmatic individuals. The strongest induced genes are interferon induced genes such as MX1, IFI44L and OAS3. Interestingly in asthmatic individuals there is an additional upregulation of inflammasome genes and other virus related genes. In healthy individuals NR4A1-2-3, molecules important for Treg functioning, are upregulated while these are downregulated in asthmatic individuals. Furthermore there is upregulation of the suppressor molecules SOCS3, CTLA-4, CD69 and ICOS in healthy, while these are downregulated in asthmatics.
Seven days after infection the interferon induced response in healthy individuals is terminated, while the other responses related to suppressive function remain upregulated. Furthermore PTGER2, a molecule that is able to dampen allergic responses, is upregulated.

Conclusion

Tregs from healthy and asthmatic individuals both show an anti-viral response after HRV infection. However there are also clear differences in response between Tregs from healthy and asthmatic individuals. These differences in response might affect Treg functions, level of inflammation, chronicity and viral clearance. Together this data suggest that Treg functions in asthmatic individuals might be altered or impaired during HRV infections.

Friday, January 25

Oral Abstract Presentation II - Asthma and asthma models
17:50 - 19:30

O05 Human Volatilome Analysis To Identify Individuals With Asthma In Clinical Settings

Mariana Valente Farraia[1], João Cavaleiro Rufo[1], Inês Paciência[1], Francisca Castro Mendes[1], Tiago Rama[2], Ana Rodolfo[2], Sílvia Rocha[3], Luís Delgado[2], André Moreira[1]

1. Faculdade de Medicina da Universidade do Porto, Porto, Portugal & Centro Hospitalar São João, Porto, Portugal, Porto, Portugal, Porto, Portugal
2. Imunologia Básica e Clínica, Departamento de Patologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal, Porto, Portugal, Porto, Portugal
3. QOPNA, Departamento de Química, Universidade de Aveiro, Portugal, Porto, Portugal, Porto, Portugal

Keywords: Exhaled Breath, Asthma, Volatilome, Volatile Organic Compounds, Diagnosis

Introduction
Exhaled breath volatile organic compounds (VOC) have shown promising results when discriminating individuals with asthma from healthy controls. This study aims to assess if the exhaled VOC analysis using an electronic nose (eNose) may be applied to identify individuals with asthma in a population with respiratory symptoms.
Method
A cross-sectional study was conducted and breath samples from 199 participants recruited from an outpatient clinic were collected and analysed using an eNose composed by 32 sensors. Lung function parameters and CARAT questionnaire to assess the control level of airways disease were performed. Information on medical diagnosis of asthma and rhinitis were retrieved for each participant. A multivariate cluster analysis model, using resistance data from the 32 sensors, was able to discriminate the VOC patterns between individuals in 2 clusters. These clusters were then compared to the clinical parameters. Adjusted generalized linear models (GLM) for confounders were used to test the developed model. The study was approved by the Ethical Committee of the University of Porto and written consent from all participants was obtained before sample collection.
Results
The study population was composed by 67.8% of individuals with a medical diagnosis of asthma. Volatilome analysis was able to significantly distinguish participants with uncontrolled asthma-like symptoms from those with controlled symptoms (p= 0.01). Individuals with symptoms of uncontrolled airways disease were discernible using the developed hierarchical cluster model.
Conclusion
In a population with respiratory diseases, the analysis of the VOC profile by eNose may be used as a fast and non-invasive complementary diagnostic agent for screening individuals in search of uncontrolled asthma-like symptoms. This may lead to an enhanced management and treatment of disease and encourages the design of confirmatory trials in which patients and clinical setting should be representative of the population where the diagnostic agent is intended to be used.

O06 Staphylococcus Aureus-Derived Serine Protease-Like Protein D Induces Allergic Asthma, Dependent On The Genetic Background Of Mice

Sharon Van Nevel, Andrea Renate Teufelberger, Natalie De Ruyck, Gabriële Holtappels, Claus Bachert, Olga Krysko

Upper Airways Research Laboratory, Ghent, Belgium

Keywords: Allergic Asthma, S. Aureus, SplD, IL-33, Airway Inflammation

Introduction
The Staphylococcus aureus-derived serine protease-like protein D (SplD) is an allergen that can induce allergic asthma in mice. Sensitization to SplD results in a Th2-biased inflammation in the airways of C57BL/6 mice, characterized by the presence of SplD-specific IgE in serum and eosinophils in the lungs. This response to SplD is dependent on the cytokine interleukin-33 (IL-33), mainly expressed in endothelial and epithelial airway cells. IL-33 plays an essential part in Th2-type immune responses by activating dendritic cells, ILC2s and Th2-cells and initiating the production of IL-4, IL-5 and IL-13. This leads to the characteristics of allergic asthma including the production of IgE, eosinophilia and goblet cell hyperplasia. Surprisingly, in contrast to C57BL/6 mice, BALB/c mice are non-responsive to SplD. The aim of the study was to analyze if IL-33 is sufficient to sensitize BALB/c mice to SplD and induce a Th2-biased inflammation.
Method
6-week old female BALB/c mice were given intratracheal applications of SplD (45 ùg) and/or IL-33 (0.2 ùg) every 48 hours for six times. 48 hours after the last application, the mice got euthanized. Inflammatory cells in bronchoalveolar lavage fluid (BALF) and lungs were analyzed by flow cytometry. Cytokine levels were measured by Luminex. Goblet cells were stained with a Periodic acid Schiff-staining. For the detection of IgE an ELISA was used.
Results
IL-33 acts in synergy with SplD resulting in strong eosinophilia in the BALF and lung tissue in BALB/c mice. Also, SplD-specific IgE and total IgE were significantly upregulated only in the group that received the combinational treatment of SplD and IL-33. Neither IL-33 nor SplD alone can induce Th2-inflammation in BALB/c mice.
Conclusion
Depending on the genetic background of the mice, the asthmatic response toward SplD was different. BALB/c mice respond with an allergic response when IL-33 was additionally given with SplD, while it has been shown previously that C57BL/6 mice develop allergic asthma upon SplD exposure without additional IL-33. These different responses could suggest an explanation why there are healthy S. aureus carriers and those carriers who develop SplD-specific IgE and allergic asthma.

O07 Preventing Airway Mucus By Delivering Allergen Via Microprojection Array Skin Patches To Mice

Nicole Van Der Burg[1], Simon Phipps[2], Alexandra Depelsenaire[3], Mark Kendall[1]

1. Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia
2. QIMR Berghofer Medical Research Institute, Brisbane, Australia
3. Translational Research Institute, Brisbane, Australia

Keywords: Microprojection Array, Allergic Airway Inflammation, Airway Mucus

Introduction
Post mortem analyses of asthmatic patients indicates that mucus plugging of the airways is one of the principle causes of death in asthma. While treatments to alleviate airway inflammation are improving, no treatments, as of yet, can specifically target nor permanently downregulate mucus production. Therefore mucus-related diseases must be treated for life using aerosol or nebulizer delivery devices. These deliveries can cause discomfort, be inconvenient to use or clean and/or require costly devices. Alternatively, several types of easily applied skin patches to deliver drugs have resulted in protected airway responses to both viral infections and allergic challenges alike. One type of skin patch that has shown great promise with a variety of airway challenges is the

microprojection array (MPA). Therefore, we hypothesised that allergy immunotherapy delivery with MPAs could protect the airways from allergen-induced inflammation and mucus production.

Method

Here we have tested a two minute dermal MPA and an epidermal MPA to delivery ovalbumin immunotherapy to the respective layers of the skin. Dermal MPAs were applied eight times to i.p. sensitised mice (ovalbumin + aluminium hydroxide) to test prevention of airway inflammation of type I hyper-sensitised mice. While epidermal MPAs were applied four – eight times to naïve mice (before i.p. sensitisation) to test prevention before type I hypersensitisation. Both groups of mice were challenged thrice via intranasal inhalation of ovalbumin before collection. Bronchial alveolar lavage fluid (BALf) was assessed for inflammatory cells and histology of lungs were stained for mucus with anti-Muc5ac.

Results

The BALf of both MPA treated groups contained significantly less inflammatory eosinophils. This was dose dependant for the epidermal MPA vaccination which worked best (60% protection) with four 0.1 µg vaccinations spaced 72 hours apart. While 100% of placebo and ovalbumin dermal MPA treated groups were protected from eosinophilia. Additionally 80% of mice applied with ovalbumin MPAs resulted in significantly less airway mucus than inflammatory groups.

Conclusion

These findings suggest application of MPAs either before or after type I hypersensitisation significantly downregulates the production of allergen-specific airway mucus. As MPAs are a cheaper, more tolerated device than aerosols, their use in the treatment of airway mucus warrants further investigation.

O08 FceRI Expression In Peripheral Blood Mononuclear Cells In The Context Of Asthma

Jonatan Leffler[1], James Read[1], Anya C Jones[1], Danny Mok[1], Elysia M Hollams[1], Ingrid A Laing[1], Peter N Le Souef[1], Peter D Sly[2], Merci M.h Kusel[1], Anthony Bosco[1], Patrick G Holt[1], Deborah H Strickland[1]

1. Telethon Kids Institute, University of Western Australia, Perth, Australia
2. Child Health Research Centre, University of Queensland, Brisbane, Australia

Keywords: Dendritic Cells, Atopic Asthma, PBMC, Flow Cytometry

Introduction

Antigen specific IgE binds the Fcε receptor I (FcεRI) expressed on several types of immune cells, including dendritic cells (DC). Activation of FcεRI influence the ability of DCs to orchestrate immune responses and may contribute to asthma development in atopic individuals. However, the extent to which DC subsets differ in FcεRI expression between atopic children with or without asthma is currently not clear.

Method

We set out to analyse the expression of FcεRI on peripheral blood mononuclear cells (PBMC) from atopic children with and without asthma, and non-atopic/non-asthmatics age-matched healthy controls.We performed multiparameter flow cytometry on PBMC from 392 children across three community cohorts and one clinical cohort based in Western Australia.

Results

We confirmed expression of FcεRI on PBMC basophils, monocytes, plasmacytoid and conventional DCs, with higher proportions of all cell populations expressing FcεRI in atopic compared to non-atopic individuals. Further, the proportion of plasmacytoid DCs that expressed FcεRI was significantly higher in atopic / asthmatic compared to atopic / non-asthmatic children, independent of serum IgE levels. The level of FcεRI expression was also significantly higher on basophils, conventional and plasmacytoid DCs on atopic / asthmatic compared to atopic / non-asthmatic children.

Conclusion

Together, our data suggest that in atopic individuals, the expression pattern of FcεRI differentiates asthmatic and non-asthmatic children. Given the significant immune modulatory effects observed as a consequence of FcεRI expression, this altered expression pattern is likely to contribute to asthma pathology in children.

Friday, January 25

Poster Session I - Topic 1: Basic and clinical immunology
21:00 - 22:00

P01 Phenotypic And Functional Landscape Of B-Cells In Essential Mixed Cryoglobulinemia

Stefania Colantuono

Department of molecular medicine, Sapienza University; Allergy Unit-Presidio Columbus, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

Keywords: Essential Mixed Cryoglobulinemia, B Cell

Introduction
Mixed cryoglobulinemia (MC) is characterized by the production of monoclonal (type II MC) or polyclonal (type III MC) rheumatoid factors (RF), which form with endogenous IgG cold-precipitable immune complexes that cause small-vessel vasculitis and multi-organ damage. Hepatits C virus is the causative agent in 90% of MC patients, usually characterized by the expansion of an anergic B cell subpopulation called CD21low B cells. Only a minority of the patients has idiopathic or essential MC (EMC) and the B cell population has been scarcely investigated so far.
Objective: to characterize the phenotypical and functional proprieties of B cells in EMC and compare them with those of HCV-related MC and from healthy donors.
Method
The B cell phenotype and function was studied in 13 patients with EMC and compared to 24 patients with HCV-MC. The proliferative response of B cells was investigated through the CFSE assay, the intracellular pERK content was measured by the BD Phos-Flow system and apoptosis was measured through annexin/7AAD staining. All the analyses were performed by flow-cytometry.
Results
EMC patient showed significant lower absolute numbers of circulating B cells compared to HCV-MC (mean ± SD: 185/mm3 ± 236 vs 529/mm3 ± 795). Interestingly percentages and absolute numbers of CD21low B cells were significantly higher in EMC compare to HD but lower than HCV-MC patients. Similarly to CD21low B cells found in HCV MC, CD21low B cells in EMC proliferated poorly in response to TLR9 stimulation, displayed dysregulated pERK signaling and were apoptosis prone.
Conclusion
Similar features of virus-specific exhaustion and anergy induced by continual antigenic stimulation observed in B cells expanded in HCV-MC are found in B cells EMC. Our findings open the question of a possible role of a still yet unknown antigen responsible for the development of EMC.

P02 Respiratory Tract Colonization By H. Influenzae And S. Pneumoniae In Common Variable Immunodeficiency: Risk-Factors And Clinical Consequence In Patients With Humoral Defect

Federica Pulvirenti[1], Romina Camilli[2], Maria Giuffrè[2], Fabiola Mancini[2], Cinzia Milito[1], Rita Cardines[2], Alessandra Ciervo[2], Annalisa Pantosti[2], Marina Cerquetti[2], Isabella Quinti[1]

1. Dpt of Molecular Medicine, Rome, Italy
2. Dpt of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

Keywords: H. Influenzae, Respiratory Tract Colonization, S. Pneumoniae, Common Variable Immunodeficiency, Mucosal Immunity

Introduction
There is limited evidence on rate of S. pneumoniae (Sp) and H. influenzae (Hi) carriage in Common Variable Immunodeficiency (CVID) and on its association with recurrent respiratory tract infections. The aim of this observational prospective study was to investigate frequency of colonization, immunological correlates and clinical consequence of carriage in CVID, the most frequent symptomatic primary antibody deficiency.
Method
Nasopharyngeal and oropharyngeal swabs were obtained from 93 adult CVID patients under Ig replacement. Sp and Hi were isolated by standard cultural methods and/or directly detected by Real-time PCR (RT-PCR). Sp isolates were serotyped by the Quellung reaction; Hi capsular type was determined by PCR. Isolates of were characterized by antimicrobial susceptibility testing. Respiratory infections observed in the six months before and after the swabs collection and antibiotic usage in the six months preceding the swab collection were recorded.
Results
The carriage prevalence by culture was 10.8% and 26.9% for Sp and Hi, respectively; 4% of CVID were co-colonized. Compared with culture, RT-PCR allowed identifying a higher carriage rate of Sp and Hi (10.8% vs 52.7%, P<0.0001 and 26.9% vs 39.8%, P=0.04, respectively). IgM serum levels<5 mg/dL and IgA serum levels<7 mg/dL were identified as risk-factor for Sp and Hi colonization and the presence of children (<6 years-old) in the house/workplace as risk-factor for Hi colonization. Other potential risk factors such as age, previous respiratory infections, bronchiectasis, antibiotic use and long-term prophylaxis were not associated with bacteria colonization. Carriers identified by culture, but not by RT-PCR, had a higher risk of upper respiratory tract infection within 60 days (HR, 4.1; 95%CI, 1.70-10.0; Log-rank test P=0.002). Most Sp isolates and 1/3 of Hi strains were resistant to macrolides/beta-lactams. Carriers of susceptible strains were never treated by beta-lactams and/or macrolides, whereas carriers of strains non-susceptible to beta-lactams and macrolides were treated for a mean of 14.4±6.6 days (P=0.05).
Conclusion
In CVID very low IgA serum level is a risk factor for carriage and colonization acts as a bacteria reservoir and as a risk factor for respiratory complications.

P03 Immunoglobulin G Subclass Deficiencies In Children And Adults: Immunological And Clinical Profile And Need For Immunoglobulin Replacement Therapy?

Jarno De Craemer[1], Laurens De Ketelaere[2], Philippe Gevaert[1], Filomeen Haerynck[3], Tessa Kerre[4]

1. Upper Airways Research Laboratory, Department of Otorhinolaryngology, Ghent University Hospital, Ghent University, Ghent, Belgium
2. Ghent University, Ghent, Belgium
3. Clinical Immunology Research Lab, Department of Pulmonary Medicine, Ghent University Hospital, Ghent, Belgium; Department of Pediatric Immunology and Pulmonology, Centre for Primary Immunodeficiency, Jeffrey Modell Diagnosis and Research Centre, Ghent University Hospital, Ghent, Belgium
4. Department of Hematology, Ghent University Hospital, Ghent, Belgium; Laboratory of Experimental Immunology, Ghent University, Ghent, Belgium

Keywords: Immunodeficiency, IgG Subclass Deficiencies, Immunoglobulin Replacement Therapy

Introduction

IgG subclass deficiencies (IgGSD) are a type of primary antibody deficiencies but don't always represent a clinically relevant disorder.
The aim of this study is to gain more insight into the immunological and clinical profile of patients with IgG2SD or IgG3SD and to assess which patients would benefit most from Ig Replacement Therapy (IRT).

Method

A retrospective study was conducted on 28 pediatric and 38 adult patients with IgG2SD and/or IgG3SD and normal total IgG, recruited from the Ghent University Hospital. Based on their medical files, we created a database including both lab results and clinical characteristics, before and after IRT. Subsequently, we compared these variables between different subsets in our patient population based on age, type of IgGSD and presence of an associated immunological abnormality, more specifically an IgA deficiency, IgM deficiency, abnormal B cell maturation or specific polysaccharide antibody deficiency (SPAD).

Results

IgA deficiency was found in 19,7% of the patients and twice as often in combination with IgG2SD than with IgG3SD. SPAD was seen in 40,9% of IgG2SD patients compared to 11,1% of IgG3SD patients. IgM deficiencies or abnormal B cell maturation were only rarely observed. At diagnosis, the majority of patients suffered from recurrent upper respiratory tract infections (URTI). Lower respiratory tract infections (LRTI) and infection-related hospitalizations were observed significantly more in patients with IgG2SD, in patients with an associated immunological defect and in children. Asthma or allergy was present in 44,1% of IgG3SD patients compared to 28% of IgG2SD patients. In total, 40 patients received IRT, which resulted in a significant increase in total IgG and IgG2, but not in IgG3. In all subgroups, a significant proportion of patients showed a decrease in infection rate.

Conclusion

Results on hospitalizations indicate that an associated immunological defect, age under 16 and IgG2SD can be linked to a more severe phenotype in IgGSD patients. Closer follow-up, more extensive diagnostics and IRT could be beneficial in these specific subgroups. This study also suggests a correlation between IgG3SD and allergy. Furthermore, results show that all patients with IgGSD who have frequent infections with need for antibiotics, benefit from IRT, even IgG3SD patients, despite not reaching normal levels of IgG3 after receiving treatment.
Results of a follow-up study, in which additional patients have been included, are currently being analyzed.

P04 HLA –B52* Is Strongly Associated With Disease Severity In Takayasu's Arteritis Patients In Serbia

Maja Stojanovic[1], Zorana Andric[2], Dusan Popadic[3], Marija Stankovic[4], Aleksandra Peric-Popadic[5], Jasna Bolpacic[5], Dragana Jovanovic[6], Rada Miskovic[5], Mirjana Bogic[5], Sanvila Raskovic[5]

1. Clinic of Allergy and Immunology, Clinical Center of Serbia; Faculty of Medicine, University of Belgrade, Belgrade, Serbia
2. Tissue Typing Department, Blood Tranfusion Institute of Serbia, Belgrade, Serbia, Belgrade, Serbia
3. Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, Belgrade, Serbia
4. Department of Pathophysiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, Belgrade, Serbia
5. Clinic of Allergy and Immunology, Clinical Center of Serbia, Belgrade, Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, Belgrade, Serbia
6. Clinic of Allergy and Immunology, Clinical Center of Serbia, Belgrade, Serbia, Belgrade, Serbia

Keywords: Takayasu Arteritis, Systemic Vasculitis, HLA Poymorphism, Vascular Disease, Biomarkers

Introduction
Takayasu arteritis (TA) is a rare systemic vasculitis that affects aorta, its major branches, and occasionally pulmonary arteries. Genetic factors, such as certain human leukocyte antigen (HLA) seem to play an important role in the development of TA. The aim of our study was to examine, for the first time, HLA polymorphisms and its correlation with clinical and demographic characteristics of TA patients in Serbia.
Method
Deoxyribonucleic acid was extracted from blood samples of 25 patients with confirmed diagnosis of TA by a fully automated system with Maxwell 16 Purification Kit. The allelic groups of HLA-A*, -B*, -C*, -DRB1* and -DQB1* loci were typed by polymerase chain reaction sequence-specific oligonucleotide probe using a LuminexTM platform. The allele frequencies were compared with a control group consisted of 1992 unrelated healthy potential bone marrow donors. To compare the differences between allele frequencies, as well as haplotype frequencies, in the control and patient groups, a 2 X 2 contingency table analysis was performed using the Fisher exact test. $p<0.05$ was considered to be statistically significant. The association of HLA-B*52 allele with clinical covariates was evaluated with ordinal logistic regression, chi square and Fisher's exact test where appropriate. p-values were corrected for multiple comparisons according to the Benjamini-Hochberg method
Results
A significant association of TA with HLA-B*52 was found [20% of patients (5/25) with 10% HLA-B*52 alleles frequency (5/50) vs 1.2% (46/3884) in healthy controls; p=0.0003962, p adj =0,011). The presence of HLA-B*52 was associated with an earlier disease onset, poorer clinical outcomes and respond to treatment. A higher frequency, but without statistical significance after p-value correction, for HLA-A*32 (p=0.012, p adj 0.2), HLA-B*15 (p=0.012, p adj 0.326), HLA-B*57 (p=0.018, p adj 0.483), HLA-C*03 (p=0.009, p adj 0.121) allelic group and DRB1*15-DQB1*05 haplotype (p=0.039, p adj 0.583) was found. In contrast to susceptibility alleles, HLA-C*03 allelic group, found in 32% (8/25) of TA patients, was present in patients with milder clinical form of the disease.
Conclusion
Our study has shown the strong association between HLA-B*52 and TA. The HLA-A*32, -B*15 and -B*57 allelic groups and DRB1*15:02-DQB1*05 haplotype, as the susceptibility factors, and HLA-C*03 as a protective allelic group in TA patients, still need to be confirmed in a larger study population.

P05 Functional Inhibitory Siglec-6 Is Upregulated In Human Colorectal Cancer-Associated Mast Cells

Yingxin Yu[1], Bart Blokhuis[1], Mara Diks[1], Ali Keshavarzian[2], Johan Garssen[1], Frank Redegeld[1]

1. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
2. Department of Internal Medicine, Division of Digestive Diseases and Nutrition, Rush University Medical Center, Chicago, United States

Keywords: Siglec-6, Human Mast Cells, Immunoreceptor Tyrosine-Based Inhibitory Motif, Colorectal Cancer, Hypoxia

Introduction
Mast cells (MC) accumulate in colorectal cancer (CRC) and the relationship between MC density and CRC progression has been well recognized. MC can be either pro-tumor or anti-tumor players, depending on the local factors present in the tumor microenvironment.

Upon malignant transformation, cancer cells express high levels of sialic acids on cell membrane or by secretion. Siglecs are a family of immunoglobulin-like receptors that bind sialic acids and each subtype has a distinct pattern of expression on immune cells. Among them, Siglec-6 is expressed predominately on MC. However, the function of Siglec-6 in MC is largely unexplored and whether it is expressed by CRC-associated MC remains unknown.

Method

Human MC were generated from peripheral CD34+ stem cells. MC activation was initiated by IgE crosslinking with or without preincubation of anti-Siglec-6 antibody. Release of beta-hexosaminidase and cytokines was quantified. To mimic the milieu of CRC, human MC were cultured with colon cancer cells or under hypoxia. Siglec-6 expression was then quantified on these conditioned MC. Furthermore, in situ expression of Siglec-6 and its ligands were measured in human CRC tissues.

Results

Siglec-6 engagement attenuated IgE-dependent MC activation, as indicated by release of beta-hexosaminidase and production of GM-CSF. Interestingly, coculture with colon cancer cells (HT29 and Caco2) induced significant upregulation of Siglec-6 on MC. In contrast, normal colon cells (CCD841) had no effect. Also, a time-dependent increase of Siglec-6 was observed when MC were incubated in hypoxia (1% O2). In situ expression of Siglec-6 was detected in CRC tissues and Siglec6+ MC were mainly found in submucosal layers. Lectin immunochemistry revealed the presence of actual ligands for Siglec-6 in human CRC tissues.

Conclusion

Together, our findings illustrate that Siglec-6 is a functionally inhibitory receptor on MC and suggest that Siglec-6 expression may be relevant for MC activity in CRC.

P06 The Role Of The Transcription Factor T-Bet In B Cell Differentiation And Function

Imran Akdemir, Bengt Johansson Lindbom

Technical University of Denmark, Copenhagen, Denmark

Keywords: B Cells, T-Bet, Interferon Signaling

Introduction

It has become clear that B cells, through their MHCII expression, can act as efficient antigen-presenting cells and thereby directly contribute to Th cell differentiation processes. It is however not clear in which contexts antigen-presentation by B cells are of particular importance or which molecular pathways that are involved in this process.

B cells are sensitive to innate and environmental signals such as TLR agonists. Such innate signaling appear to underlie the heterogeneity within the peripheral B cell pool. We have recently demonstrated complementary roles for B cell intrinsic signaling by type I and type II IFNs in driving B cell responses of the IgG2a isotype (manuscript in preparation). Our results indicate that B cells that develop in response to this cytokine combination are similar to a recently described subset of B cells that require the transcription factor T-bet for their development, display an enhanced antigen-presenting capacity and appears in a number of immune-mediated disorders.

Method

Mouse models include CD19Cre x ifnarfl/fl, CD4Cre x Bcl6 fl/fl, and T-bet reporter mice. Mice are immunized with OVA and TLR ligands. CD4 T cell responses are studied by adoptive transfer technique.

Multi-parameter flow cytometry is the key methodology in this project. Antigen-specific B and T cells are either analysed directly by flow cytometry or sorted and subjected to qPCR. Human PBMCs are analysed by flow cytometry.

Results

T-bet and CD11c are co-expressed in B cells both in mouse and human T-bet+ B cells can be found in different compartments of B cells in mouse T-bet can be induced in vitro by

IFN-gamma in B cells activated by anti-IgM and R848 T-bet+ B cells can be found in different compartments in human PBMCs but they are primarily associated with IgG1, IgG3 and IgA1 isotypes

Conclusion

We observed CD11c+ T-bet+ B cells both in mouse and human as reported in the literature
We observed different phenotypic "subsets" of T-bet expressing B cells in the mouse
We can induce T-bet by TLR7 ligand and IFN-gamma in vitro as reported in the literature
In human, T-bet expressing B cells can be found in different subsets of B cells (memory, naïve subsets)
In the memory B cell compartment, T-bet expression appears to be primarily associated with the IgG1, IgG3 and IgA1 isotypes (and more or less absent from IgG2 and IgA2 memory) in human
We will try to dissect the role of T-bet expressing B cells in driving pathogenic T cell responses in mouse models and in IBD patient

P07 Stress Hormones, Cell Immunity And Genetic Polymorphism Of Protein Coding GABRA6 Gene Among Different Professional Groups

Mariya Ivanovska[1], Petya Gardjeva[2], Dora Terzieva[3], Nonka Mateva[4], Hristo Ivanov[5], Ivan Zheljazkov[5], Aleksandar Linev[5], Marianna Murdjeva[1]

1. Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University; Divison of immunological assessement of post-traumatic stress disorder, TCEMED, Plovdiv, Bulgaria
2. Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria
3. Department of Clinical Laboratory, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria
4. Department of Medical Informatics, Biostatistics and E-learning, Faculty of Public health, Medical University, Plovdiv, Bulgaria
5. Section Genetics at the Department of Pediatrics and Medical Genetics, Medical Faculty, Medical University, Plovdiv, Bulgaria

Keywords: Stress Hormones, Immunity, Genetic Polymorphism

Introduction

To study the effects of stress hormones (cortisol and noradrenaline) on immune system and genetic polymorphism of protein coding GABRA6 (Gamma-AminoButyric acid type A Receptor Alpha6 subunit) gene in medical students, medical doctors and yoga practitioners.

Method

A total of 81 participants were studied: 29 yoga practitioners (control group), 25 students during exam and 27 medical doctors working under chronic stress. Absolute numbers and percentage of T, B and NK cells, serum cortisol, plasma noradrenaline and genetic polymorphism of GABRA6 were examined by flow cytometry, chemiluminescence, ELISA and PCR. The statistical analysis was performed with SPSS17.

Results

Although within the reference range, medical doctors under chronic stress had lowest values of cellular immunity, students - average and yoga practitioners - highest. In doctors with chronic stress, the established high noradrenaline concentrations in n=25 (92.59%) correlated with decreased total absolute numbers of T and NK cells ($p \leq 0.05$). In medical students elevated serum cortisol levels were found in 32% (n=8) and at medical doctors only in 7.40% (n=2). It was found that 85.1% (n=23) of 27 tested (yogins=10, doctors=7, students=10) were with T1521C polymorphism of GABRA6 associated with changes in ACTH and cortisol levels.

Conclusion

Possibly, due to the presence of compensatory mechanisms in physicians under chronic

stress conditions, no increased serum cortisol was detected. Based on the study, we can speculate that T1521C polymorphism of GABRA6 is not really associated with an increased activity of the HPA axis (proved in group of medical doctors) and suppressed cell immunity.

P08 Wasp Venom Allergy In A Patient With Systemic Mastocitosis: A Challenging Therapeutic Approach

Maria Luís Marques[1], Leonor Cunha[1], Esmeralda Neves[2,3], Margarida Lima[4,3], Helena Falcão[1]

1. Serviço de Imunoalergologia, Centro Hospitalar e Universitário do Porto, Porto, Portugal
2. Serviço de Imunologia, Centro Hospitalar e Universitário do Porto, Porto, Portugal
3. Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas da Universidade do Porto, Porto, Portugal
4. Consulta Multidisciplinar de Linfomas Cutâneos e Mastocitoses e Laboratório de Citometria do Serviço de Hematologia Clínica,Centro Hospitalar e Universitário do Porto, Porto, Portugal

Keywords: Mastocitosis; Wasp Venom Allergy; Omalizumab

Introduction

Patients with systemic mastocytosis (SM) may develop life-threatening reactions after Hymenoptera stings. Hymenoptera venom immunotherapy (VIT) can be combined with omalizumab (anti-IgE recombinant humanized monoclonal antibody) to suppress systemic reactions developing due to VIT. There are promising reports of the use of omalizumab as add-on therapy in patients with systemic mastocytosis and recurrent anaphylaxis during VIT. The literature reveals a wide range of responses between individuals in terms of dosage and duration of therapy with omalizumab.

Case description

We report a case of a 61 year-old-man with indolent SM without skin lesions, who experienced three episodes of severe anaphylaxis after a wasp sting. Sensitization to wasp venom was confirmed with serum IgE specific for wasp venom (1.10 kUA/L), and recombinant wasp venom allergens (rVes v1 1.59 kUA/L, rVes v5 0.01 kUA/L), and conventional immunotherapy for wasp was initiated in combination of omalizumab, considering the high risk of severe reaction during VIT. The VIT protocol is being well tolerated, and the patient is already in maintenance phase (50µg +50µg of VIT monthly). Three months after initiating VIT, the patient was stung by a wasp and developed only a slight local reaction, which resolved spontaneously. This result confirmed the success of VIT. However, since the first administrations the patient reported arthralgias and had an episode of gout, which can be related to omalizumab. The discontinuation of therapy with omalizumab is being weighted.

How this report contributes to current knowledge

This case suggests that omalizumab may be an useful pretreatment medication to prevent reactions during immunotherapy in patients with SM, but there are still some questions remaining regarding the adverse effects and discontinuation of this therapy.

P09 Modulation Of Vascular Endothelial Growth Factor And Matrix Metalloproteinase-9 Production By Metoprolol In Human Hematopoietic Cells

Fatemeh Hajighasemi, Baran Hajatbeigi

Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran

Keywords: Metoprolol, Hematopoietic, Cells, MMP-9, VEGF

Introduction
Metoprolol (a selective cardio-β1-blocker) has been broadly used in treatment of cardiovascular diseases. Moreover anti-inflammatory properties of metoprolol have been demonstrated. Also evidence proposes that epigenetic modifications of adrenergic beta-1 receptor are influential factors in metoprolol's efficiency. Matrix metalloproteinases (MMPs), a large group of enzymes degredating the extracellular matrix, and vascular endothelial growth factor (VEGF), as a cytokine, are involved in some pathological states including allergic contact dermatitis and asthma. VEGF and MMP-9 have an important role in inflammation. Besides epigenetic modifications of VEGF and MMP-9 expression has been revealed. In this study effect of metoprolol on VEGF and MMP-9 production in leukemic U937 and Molt-4 cells has been assessed in vitro.
Method
Human U937 and Molt-4 leukemic cells were cultured in complete RPMI-1640 medium supplemented with 10 % FBS. Then the cells at exponential growth phase were stimulated with PMA at optimum dose and incubated with different concentrations of metoprolol (1-1000 µg/ml) for 24 hours. Afterward the amounts of VEGF and MMP-9 in cell culture supernatant were determined by ELISA assay.
Results
Metoprolol significantly decreased the PMA- stimulated VEGF/MMP-9 production in U937 and Molt-4 cells dose-dependently.
Conclusion
Our results suggest that metoprolol could be a potential VEGF/MMP-9 inhibitor. So anti-inflammatory effect of metoprolol, reported by others, may be partly due to its inhibitory effects on VEGF/MMP-9 secretion. Accordingly metoprolol might be useful as a novel therapeutic candidate for inflammatory- mediated disorders such as some allergies in which VEGF/MMP-9 are over-expressed. Moreover as metoprolol's efficacy is controlled by epigenetic modifications of adrenergic beta-1 receptor and that epigenetic modifications of VEGF and MMP-9 expression have been shown, the inhibitory effects of metoprolol on VEGF /MMP-9 secretion may be somewhat due to epigenetically–induced anti-inflammatory properties of metoprolol or might be mediated via epigenetically modifications of VEGF/MMP-9 expression by this drug. Since epigenetherapy could be a promising novel treatment approach for myocardial infarction and also is potentially useful for modulation of inflammatory genes, other studies on metoprolol role in epigenetic modification of VEGF /MMP-9 expression as well as other inflammatory genes are warranted.

P010 Th17- And Th22- Related Cytokines In Arterial And Venous Blood Of Patients Affected By Systemic Sclerosis (SSc) With And Without Digital Ulcers (DU).

Stefania Nicola, Giovanni Rolla, Luisa Brussino

Università degli Studi di Torino - Dipartimento di scienze mediche - AO Ordine Mauriziano Umberto I Torino, Torino, Italy

Keywords: Th17, Cytokines, Systemic Sclerosis, Digital Ulcers, IL22

Introduction
SSc is a chronic connective tissue disease that often sets on with Raynaud’s phenomenon and repeated ischemia-reperfusion cycles, resulting in DU in 50% of patients. Due of hypoxia, many cells release cytokines and growth factors with a paracrine action on the ulcer site. The unbalance of Th1 cytokines in SSc is known, and Th17 as Th22 cytokines were found increased in serum and skin biopsies of SSc patients, but few studies analyzed the pattern in arterial (AB) and venous blood (VB) of SSc patients with DU. The aim of our study was to evaluate Th17 (IL1b, IL6, IL17, IL21, IL23, TGFb) and Th22 (IL22) cytokines,

TNFa, GMCSF and Endothelin1 (ET1) in AB and VB of SSc patients with or without DU, and to correlate cytokines and scores of skin and vascular involvement.

Method

All consecutive patients with SSc attending to our hospital in 2014-2015 were enrolled in the study (Fig.1). All patients, divided in two subgroups based on the presence of DU or not, underwent arterial and venous cytokines sampling, analyzed using a multiplex immunoassay with a xMAP technology (Bio-Rad,USA), nailfold videocapillaroscopy (NVC) evaluated with a qualitative score (Cutolo 2007) and modified Rodnan skin score (mRSS) (Khanna 2017). Statistical analysis was performed with STATA 10s using non-parametric tests for paired samples, considering only $p<0.05$. Correlations were done with Spearman's rank coefficient.

Results

29 patients affected by SSc with or without DU were enrolled (Tab.1). In the SSc group, IL22, IL23, IL1b, TGFb and IL6 were significantly higher in VB compared to AB, while GMCSF was higher in AB (Tab.2). Patients with DU showed significant higher concentration in VB of IL1b, IL6, IL22 and TGFb, whether GMCSF was significantly higher in AB (Tab.3). No differences were found in patients without DU. In VB, NVC positively correlated with IL22, IL23, IL17 and negatively with ET1; mRSS negatively correlated with IL21. In AB, NVC positively correlated with TNFa (Tab.4).

Conclusion

Our results showed elevated Th17 cytokines levels in VB compared to AB of SSc patients with DU, normal in those without DU, suggesting a local inflammation and a production of these cytokines on the ulcer site. Moreover, the higher levels of TNFa and GMCSF in AB of patients with DU support the attempt to repair the hypoxia damage underlying the fibrosis mechanism, and the correlation between Th17 cytokines, NVC and ET1 agrees with the potent pro-fibrotic stimulus at the onset of disease, which decreases as the SSc progresses.

Demographic data of Patients affected by SSc	
Patients	N. 29
Gender	28 females and 1 male
Mean age	64.5 years (range: 28–80)
Digital ulcers (DU)	20 patients presenting DU, 9 without DU.

Table 1 - Demographic data of Patients affected by SSc with and without DU

Cytokines	**Venous Blood concentration** **Median (pg/ml) [IC 95%]**	**Arterial Blood concentration** **Median (pg/ml) [IC 95%]**	**p**
TNF-a	2.27 [2.15 – 4.64]	5.15 [0.06 – 28.87]	n.s.
GM-CSF	140.90 [140.30 - 140.93]	141.20 [140.91 - 141.26]	0.001
IL-23	8.84 [6.71 – 9.84]	5.73 [1.085 - 12.07]	0.005
IL-1b	0.18 [0.22 - 0.58]	0.14 [0.12 - 0.17]	0.008
IL-6	2.45 [2.67 - 8.40]	1.55 [1.40 - 3.30]	0.004
IL-17	1.08 [0.84 - 1.13]	1.08 [0.88 - 1.24]	n.s.
IL-21	23.38 [17.16 – 24.28]	23.38 [21.44 - 26.53]	n.s.
IL-22	4.52 [3.63 - 4.63]	3.77 [3.41 - 5.25]	<0.001
TGF-b	6.62 [6.51 – 10.68]	4.98 [3.48 - 6.95]	0.041
ET-1	15.77 [13.13 - 19.65]	16.24 [12.68 - 18.28]	n.s.

Table 2 - Comparison between venous and arterial blood cytokines' concentration in patients affected by systemic sclerosis, regardless of the presence of DU or not.

Cytokines	**Venous Blood concentration** **Median (pg/ml) [IC 95%]**	**Arterial Blood concentration** **Median (pg/ml) [IC 95%]**	**p**
TNF-a	8.51 [0.21 – 8.72]	28.81 [0.06 – 28.87]	n.s.
GM-CSF	140.88 [140.26 - 140.99]	141.18 [140.81 - 141.28]	<0.001
IL-23	10.73 [7.14 - 11.62]	10.73 [10.58 - 11.68]	n.s.
IL-1b	0.20 [0.17 - 0.64]	0.14 [0.11 - 0.16]	0.024
IL-6	3.71 [2.52 - 11.03]	1.64 [1.22 - 3.98]	0.012
IL-17	1.08 [0.78 - 1.18]	1.12 [0.94 - 1.33]	n.s.
IL-21	23.38 [17.81 - 27.00]	23.38 [21.42 - 27.01]	n.s.
IL-22	4.64 [4.13 - 5.30]	3.62 [3.33 - 4.60]	0.006
TGF-b	7.10 [6.31 - 13.10]	7.02 [6.44 - 7.06]	0.046
ET-1	14.39 [13.51 - 16.38]	15.88 [11.38 - 17.40]	n.s.

Table 3 - Comparison between venous and arterial blood cytokines' concentration in patients affected by systemic sclerosis presenting digital ulcers.

Cytokines	**Nailfold videocapillaroscopy**		**mRSS**	
Venous blood				
IL-17	ρ = 0.465	p = 0.039		
IL-21			ρ = -0.427	p = 0.050
IL-22	ρ = 0.460	p = 0.041		
IL-23	ρ = 0.411	p = 0.042		
ET-1	ρ = -0.437	p = 0.044		
Arterial blood				
TNF-a	ρ = 0.460	p = 0.043		

Table 4 – Correlations between scores of skin involvement and blood cytokines concentration in patients presenting DU.

Study protocol exclusion criteria	
Smoking	Asthma
COPD	History of cancer
Current oral/inhaled corticosteroids	Immunosuppressive drug therapy
Current or recent (last 8 weeks) systemic or airway infection	Other autoimmune diseases

Friday, January 25

Poster Session I - Topic 2: Innate immunity and epithelial barriers
21:00 - 22:00

P011 Characterization Of Alum-Induced NET-Formation In Human Neutrophils

Manuel Reithofer[1], Dominika Polak[1], Claudia Kitzmüller[1], Georg Greiner[2], Barbara Bohle[1], Beatrice Jahn-Schmid[1]

1. Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
2. Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

Keywords: Alum, NETs, Adjuvanticity, Immuntherapy

Introduction
Alum is the most widely used vaccine adjuvant, especially in allergen-specific immunotherapy. However, the mechanism behind its adjuvanticity is not totally solved. In mice, host-derived DNA has been reported to be involved in the adjuvant effect of alum, but the cellular source was not clearly defined. Neutrophils are the first cells at the site of injection and have the ability to simultaneously release intracellular DNA and granular material, so-called neutrophil extracellular traps (NETs). This modified DNA may represent danger-associated molecular patterns (DAMPs) playing a role in the initial immunse response.
Method
We investigated alum-induced NET-formation in human neutrophils and its underlying pathway, mainly based on confocal microscopy and plate-reader assays to quantify released DNA. Neutrophils were stimulated with alum or PMA and ionomycin as positive controls. The underlying signalling pathway was studied using specific inhibitors. Furthermore, extracellular flux measurements were performed to evaluate metabolic requirements.
Results
Strong NET-formation was induced by all stimuli as visualized by confocal fluorescence microscopy showing co-localization of extracellular DNA and different granular proteins. In addition, increased neutrophil elastase activity was found in cultures of neutrophils stimulated with alum. Ionomycin and alum induced mitochondrial reactive oxygen species (mROS), whereas PMA triggered cytoplasmatic NADPH oxidase-dependent ROS. Alum induced rapid DNA-release similar to ionomycin and dependent on phagocytosis, extracellular calcium and NFκB-signalling. Similar to crystal-induced NET-release a significant dependence on necroptosis signalling was found. During the process of NET-formation, increased glycolysis, as well as mitochondrial respiration was observed.
Conclusion
Together, alum potently induced rapid, mROS-dependent NET-release in human neutrophils in vitro, utilizing energy from glycolysis and mitochondrial respiration. These NETs may represent danger-associated molecular patterns involved in the initial immune response to alum-adjuvated vaccines and may play a role in subsequent T cell polarization.

P012 Electrical Impedance Spectroscopy For The Assessment Of Skin Epithelial Barrier Defects

Arturo O. Rinaldi[1], Anita Dreher[1], Hideaki Morita[1], Marja Gautschi[2], Kristina Tsekova[2], Simon Grant[3], Per Svedenhag[3], Matthias Möhrenschlager[2], Cezmi A. Akdis[1]

1. Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland
2. Hochgebirgsklinik Davos (HGK), Davos, Switzerland

3. SciBase AB, Sundbyberg, Sweden

Keywords: Epithelial Barrier, Atopic Dermatitis, Electrical Impedance, Transepidermal Water Loss

Introduction
Several skin and mucosal inflammatory disorders, such as atopic dermatitis (AD), have been associated with an impaired epithelial barrier function, which allows allergens, pollutants or microbes to enter the tissue and activate the immune response. The aim of this study was to establish a method to directly assess the in vivo status of epithelial barrier function by electrical impedance (EI) spectroscopy.
Method
Epithelial barrier of mice was damaged by epicutaneous application of proteases and cholera toxin and by tape stripping. After transmitting a harmless electrical signal through the skin, electrical impedance is measured by using NeviSense (Scibase) device. EI and transepidermal water loss (TEWL) were measured before and after the application. Immune histological analysis (IHC) and quantitative RT-PCR were performed in skin biopsies of the mice to evaluate the epithelial barrier. In addition, EI and TEWL were measured in AD patients during 21 days of clinical treatment at High Altitude Clinic, Davos.
Results
Already 1 hour after the treatment with proteases, a dose dependent reduction of EI was detected. Simultaneously, an increase of TEWL was observed, showing a significant negative correlation with EI, demonstrating that changes of EI were directly linked to epithelial barrier defects. 24 hours after the treatment, EI showed a tendency to increase to control levels, suggesting a restoration of the epithelial barrier. Epithelial barrier disruption was confirmed by histological analysis, which showed an impaired stratum corneum and higher cellular infiltration after papain application, and by IHC and qPCR, which showed downregulation of filaggrin and other molecules involved in the barrier function. Similar results were observed after tape stripping and cholera toxin application. In the human study, unaffected skin of AD patients showed a significantly decreased EI values compared to healthy subjects and AD lesions were characterized by a decrease of EI and an increase of TEWL compared to non-lesional skin. Moreover, AD lesional skin showed an increase of EI and a decrease of TEWL during the 21 days treatment and SCORAD (the clinical score for assessing the severity of AD) showed a negative correlation with EI and positive correlation with TEWL.
Conclusion
EI spectroscopy can be used to detect epithelial barrier defects in the skin and used for monitoring patient and treatment effects as a rapid and non invasive in vivo diagnostic method for atopic dermatitis.

P013 The Effect Of Bacteriophages On Innate Lymphoid Cells

Anna Globinska[1], Pattraporn Satitsuksanoa[1], Nina Chanishvili[2], Nikolaos Papadopoulos[3], Tadech Boonpiyathad[1], Mübeccel Akdis[1], Cezmi Akdis[1]

1. Swiss Institute of Allergy and Asthma Research, Davos, Switzerland
2. Giorgi Eliava Institute of Bacteriophagy, Microbiology and Virology, Tbilisi, Georgia
3. University of Athens, Allergy Department, 2nd Pediatric Clinic, Athens, Greece

Keywords: Bacteriophages, Innate Lymphoid Cells

Introduction
Bacterial viruses (phages) colonize all niches of the human body and exert selective pressure on their bacterial hosts. Despite the abundance of phages throughout the body, little is known about their interactions with the human immune system. Transcriptomic profiling of the immune responses induced by phages in peripheral blood mononuclear

cells (PBMCs) revealed activation of both pro- and anti-inflammatory responses, suggesting that phages may be involved in shaping the immune system. However, the complex nature of these interactions is scarce. Hence, we aimed to characterize the immunomodulatory potential of phages and investigate the effect of phages on innate lymphoid cells (ILCs).

Method

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors using density gradient centrifugation. PBMCs at a density of 10^6 cells/ml were cultured in the presence of Staphylococcal phages (10^6 PFU) or left unstimulated for 24h, 48h and 72h. Immune response-related gene expression was measured by real time qPCR. Concentration of proteins in cell culture supernatants was assessed using Multiplex Immunoassay. Cell proliferation and viability were evaluated by flow cytometry using Cell Trace Violet and Zombie Yellow, respectively. Multicolor flow cytometry analysis was used to identify different ILC subsets – ILC1, ILC2, ILC3.

Results

Increased expression of interleukin (IL)-6, IL-8, macrophage chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1β (MIP-1β) and tumor necrosis factor-α (TNF-α) mRNAs were observed in PBMCs exposed to 10^6 PFU of S. aureus phages at 24h. In parallel with an increased mRNA expression, the concentrations of IL-6, IL-8, MCP-1, MIP-1β and TNF-α were significantly higher in cell culture supernatants at 48h after stimulation with 10^6 and 10^5 PFU of S. aureus phages, whereas no induction of RANTES, IL-4, IL-5, IL-13, IL-15, TGF-β and IFN-γ was observed. Viability of PBMCs did not change and there was no effect of S. aureus phages on proliferation of CD3+ and CD19+ cells at any time point. There was a tendency towards decreased frequency of ILC1 within total (Lin-CD127+) ILCs upon stimulation with S. aureus phage (10^6 PFU). In contrast, an increase in the frequency of ILC2 was observed upon stimulation with phages, whereas phages seemed not to affect the frequency of ILC3.

Conclusion

Our results suggests innate immune response stimulation and immunomodulatory potential of phages.

P014 Oral Mucosa Remodeling Occurs In Severe Allergic Patients

Javier Sanchez-Solares[1], Marisa Dolset[1], Gonzalo Hormias[1], Teresa Carrillo[2], Carmen Moreno[3], Leticia Mera[1], Maria Marta Escribese[4], Domingo Barber[4], Cristina Gomez-Casado[4]

1. Institute of Applied Molecular Medicine (IMMA), San Pablo CEU University, Madrid, Spain
2. Hospital Universitario de Gran Canaria, Allergology Service, Las Palmas De Gran Canaria, Spain
3. Hospital Universitario Reina Sofia, Allergology Service, Córdoba, Spain
4. Insitute of Applied Molecular Medicine (IMMA); Deparment of basic medical sciences, medical school, CEU San Pablo Univeristy, Madrid, Spain

Keywords: Oral Mucosa, Remodeling, Food Allergy

Introduction

In a previous study, we demonstrated that severe grass pollen allergic patients from a region with a high grass pollen load undergo oral mucosal remodeling and develop profilin-mediated food allergy. This suggests that oral mucosa is an immunocompetent site and that it may have a role, not only in the progression of respiratory to food allergic reactions, but also in the development of complex syndromes or the immunological basis of sublingual immunotherapy. However, oral mucosa features in allergic inflammation remain largely unexplored.

In this study, we aim to characterize oral mucosa in severe respiratory allergic patients who do not present food allergy.

Method
We recruited two groups of patients: severe respiratory allergic patients to olive pollen (n=5), and patients who experienced anaphylaxis to house dust mites (HDM) (n=6) from areas where there is an elevated exposure to the respective allergens. We characterized histologically oral mucosal features in biopsies taken from the cheek lining of these allergic patients and compared with a group of non-allergic controls (n=5). Fibrosis, epithelial cell junctions, angiogenesis and immune cell recruitment were assessed with appropriate antibodies and histological staining.
Results
Both allergic groups displayed: 1) A significantly decreased expression of epithelial cell junctions; 2) Increased collagen deposition (fibrosis), when compared to controls. No significant differences were found in angiogenesis. Accordingly, minor differences were found in immune cell recruitment.
Conclusion
Oral mucosal remodeling occurs independently of food allergy, associated to severe respiratory manifestations, and regardless of the allergen involved.

P015 Novel Evidence That BAFF Directly Regulate Epithelial Barrier Function

Andrzej Eljaszewicz[1], Paulina Wawrzyniak[2], Urszula Radzikowska[3], Dries Van Elst[2], Marlena Tynecka[4], Kamil Grubczak[4], Milena Sokolowska[2], Cezmi A Akdis[2], Marcin Moniuszko[5]

1. Medical University of Bialystok, Swiss Institute of Allergy and Asthma Research, Bialystok, Poland
2. Swiss Institute of Allergy and Asthma Research, Davos, Switzerland
3. Swiss Institute of Allergy and Asthma Research; Medical University of Bialystok, Davos, Switzerland
4. Medical University of Bialystok, Bialystok, Poland
5. Medical University of Bialystok, Bialsytok, Poland

Keywords: BAFF, Epithelial Cells, Cytokines

Introduction
B-cell activation factor (BAFF), a member of tumor necrosis factor superfamily, is playing a crucial role in normal B cell development and function and promote survival and proliferation of malignant B and acute myeloid leukemia blasts. Furthermore, BAFF was shown to suppress Th2 dependent allergic airway inflammation. However, to date, the direct effect of BAFF on Th2 cytokine stimulated bronchial epithelial cells has not been elucidated. Therefore, we aimed here to understand the effects of BAFF on the function of Th2 cytokine stimulated bronchial epithelial cells.
Method
We used in vitro differentiated human bronchial epithelial cells from asthmatic and nonasthmatic donors.
Results
BAFF may interact directly with three membrane receptors, namely BAFF-R (B-cell activating factor receptor), TACI (Transmembrane activator and CAML interactor) and BCMA (B-cell maturation antigen). Therefore, first, we confirmed expression of receptors as mentioned above in differentiated human bronchial epithelial cells (hBEC) by qPCR and western blot. Next, we analyzed whether Th2 related cytokines may influence BAFF-R, TACI and BCMA expression. Finally, we stimulated hBEC with IL-4 alone or IL-4 in the presence of BAFF. Interestingly we found that BAFF may regulate mucin, proinflammatory cytokines, and chemokine, but not tight junction protein expression.
Conclusion
In conclusion, we showed novel evidence that BAFF signaling in differentiated hBEC may limit proinflammatory cytokine and chemokine production, but do not affect tight junction protein expression.

P016 Bacillus Calmette–Guérin Immunotherapy Boosts Innate Immunity In Recurrent Respiratory Papillomatosis

Evelina Vetskova, Maria Nikolova

National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Keywords: HPV, BCG, Innate Immunity,

Introduction

Recurrent respiratory papillomatosis (RRP) is the most common benign neoplasm of the larynx caused by human papillomavirus HPV-6 and HPV-11 and characterized by recurrent proliferation of squamous papillomas within the respiratory tract. RRP is a major clinical problem because of significant airway obstruction, extremely high frequency of relapses and the possibility of malignant transformation into squamous cell carcinoma. Bacillus Calmette–Guérin (BCG) immunotherapy has been successfully used in the treatment of several malignancies. The present study investigates the effects of BCG therapy in RRP patients.

Method

Blood samples from RRP patients (n = 35) subjected to combined microsurgery / BCG-immunotherapy were studied by multicolor flow cytomerty in comparison to RRP patients subjected to surgical treatment only and to healthy controls.

Results

RRP patients are characterized by decreased levels of cytokine – producing plasmacytoid dendritic cells (pDCs) and activated (mature) DCs compared to healthy controls (% ± SEM: pDCs =2.9 ± 1.4 versus 9.3 ± 1.4; mDCs = 30.8 ± 1.5 vs. 53.6 ± 1.5, $p < 0.05$). In addition, the population of CD14 / CD16+ HLA-DR++ monocytes is significantly decreased in RRP patients compared to the control group (3.4 ± 0.7 vs. 8.4 ± 2.1, $p < 0.05$). BCG immunotherapy increased significantly the levels of pDCs (9.0 ± 1.8 vs 3.1 ± 1.1, $p < 0.05$) and mature DCs (55.0 ± 3.2 versus 31.3 ± 1.2, $p < 0.05$) at 12 months after the beginning and normalized to reference values at the end of the therapy. The share of cytokine-producing CD56hi NK cells which was significantly reduced in untreated patients is increased significantly during BCG immunotherapy and reached the values typical for healthy subjects at 12 months after the start of therapy (5.9 ± 0.3 vs. 1.1 ± 0.8, $p < 0.05$). The immunotherapy with BCG resulted in a statistically significant increase of CD14 / CD16+ HLA-DR++ monocytes at 12 months (7.1 ± 1.3 versus 3.2 ± 0.9, $p < 0.05$), reaching the reference range for healthy subjects at 20 months after the start of therapy.

Conclusion

These results indicate that BCG stimulates the innate immunity through increasing the virus-recognizing and antigen-expressing potential of dendritic cells, monocytes and NK cells leading to activation of the specific antiviral response in RRP patients.

P017 Activation And Maturation Differences Between Myeloid And Monocyte-Derived Dendritic Cells In Patients With Immediate Allergic Reactions To Betalactams

Rubén Fernández-Santamaría[1], Alba Rodríguez-Nogales[1], Francisca Palomares[1], Adriana Ariza[1], María José Rodríguez[1], Miguel González-Visiedo[1], Ángela Martín-Serrano[1], Ana Molina[1], Cristobalina Mayorga[1], María José Torres[2], Tahia Fernández[1]

1. Research Laboratory, IBIMA-Regional University Hospital of Malaga-UMA, Málaga, Spain
2. Allergy Unit, Regional University Hospital of Malaga-IBIMA-UMA, Málaga, Spain

Keywords: Dendritic Cells, Drug Allergy, Betalactams

Introduction
Myeloid dendritic cells (mDCs) have a sentinel activity in peripheral blood, capturing and processing antigens. Nevertheless, most studies use monocyte-derived dendritic cells (moDCs) in in vitro assays to study the specific drug recognition by the immune system, because monocytes can be easily isolated in a higher number from peripheral blood mononuclear cells (PBMCs). Nevertheless, some research suggest that moDCs are more similar to monocytes than circulating DCs. For this reason, the main objective of this research was to analyse the differences between mDCs and moDCs activation and maturation patterns after the specific recognition of amoxicillin (AX) and clavulanic acid (CLV).
Method
PMBCs were obtained from 10 healthy subjects and from 10 patients with immediate allergic reaction to AX and CLV. mDCs and monocytes were isolated from PBMCs. The latter were cultured with GM-CSF and IL-4 to obtain moDCs. mDCs and moDCs were cultured with the culprit drug. Flow cytometry was used to analyse the overexpression of different activation and maturation markers (CCR7, CD40, CD80, CD83 and CD86). Results were represented as maturation index (MI).
Results
moDCs and mDCs from allergic patients to both drugs show higher expression of maturation and activation markers compared to controls after culture them with the culprit drug. Nevertheless, mDCs from allergic patients show more differences with controls than when moDCs were used. Higher expression of CCR7, CD40 and CD86 were found in mDCs compared to moDCs from allergic patients to AX (P=0.006, P=0.02, P=0.03 respectively). In the same way, mDCs from CLV allergic patients show higher expression of CCR7, CD40 and CD86 compared with moDCs (P=0.04, P=0.01, P=0.02 respectively). Any difference was detected in the analysis of CD80 and CD83.
Conclusion
mDCs show higher expression of activation and maturation markers compared to moDCs. These data suggest that the analysis of maduration and activation markers of mDCs could be useful to the study the selective recognition of betalactams by DCs, providing more realistic and accurate results than when moDCs are used.

P018 Activated Intestinal Epithelial Cells Conditioned With 2'-Fucosyllactose And CpG ODN Might Instruct MoDC To Drive Th1 Differentiation

Veronica Ayechu-Muruzabal[1], Atanaska I. Kostadinova[1], Saskia A. Overbeek[1], Bernd Stahl[2], Johan Garssen[1], Belinda Van'T Land[3], Linette E.m. Willemsen[1]

1. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
2. Nutricia Research, Utrecht, The Netherlands
3. Department of Paediatric Immunology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

Introduction
Human milk is composed of diverse and complex oligosaccharides (HMOS). A mixture of short chain galacto- and long chain fructo-oligosaccharides 9:1 (GF) mimicking some structural and functional capacities of HMOS previously showed to promote Th1 and regulatory type immune polarization in the presence of CpG in an in vitro co-culture model. In the current study, the immunomodulatory capacities of 2'-Fucosyllactose (2'FL) were compared to GF and to a 1:1 mixture of 2'FL and GF. Additionally, the ability of 2'FL-exposed intestinal epithelial cells (IEC) to instruct immature monocyte derived dendritic cell (moDC) function was evaluated.
Method
HT-29 cell line, grown in transwells and co-cultured with anti-CD3/CD28 activated peripheral blood mononuclear cells (PBMC), was apically exposed to 2'FL, GF or 2'FL/GF (0.25, 0.5, or 1.0 w/v%) either or not combined with CpG ODN M362 (0.5 uM). IEC were

washed and co-cultured with moDC. moDC were then used in an allogeneic assay where their capacity to induce naïve CD4+ T-cell differentiation was evaluated.

Results

In presence of CpG, GF as well as 2'FL and 2'FL/GF enhanced IFN-gamma and IL-10 secretion of activated PBMC co-cultured with IEC compared to CpG alone ($p<0.05$), while IL-13 and IL-5 remained low. IEC-derived galectin-3, TGF-beta1 (both $p<0.001$), galectin-9 and galectin-4 (both $p<0.05$) of CpG-exposed cells was further increased by GF, 2'FL and/or 2'FL/GF compared to CpG alone. Only moDC co-cultured with activated IEC conditioned with 2'FL and CpG increased IFN-gamma secretion by CD4+ T-cells ($p<0.05$).

Conclusion

These data imply that, similar to GF, exposure of IEC to 2'FL alone or 2'FL/GF combined with CpG polarize the immune response towards a Th1 and regulatory type. IEC-derived galectins might be involved in the immunomodulatory effects. moDC exposed to 2'FL and CpG-conditioned IEC instructed Th1 differentiation, suggesting that 2'FL can shape the adaptive immune response by affecting IEC function.

Friday, January 25

Poster Session I - Topic 3: Allergens and allergic inflammation
21:00 - 22:00

P019 Structure Based Epitope Grafting As A Tool For Exploring The Conformational IgE Epitopes Of The Major Birch Pollen Allergen, Bet V 1

Stefanie Schmalz, Christian Radauer

Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Introduction
Knowledge of the epitopes bound by allergen specific IgE may aid in predicting symptom severity, cross-reactivity and efficacy of allergen immunotherapy. Most conformational IgE epitopes of the major birch pollen allergen, Bet v 1, have not been characterized, yet. We aimed to identify relevant IgE epitopes by grafting epitope sized surface patches of Bet v 1 onto TTHA0849, a non-IgE-binding structural homologue from Thermus thermophilus.
Method
Based on a structural alignment, surface-exposed residues of TTHA0849 were replaced by corresponding ones of Bet v 1 while preserving the hydrophobic core. Thereby, we created 14 chimeric proteins (TB1-TB14), each carrying a different Bet v 1-derived surface patch. Codon-optimized synthetic genes were expressed in Escherichia coli as 6xHis-tagged proteins and purified by metal chelate affinity chromatography. The chimeras were characterized via SDS-PAGE, matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS), circular dichroism (CD) spectroscopy and dynamic light scattering.
Results
Until now, three chimeras (TB1,TB2 and TB3) were expressed as soluble proteins. Purification yielded 13 mg, 58 mg and 16 mg from 1 liter of bacterial culture. MALDI-MS analysis revealed that the chimeras matched their theoretical masses. The CD spectra showed mixed alpha-beta structures indicating correct folds of the chimeras. Dynamic light scattering of TB2 showed <2% aggregation.
Conclusion
Our preliminary data indicate that the structure based design of single-epitope carrying chimeric proteins yielded soluble, folded proteins which will be used in IgE binding assays to characterize the epitope repertoires of sera from birch pollen allergic patients.
Supported by: the Austrian Science Fund (FWF) grant P 30936-B30.

P020 Adolescent Preconceptional Smoking Alters The Body Weight Of The Next Generation In A Sex-Specific Manner

Barbara Hammer[1], Natalia El-Merhie[1], Sabine Bartel[1], Cecilie Svanes[2], Susanne Krauss-Etschmann[1]

1. Research Center Borstel - Leibniz Lung Center, Borstel, Germany
2. Centre for International Health University of Bergen, Department of Occupational Medicine Haukeland University Hospital Bergen, Bergen, Norway

Keywords: Smoking, Adolescence, Mouse Model, Asthma

Introduction
A recent multicenter epidemiological study suggested that paternal smoking during adolescence can increase the risk to develop early-onset non-allergic asthma in children. The effects on offspring were still observed even when fathers had quit smoking years

before the child was born. However, the underlying mechanisms are currently unexplored. Therefore we aimed to develop a murine model of adolescent smoking in order to investigate the parental and offspring´s phenotype.

Method

Male and female C57BL/6 mice (21-day-old) were exposed to mainstream cigarette smoke (CS) (research cigarettes 3R4F) for 2 weeks to 1puff/min (6 cigarettes) and 4 weeks to 4puffs/min (24 cigarettes) for 1 hour per day for 5 days/week. Thereafter, smoke-exposed animals were mated with air-controls. The body weight (BW) of offspring was recorded daily.

Results

CS-exposed male and female parents showed a decrease in weight gain compared to air-controls. 50% of control females became pregnant in comparison to only 25% of pregnant females in the CS-exposed group, where sperm counts were normal in males. CS-exposed fathers showed neutrophilia in BAL and total cell count of the thymus was decreased. Male offspring from smoking fathers demonstrated an increase in BW, validated by a significant increase in weight gain compared to air-controls. Male offspring from smoking mothers had significantly lower BW only on postnatal day 3 (PND3) compared to air-controls. Female offspring from smoking mothers had a decreased BW, strengthened by a lower weight gain, whereas female offspring from smoking fathers showed normal BW.

Conclusion

Despite normal sperm count, we observed a decrease in pregnancy rate in adolescent smoking mothers. In offspring, we were able to detect an altered BW in offspring from adolescent smoking fathers and mothers, following sex-specific patterns. We hypothesize that sperm microRNA (miRNA) could be altered; therefore, we will analyze miRNA patterns in murine sperm cells of smoked-exposed fathers to obtain insight into epigenetic changes in miRNA expression. Future experiments will include an asthma model performed with murine offspring from adolescent smoking fathers and mothers.

P021 Mast Cell Heterogeneity Landscape In Fetal Skin: An Effector Player In Pediatric Allergies?

Rasha Msallam, Hassen Kared, Xiao Meng Zhang, Jospehine Lum, Jinmiao Chen, Florent Ginhoux

Singapore Immunology Network (SIgN), Singapore, Singapore

Keywords: Mast Cells, Fetus, Skin, Pediatric Allergies.

Introduction

Pediatric Allergies (PA) have shown a high prevalence in the last decade worldwide. From newborn babies to teenagers, patients might suffer from allergic reactions (such as asthma, skin inflammation, cutaneous anaphylaxis allergies or food allergies). Symptoms could vary from mild to life threatening symptoms. Hence, PA add a new layer of complexity to allergic diseases definition, classification, and treatment protocols. Few information is known about the multi-factorial circumstances that could play a role in elicitation PA during pregnancy and/ or during early life after birth. On the other hand, Mast cells (MC) have been widely studied as major immunological players in allergic reactions in adults, but their phenotype during development is still poorly investigated. Our project aims to decipher the phenotype and heterogeneity of fetal MC in mouse and human skin during pregnancy and after birth.

Method

Single-cell transcriptomis (scRNA-seq) and multi-color fluorescence flow cytometry (Symphony) analysis of fetal human skin MC at 14 weeks, 16 weeks and 21 weeks estimated gestational age (EGA) in comparison to adult human skin MC.

Results

Murine MC exhibit a significant heterogeneity in adult skin, that was confirmed in fetal and adult human skin as well. Furthermore, at 16 weeks estimated gestational age (EGA), fetal

MC show an interesting heterogeneity, expressing activation markers such as CD63 and functional/maturation markers such as FceR, the latter known to be involved in allergic reaction, through activation of MC degranulation. These observations suggest that fetal MC could be triggered even before birth.

Conclusion

Our results unraveled a possible link between fetal MC development, maternal environment during gestation and PA initiation; and provide unprecedented resource for further understanding the biology of PA by identification bio-targets to control activated neonatal MC, to reduce risk factors that could involve in PA development.

P022 Longitudinal Immunoglobulin Heavy-Chain Repertoire Profiling Of Memory B Cell, Plasmablasts And Plasma Cells From Peripheral Blood Of Individuals With Birch Pollen Allergy

Artem Ilyich Mikelov[1], Maria Andreevna Turchaninova[2], Ekaterina Aleksandrovna Komech[2], Dmitry Borisovitch Staroverov[2], Yuri Borisovitch Lebedev[2], Dmitriy Mikhailovitch Chudakov[1], Ivan Vladimirovitch Zvyagin[2]

1. Skolkovo Institute of Science and Technology, Moscow, Russia
2. Shemiakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia

Keywords: Birch Pollen Allergy, IGH Repertoires By High-Throughput Sequencing, Memory B-Cells, Plasmablasts, Plasma Cells

Introduction

Mechanisms underlying allergy-related immunological memory development and maintenance still remain obscure. IgE produced by B-cell lineage cells is the known causative agent triggering clinical manifestations. The cell subsets, responsible for production of IgE and its persistence in human body are not well characterised. Little is known about the structure and seasonal dynamics of B-cell receptor repertoire of allergic individuals. In this study we aimed to characterize IGH repertoires of key cell subsets of B-cell lineage from the peripheral blood of donors, susceptible to birch pollen allergy.

Method

Fluorescence activated cell sorting was used to isolate Memory B-cells (CD20+,CD19+,CD27+), Plasmablasts (CD20+,CD19-,CD27 High+, CD138-) and Plasma Cells (CD20+,CD19 Low-,CD27+, CD138+) were isolated from the peripheral blood of 4 donors, allergic to birch pollen, and two healthy volunteers at three time points during 1 year, including 2 off and 1 in birch pollination season.

State-of-the-art technique was used for IGH cDNA library preparation, utilizing molecular barcodes for error correction and data normalization. This allowed recovery of high-quality full-length IGH repertoires, retaining isotype information.

Results

Clonal groups with IgE clonal sequence were detected in 3 of 4 allergic and 1 of 2 healthy donors. IgE-containing clonal groups in repertoires of included members from multiple cell subsets of allergic individuals, which was not the case for healthy donors. Such clonal groups also contained IgG, IgM and IgA members, and persisted in several time points.

In memory B cell subset of 4 out of 6 donors we found clonal sequences of IgE isotype. These IGH clonotypes were hypermutated at the rates, similar to those of IgG and IgA clonotypes from the same clonal groups.

Conclusion

Clonal groups with IgE members in B-cell receptor repertoires from peripheral blood of allergic donors have complex structure: they are found in multiple cell subsets, isotypes and persist for at least a year. This indicates that allergy-related immunological memory in humans has multiple back-ups even at the level of B-cell lineage, pinpointing the need for development of combination therapies affecting multiple targets.

IgE Memory B-cells, observed in this study, may serve the most direct progenitors for IgE-secreting cells. Nevertheless, their extremely low prevalence in the peripheral blood does not allow to assert that this is the dominant way of maintaining long-term IgE memory.

P025 Characterization Of Amoxicillin- And Clavulanic Acid-Specific T-Cells In Patients With Amoxicillin-Clavulanic Acid Hypersensitivity Reactions

Adriana Ariza[1], María Isabel Montañez[2], Arun Tailor[3], Monday O Ogese[3], Tahia D Fernández[1], María José Torres[4], Dean J Naisbitt[3]

1. Research Laboratory, IBIMA – Regional University Hospital of Malaga – University of Malaga, Málaga, Spain
2. Research Laboratory, IBIMA – Regional University Hospital of Malaga – University of Malaga - BIONAND, Málaga, Spain
3. Dept. Molecular & Clinical Pharmacology, MRC Centre for Drug Safety Science, University of Liverpool, Liverpool, United Kingdom
4. Allergy Unit, IBIMA – Regional University Hospital of Malaga – University of Malaga, Málaga, Spain

Keywords: Amoxicillin, Clavulanic Acid, T-Cells, Hypersensitivity

Introduction

Amoxicillin (AX) is the most common cause of drug hypersensitivity mediated by a specific immunological mechanism, which is often prescribed alongside clavulanic acid (Clav). Sensitivity of in vitro testing is low, probably due to the use of structures not optimally recognized. In this study we generate and characterize AX- and Clav-specific T-cell clones to be used as tool to study the immunological recognition of new structures.

Method

Drug-specific T-cell clones were generated from peripheral blood mononuclear cells by serial dilution and repetitive mitogen stimulation. Antigen specificity was assessed by measurement of proliferation ([3H]-thymidine incorporation) and cytokine release (ELISpot).

Results

110 AX-specific and 96 Clav-specific T-cell clones were generated from 7 patients. Proliferation of AX- and Clav-specific clones was dose-dependent, no cross-reactivity between AX and Clav was observed and they required the presence of drug and antigen presenting cells to proliferate. Drugs were presentend to CD4+ T-cells by MHC-II and to CD8+ by MHC-I. The highest level of cytokine secreted was IFN-gγγ, followed by IL-13, IL-5 and IL-10.

Conclusion

AX- and Clav-specific T-cell clones can be generated from AX-Clav hypersensitivity patients. They are activated only in the presence of antigen presenting cells, supporting the hapten hypothesis for the recognition and presentation of betalactam antibiotics. The specific T-cell clones generated are an immunologically characterized tool that can be used for the analysis of new chemical structures to be included in in vitro diagnostic tests.

P026 Post-Translational Modifications Of Major Allergens Bet V 1 And Ara H 2 Expressed In Nicotiana Benthamiana Plants

Öykü Üzülmez, Vanessa Mayr, Angelika Tscheppe, Chiara Palladino, Heimo Breiteneder

Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Keywords: Recombinant Allergens, Plant Biotechnology, Post-Translational Modifications

Introduction

Peanut proteins, especially its major allergen Ara h 2, are a frequent cause of food-induced anaphylaxis. In-vitro diagnostic tests are performed with recombinant allergens, which are produced in bacteria and yeast systems. The production plant-derived allergens with post-translational modifications (PTMs) in N. benthamiana is an alternative to obtain natural-like products. To establish this expression system, we initially used the major birch pollen allergen Bet v 1, a protein that harbours no PTMs.

Method

Agrobacterium tumefaciens, contains a Ti plasmid enabling the delivery of T-DNA into plant cells. We used two tobacco mosaic virus (TMV)-based provectors that harboured either T-DNAs encoding Bet v 1 or Ara h 2 on a 3'-module, or viral proteins on a 5'-module. A third provector delivered the phiC31 integrase for recombining the 3'- and 5'-modules. Plants were infiltrated under vacuum while submerging leaves into a suspension of agrobacteria transformed with the respective plasmids. The mRNA synthesis of the allergens was achieved after a successful recombination of the subgenomic promoter and allergen sequences.

Results

The recombinant allergens Bet v 1 and Ara h 2, both including a C-terminal hexa-histidine tag, were extracted from the leaves and isolated using Ni-NTA loaded beads. The identity of the purified allergens was confirmed via mass spectrometry and they were tested for obtained PTMs. The recombinant allergens were detected by both monoclonal antibodies and IgE from allergic patients' sera.

Conclusion

We showed that this plant expression system produced functional allergens and attaches PTMs. Supported by Austrian Science Fund doctoral program W1248-B30.

Saturday, January 26

Oral Abstract Presentation III - Allergens and tolerance induction
09:20 - 11:00

O09 A New Hypoallergenic Ara H 2 Mutant For Potential Use In AIT-In Vitro And In Vivo Studies

Angelika Tscheppe[1], Dieter Palmberger[2], Christian Radauer[1], Leonie S. Van Rijt[3], Merima Bublin[1], Christine Hafner[4], Wolfgang Hemmer[5], Vanessa Mayr[1], Chiara Palladino[1], Adrian Logiantara[3], Ronald Van Ree[3], Reingard Grabherr[2], Heimo Breiteneder[1]

1. Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria
2. Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
3. Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
4. Department of Dermatology, University Hospital St. Pölten, Karl Landsteiner University of Health Sciences, St. Pölten, Austria
5. Floridsdorf Allergy Center, Vienna, Austria

Keywords: Ara H 2, Hypoallergenic Mutant, In Vitro Studies, In Vivo Studies

Introduction
Derivatives of the major peanut allergen Ara h 2 with reduced IgE-binding capacities are promising candidates for allergen specific immunotherapy. Little is known about the role of conformational and linear IgE epitopes. Therefore, we aimed to define the patient-specific IgE epitope profiles.
Method
A mutant (mt) lacking most linear epitopes and the wild-type protein (wt) were expressed in the baculovirus-insect cell system. Purified allergens and the natural protein (n) were reduced and alkylated (red/alk). IgE-binding was tested by ELISA using 55 patient´ sera. Basophile activation tests (BATs) were performed with 9 Ara h 2-sensitized patients. Furthermore proteins were analyzed for their ability to interact with T-cells. To determine the anaphylactic potency of the different proteins C3H/HeJ mice were sensitized orally with nAra h 2 and challenged intraperitoneally with the various proteins.
Results
IgE-binding to red/alk Ara h 2 was reduced in a patient specific manner by up to 50% due to the loss of conformational epitopes ($p<0.001$). Likewise, IgE-binding was reduced by up to 70% when linear epitopes were mutated ($p<0.001$). Patients with high levels of Ara h 2 specific IgE tended to recognize primarily linear epitopes ($r=0.305$, $p=0.025$), while patients with low levels of Ara h 2 specific IgE recognized mainly conformational epitopes ($r=-0.5$, $p=0.0001$). BATs showed the lowest activation upon stimulation with the red/alk mutant. All proteins stimulated proliferation of T-cells from peanut allergic patients to similar levels. Mice reacted with anaphylaxis upon challenge with mt, wt and n but not with the red/alk proteins.
Conclusion
Our results indicate that both epitope types are important for IgE-binding in a patient specific manner. In contrast to previous findings, full destruction of the 3D structure is required when designing safe hypoallergens.

O010 Polymerized Allergoids Coupled To Non-Oxidized Mannan (PM) Drive Monocyte Differentiation Into Tolerogenic Dendritic Cells And Anti-Inflammatory Macrophages

Cristina Benito-Villalvilla[1], Mario Pérez[1], José Luis Subiza[2], Oscar Palomares[1]

1. Complutense University of Madrid, Madrid, Spain
2. Inmunotek, SL., Alcalá De Henares, Madrid, Spain

Keywords: Immunotherapy, Vaccine, Dendritic Cell, Macrophage, Tolerance

Introduction

Allergen-specific immunotherapy (AIT) is the single curative treatment for allergy, but it still faces problems related to efficacy, security, duration and patient compliance. Recent studies demonstrated that glutaraldehyde-polymerized grass pollen allergoids coupled to non-oxidized mannan (PM) represent next generation vaccines for AIT targeting dendritic cells (DCs) and promoting the development of forkhead box P3 (FOXP3)+ regulatory T (Treg) cells. The aim of this study has been to investigate the impact of PM over the monocyte differentiation process into DCs and macrophages (MØ).

Method

Monocytes were differentiated in the presence of IL-4 and GM-CSF to obtain human monocyte derived DCs (hmoDCs), with GM-CSF to obtain GM-MØ, or with M-CSF to obtain M-MØ. PM was added at days 0 and 4 of the differentiation to obtain PM/hmoDCs or PM/GM-MØ. The expression of surface markers and cytokine signature were determined by flow cytometry, qPCR or ELISA. Allogeneic cocultures of PM/hmoDCs and naïve CD4+ T cells were performed to analyse T cells polarization. FOXP3+ Treg cells were quantified. Blocking and pharmacological inhibition experiments were performed in PM/hmoDCs.

Results

PM promote the differentiation of human monocytes into tolerogenic DCs characterized by a significantly lower cytokine production after LPS stimulation (TNF-α, IL-6 and IL-1β), higher IL10/TNF-α, IL-10/IL-6 and IL-10/IL-1β ratios, and a higher expression of the tolerogenic molecules PDL1, IDO, SOCS3 and IL10 than hmoDCs generated in the absence of PM. PM/hmoDCs also show a higher capacity to promote the generation of FOXP3+ Treg cells than hmoDCs generated in the absence of PM. Blocking experiments suggest that the inhibition of indoleamine-2,3-dioxygenase (IDO) reduces the induction of FOXP3+ Treg cells by PM/hmoDCs. Furthermore, human macrophages differentiated in the presence of PM and GM-CSF acquired an immunosuppressive-like profile similar to the profile of M-MØ. PM/GM-MØ are characterized by a remarkable production of IL-10 after LPS stimulation and a high expression of CD163, CCL2, IL10 and CD14 macrophage markers.

Conclusion

Our results demonstrate that polymerized allergoids coupled to non-oxidized mannan modulate monocyte differentiation by promoting tolerogenic dendritic cells and anti-inflammatory macrophages, which might also well contribute to the generation of healthy immune responses to allergens induced by these next-generation vaccines.

O011 A Novel Pectate Lyase Allergen Hel A 6: Characterization And In Silico Multi-Epitope Vaccine Designing

Nandini Ghosh, Swati Gupta Bhattacharya

Bose Institute, Kolkata, India

Keywords: Pectate Lyase, Purification, Immuno-Proteomics, Cross Reactivity, In Silico Vaccine

Introduction

Pectate lyase is an important group of pollen allergens comprising 4.1% of all allergens

reported so far. They are distributed among the members of Cupressaceae and Asteraceae family. The present study is aimed to purify and characterize a novel pectate lyase allergen from sunflower (Helianthus annuus L.) and to study its cross reactivity with other pectate lyase allergens of Asteraceae family. This allergen is designated as Hel a 6 (WHO/IUIS). An in silico approach was taken to design vaccine against all pectate lyase allergens.

Method

Natural Hel a 6 was purified from sunflower pollen by anion exchange and gel filtration chromatography. The identity of the purified protein was confirmed by mass spectrometry and further by pectate lyase enzyme assay using pectin as a substrate and salicylic acid as a potential inhibitor. Enzyme activity was measured at different temperature, pH and Ca2+ concentration. Dot blot, ELISA and active histamine release assay confirmed the allergenicity of purified Hel a 6. Its secondary structure, thermal denaturation and pH stability were determined by CD spectroscopy. The cross reactivity of Hel a 6 with Amb a 1 and Art v 6 was studied by inhibition ELISA, inhibition western blot and direct histamine release upon cross sensitization by Amb a 1 and Art v 6. In silico approach was taken to identify probable cross reactive epitopes and potential multi-epitope vaccine molecule was designed.

Results

Natural Hel a 6, showing extensive homology with pectate lyase of H. annuus in MS/MS, was purified with ~95% purity. Around 63% of sunflower sensitized patients showed IgE reactivity towards Hel a 6. Patients showed highest level (50%-78%) of histamine release at 100 ng/ml of allergen. Enzyme activity was highest at 60°C, pH 8.0 and 0.2 mM Ca2+ with Vmax 0.33µM/Min and Km 0.2 µM. The protein surface showed predominance of α helical conformation. The unfolding of protein secondary structure was complete at 75°C which was partially refolded after cooling. Little change in secondary structure was observed at different pH. Inhibition ELISA showed 50% - 80% cross reactivity of Hel a 6 with Amb a 1 and Art v 6. Cross reactivity was also proved by inhibition blot and 40%-60% histamine release upon cross sensitization. Computational studies depicted 3 cross reactive epitopes which were used for in silico vaccine designing.

Conclusion

The present study characterized a novel cross reactive pectate lyase allergen from sunflower and showed a path towards vaccine designing.

O012 Development Of A Potential New Vaccine Candidate For House Dust Mite Allergen Immunotherapy By Destroying IgE-Binding While Preserving Immunogenicity Of The Major Allergen Der P 2.

Lisa Pointner[1], Heidi Hofer[1], Josef Laimer[1], Tamara Weidinger[1], Christof Ebner[2], Peter Briza[1], Peter Lackner[1], Michael Wallner[1], Fatima Ferreira[1], Michael Hauser[1]

1. Department of Biosciences, University of Salzburg, Salzburg, Austria
2. Allergie-Ambulatorium, Vienna, Austria

Introduction

Allergic reactions to house dust mites (HDM) represent the primary cause of indoor allergies in industrialized countries. Der p 2 is one of the major allergens from the European HDM Dermatophagoides pteronyssinus eliciting allergic rhinitis and asthma. To date, the only curative treatment for allergic diseases is allergen immunotherapy (AIT). However, the administration of allergen extracts frequently causes side effects and may induce new sensitizations. Researching for new safe and efficient vaccine candidates is therefore critical to avoid disadvantages accompanying AIT.

Method

A hypoallergenic variant of Der p 2 was designed by site-directed mutagenesis on the surface of the allergen. The mutant, rDer p 2-8x, was produced recombinantly in E. coli, purified to homogeneity and physicochemical properties were characterized using CD and FTIR technologies. Allergenicity of the variant was investigated by mediator release assays

with serum samples of HDM allergic donors. Additionally, the immunological behaviour in vivo was analysed by ELISpot with splenocytes of immunized mice.

Results

Results from CD and FTIR measurements showed that rDer p 2-8x displayed almost identical secondary structural elements as the recombinant wild-type (WT) allergen, rDer p 2. Moreover, rDer p 2-8x revealed a significant reduction in its IgE-binding capacity compared to rDerp 2. Finally, despite the surface mutations on the molecule, in vivo experiments demonstrated a cross-reactive IgG response, though, IgE cross-reactivity was very weak, suggesting that rDer p 2-8x is a stronger immunogen than its WT counterpart.

Conclusion

This study demonstrates that the site-directed mutagenesis strategy successfully modified the allergen to reduce its allergenicity without altering its folding or its stability and keeping its immunogenicity intact. This allergen variant could therefore be a potential interesting vaccine candidate for the treatment of dust mite allergic patients.

Saturday, January 26

Oral Abstract Presentation IV - Epithelium and microbes
17:50 - 19:30

O013 Heligmosomoides Polygyrus Infection Induces Anti-Viral Gene Expression In The Lung Epithelium And Immune Cells

Matthew Burgess[1], Amanda Mcfarlane[2], Hannah Mayr[3], Karla Berry[1], Henry Mcsorley[1], Jurgen Schwarze[1]

1. University of Edinburgh, Edinburgh, United Kingdom
2. University of Glasgow, Glasgow, United Kingdom
3. Medical University of Vienna, Vienna, Austria

Keywords: RSV-Infection, Helminths, Myeloid Cells, Type-I Interferon

Introduction
Infant respiratory viral infections are a major cause of infant hospitalisation and a risk factor in the development of persistent wheeze, airway allergic responses and ultimately asthma.
We have recently shown that ongoing infection in mice with the gut helminth Heligmosomoides polygyrus (H. polygyrus) protects against respiratory syncytial virus (RSV) infection, reducing viral load, associated immunological changes and airway impairment. This protective effect is dependent upon the induction of type-I interferons in the gut and/or the lung and the presence of normal gut microbiota.
This work now asks how a strictly enteric gut infection signals immune changes to the lung and which cells respond to this signal by the expression of interferons or interferon stimulated genes (ISGs).
Method
Naïve 8-12 week old mice were infected with 200 L3-stage H. polygyrus larvae or H2O sham infections. 10 days post infection (dpi) blood was collected by cardiac puncture and processed for serum separation. Lungs and hind limb bones were dissected and processed for histological analysis or RNA extraction.
Diluted serum was administered to naïve mice and 24 hours later mice culled for lung RNA isolation or infected with RSV. RSV infected animals were culled 4 dpi and lungs dissected for plaque assays to measure viral load.
Bone marrow from femurs and tibia were assessed for colony forming potential with Methocult colony assay plates and colonies microscopically identified and confirmed by Giemsa stain.
Results
Intravenous serum transfer from mice 10 days after H. polygyrus infection to naïve mice induced similar increases in interferon beta and ISG expression as seen in H. polygyrus infection, and reduced peak viral load after subsequent RSV infection.
This induction of anti-viral genes is observed across both the lung epithelial cells and immune populations including interferon beta positive lung macrophages. We hypothesised that this antiviral myeloid state originates systemically and found elevated interferon beta levels in the bone marrow of H. polygyrus infected animals. Furthermore, the bone marrow exhibited elevated myelopoiesis driving an increase in circulatory monocyte populations and in recruited monocytes in the lung.
Conclusion
These results suggest that during H. polygyrus infection serum borne factors induce an anti-viral state in the lung epithelium and circulatory monocytes allowing these cells to mount a rapid and protective response to RSV-infection.

O014 JAK1/3-Inhibition Preserves Epidermal Morphology In Full Thickness 3D Skin Models Of Atopic Dermatitis And Psoriasis

Karlijn Clarysse, Inge Kortekaas, Jan Gutermuth

University hospital Brussels, Jette, Belgium

Introduction
Janus kinase (JAK) inhibition may be a promising new treatment modality for inflammatory (skin) diseases. However, little is known about direct effects of kinase inhibitors on keratinocyte differentiation and function as well as skin barrier formation.
Method
3D skin equivalents of both diseases were developed and concurrently pretreated with tofacitinib. To induce AD, 3D skin equivalents were stimulated with recombinant human IL-4 and IL-13. Psoriasis-like conditions were induced by incubation with IL-17A, IL-22 and tumor necrosis factor α (TNFα). The activation of signal transducer and activator of transcription (STAT)1, STAT3 and STAT6 was assessed by western blot analysis. Microarray analysis and quantitative real-time PCR were used for gene expression analysis.
Results
Tofacitinib pretreatment preserved epidermal morphology and reduced STAT3 and -6 phosphorylation of AD-like and STAT3 phosphorylation of psoriasis-like culture conditions in 3D skin models compared to sham-controls. Filaggrin expression was fully maintained in the AD-like models, but only partially in psoriasis-like conditions after pretreatment with tofacitinib. In addition, tofacitinib upregulated DSC1, FLG and KRT1. Using gene expression analysis, downregulation of POSTN and IL24 was observed in AD-like conditions whereas downregulation of IL20 and IL1B was observed in psoriasis-like conditions.
Conclusion
JAK1/3 inhibition counteracted cytokine-induced AD- and psoriasis-like epidermal morphology and enhanced keratinocyte differentiation in 3D skin models. This effect was more pronounced in the AD-like models compared to the psoriasis-like 3D skin models.

O015 The Gut-Lung Axis Backwards: Allergic Airway Diseases Modulate The Microbial Composition In The Gut

Elke Korb[1], Katharina Ambroz[1], Mirjana Drinic[1], Christian Zwicker[1], Tatjana Svoboda[2], Murat Bagcioglu[2], Monika Ehling-Schulz[2], Buck T. Hanson[3], Craig Herbold[3], Alexander Loy[3], Stefanie Widder[4], Ursula Wiedermann[1], Irma Schabussova[1]

1. Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria
2. Institute of Microbiology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria
3. Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, Vienna, Austria
4. Department of Medicine I, Medical University of Vienna, Vienna, Austria

Keywords: Gut-Lung Axis, Microbiome, Allergic Airway Inflammation, Tolerance Induction

Introduction
In industrialized countries, the prevalence of allergic airway diseases is constantly rising. The modified microflora hypothesis suggests a link between the gut microbiome and the susceptibility to develop allergies. Bacterial dysbiosis in the gut has been shown to influence immune responses in distant organs, such as the lung. In this project we aim to determine whether this cross-talk via the gut-lung axis acts bi-directionally.
Method
We tested the impact of ovalbumin-induced allergic airway inflammation and orally-

induced allergen-specific tolerance on the composition and function of the intestinal microbiome in mice. The microbial communities in cecal and fecal samples were assessed with Fourier transform infrared spectroscopy (FT-IR) and Illumina MiSeq sequencing of 16S rRNA gene amplicons. Additionally, the metabolic pattern in serum was analyzed with hydrophilic interaction chromatography/mass spectrometry (HILIC-MS).

Results

FT-IR measurements indicated shifts in the gut microbiome of tolerized and sensitized mice compared to naïve mice. Analysis of 16S rRNA gene sequence data indicated an increase in relative abundances of the families Prevotellaceae and Ruminococcaceae, while Bacteroidaceae were decreased in feces of allergic mice compared to tolerized mice and sham controls. HILIC-MS revealed a distinct metabolite pattern in serum of sensitized mice, which exhibited reduced levels of L-carnitine and its alkylated forms compared to sham-controls.

Conclusion

Precise characterization of the mechanism of the lung-gut axis, the communication between these distant mucosal tissues and the impact of this interaction on shaping the immune system might pave the way for characterization of novel intervention strategies to prevent or treat allergic diseases.

O016 Lactobacillus Casei AMB-R2 Restores Nasal Epithelial Barrier Integrity In Chronic Rhinosinusitis By Increasing The Expression Of Tight Junctions

Katleen Martens[1], Brecht Steelant[1], Ilke De Boeck[2], Benoit Pugin[1], Sven F Seys[1], Olivier Vanderveken[3], Sarah Lebeer[2], Peter W Hellings[4]

1. KU Leuven, Leuven, Belgium
2. UAntwerpen, Antwerpen, Belgium
3. UAntwerpen - UZ Antwerpen, Antwerpen, Belgium
4. KU Leuven - UZ Leuven, Leuven, Belgium

Keywords: Lactobacilli, Chronic Rhinosinusitis, Eptihelial Integrity, Tight Junctions,

Introduction

Epithelial barrier dysfunction is demonstrated in patients with chronic rhinosinusitis with nasal polyps (CRSwNP). Lactobacilli can restore epithelial barrier dysfunction, though its effect on barrier function in CRSwNP has not been studied. In this study we wanted to evaluate the barrier restoring capacity of Lactobacillus casei AMB-R2 (AMB-R2) in CRSwNP.

Method

Sinus tissue and nasal swaps from patients with CRSwNP (n=14) were collected during functional endoscopic sinus surgery. Bacterial DNA from nasal swaps was isolated for Illumina MiSeq sequencing to determine relative abundance of lactobacilli. Sinus tissue was mounted in Ussing chambers to evaluate epithelial integrity by measuring trans-tissue resistance (TTR). Nasal epithelial cells (NECs) from controls and CRSwNP patients (n=6/group) were stimulated with AMB-R2 and epithelial integrity was evaluated by measuring trans-epithelial resistance (TER). BALBc mice (n=5/group) were endonasally pretreated with AMB-R2 prior to 3 consecutive applications of IL-4 to induce barrier dysfunction. FITC-dextran 4 kDa was applied endonasally to evaluate mucosal permeability.

Results

TTR of sinus tissue from CRSwNP patients was significantly decreased (10±0.8 vs. 32 ±4, $p<0.0001$) compared to controls. Relative abundance correlated positively with the TTR in CRSwNP patients ($r= 0.5729$; $p<0.05$). Stimulation with AMB-R2 significantly increased TER in CRSwNP cultures by increasing mRNA expression of ZO-1, occludin and claudin-4. In vivo, pretreatment with AMB-R2 prevented IL-4 induced barrier dysfunction ($p<0.01$) compared to positive control.

Conclusion

The sino-nasal epithelial barrier is disrupted in CRSwNP, which is associated with a decreased relative abundance of lactobacilli. Incubation with AMB-R2 restores nasal epithelial barrier integrity in CRSwNP in vitro and in vivo.

Saturday, January 26

Poster Session II - Topic 4: Mechanisms and treatment of food allergy
21:00 - 22:00

P027 Efficacy And Safety Of Low-Dose Oral Immunotherapy For Patients With Wheat-Induced Anaphylaxis

Ken-Ichi Nagakura, Motohiro Ebisawa

Sagamihara national hospital, Sagamihara, Japan

Keywords: Anaphylaxis, Oral Food Challenge, Oral Immunotherapy, Wheat Allergy

Introduction
Only few studies have investigated oral immunotherapy (OIT) for patients with anaphylaxis, particularly those with wheat allergy. In this study, we examined the validity of low-dose OIT for patients with anaphylactic wheat allergy.
Method
Eligible subjects were aged 5-18 years with a history of wheat anaphylaxis and confirmed reactions during oral food challenge (OFC) against 53 mg of wheat protein. In the OIT group, patients were hospitalized for a 5-day build-up phase. Patients gradually increased wheat ingestion until 53 mg/day, then ingested 53 mg of wheat protein daily at home. One year later, they underwent OFC of 53 and 400 mg after OIT cessation for 2 weeks to confirm sustained unresponsiveness (SU). Patients with symptoms after ingesting 53 mg or less and who eliminated wheat were defined as the historical control group.
Results
Sixteen subjects (median age of 6.7 years) with wheat anaphylaxis received the low-dose OIT and 11 subjects (median age of 6.4 years) were categorized into the historical control group. Median wheat- and ω-5 gliadin-specific IgE levels were 292 kUA/L and 7.5 kUA/L, respectively, in the OIT group and 42 kUA/L and 3.5 kUA/L, respectively, in the control group. Within 1 year, 88% achieved desensitization to 53 mg in the OIT group. After 1 year, 69% and 9% patients passed the 53 mg OFC and 25% and 0 achieved SU to 400 mg in the OIT and control groups, respectively ($p = 0.002$ and 0.07, respectively). (Table 1) Wheat- and ω-5 gliadin-specific IgE levels significantly decreased to 154 and 4.2 kUA/L, respectively, at 1 year, and wheat- and ω-5 gliadin-specific IgG and IgG4 levels significantly increased at 1 month in the OIT group. (Figure 1) By contrast, wheat- and ω-5 gliadin-specific IgE levels were unchanged in the historical control group. There were no adverse reactions requiring intramuscular adrenaline during OIT protocol.
Conclusion
Low-dose wheat OIT induced immunological changes and could achieve SU in patients with anaphylaxis.

	OIT group (n=16)	Historical control group (n=11)	*p* value
Desensitization to 53 mg of wheat protein	14 (87.5%)	-	-
Passing the OFC to 53 mg of wheat protein	11 (68.8%)	1 (9.1%)	0.002
SU to 400 mg of wheat protein	4 (25.0%)	0 (0%)	0.07

P028 Impact Of Mouse Feed Composition On Immune Response And Food Allergy Development

Nazanin Samadi, Eleonore weidmann, Martina Klems, Klara Seppova, Davide Ret, Eva Untersmayr

Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Keywords: Food Allergy; Diet; Mouse Chow; Experimental Mouse Model; Oral Immunizations; Polyunsaturated Fatty Acids; Soy

Introduction
Our diet is known to substantially influence the immune response not only by support of mucosal barriers, but also via direct impact on immune cell. Thus, it was of great interest to compare the immunological influence of two different mouse chows with substantial differences in micro-, macronutrient, lipid and vitamin content on the food allergic response in our previously established mouse model.

Method
As the two mouse chows of interest, we used the feed previously used in animal facilities at the Medical university of Vienna (soy + low fatty acid (FA) feed) and compared it to the mouse chow in current use (soy free + high FA feed) in an established protocol of immunizations using Ovalbumin (OVA) as a model allergen under concomitant gastric acid suppression.

Results
In the animals receiving soy + low FA feed, OVA-specific IgE, IgG1, IgG2a antibody levels were significantly enhanced in comparison to the animals receiving soy free + high FA feed. Moreover, food allergy was evidenced only in sensitized mice under soy + low FA feed by a drop of body temperature. In contrast, mice on soy free + high FA feed being protected from IgE development under OVA sensitization had significantly higher levels of IL-10.

Conclusion
In conclusion, soy + low FA feed was auxiliary during sensitizations, while soy free + high FA feed supported oral tolerance development and food allergy prevention.

P029 In Vitro And In Vivo Evaluation Of A Combination Of Dietary ScGOS:LcFOS And N-3 PUFA In Prevention Of Cow's Milk Allergy

Kirsten Szklany[1], Aletta D. Kraneveld[2], Veronica Ayechu-Muruzabal[1], Mara Diks[1], Johan Garssen[3], Leon M. J. Knippels[3]

1. Division of Pharmacology, Utrecht Institute of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
2. Division of Pharmacology, Utrecht Institute of Pharmaceutical Sciences, Faculty of Science, Utrecht University and Institute for Risk Assessment Sciences, Faculty of Veterinary Sciences, Utrecht University, Utrecht, The Netherlands
3. Division of Pharmacology, Utrecht Institute of Pharmaceutical Sciences, Faculty of Science, Utrecht University and Danone Nutricia Research, Utrecht, The Netherlands

Keywords: Cows Milk Allergy, Dietary Intervention, Prebiotics, Omega-3 Fatty Acids,

Introduction
A specific mixture of prebiotics short-chain galacto- and long-chain fructo-oligosaccharides (GF) and omega-3 poly unsaturated fatty acids (PUFA) like eicosapentaenoic acid (EPA) and docohexaenoic acid (DHA) have immunomodulatory capacities and are shown to reduce allergic symptoms in a cow's milk allergy (CMA) model. We evaluated

immunomodulatory effects of a combination of GF and PUFA in an intestinal epithelial cell (IEC)–peripheral blood mononuclear cells (PBMC) co-culture model and assessed the effects in vivo.

Method

Human IEC were co-cultured with anti-CD3/CD28 activated PBMCs and exposed for 24hr to 0,5% GF (9:1), 2μM EPA, 10μM DHA and combinations hereof in presence of the TLR9 ligand CpG. IFNγ, TNFα, IL13 and IL10 levels were measured in the supernatant. C3H/HeOuJ mice received control or enriched diet with 1% GF (9:1), 6% PUFA or 1% GF and 6% PUFA combined. Mice were sensitized to cow's milk whey protein. Clinical parameters were measured, and isolated splenic lymphocytes were antigen-specifically stimulated to assess IL5, IL13 and IFNγ responses.

Results

IFNγ and IL10 levels in the IEC-PBMC co-culture were unaffected by individual components, however, GF and EPA, increased significantly the IFNγ and IL10 levels compared with CpG only. The IL13 and TNFα levels were unaffected by the tested conditions. In vivo, body temperature of allergic mice decreased significantly after intradermal challenge. Only in GF-treated mice the temperature was significantly higher compared with allergic control mice. Also, the temperature was significantly higher in GF-treated compared with the combination of GF and PUFA-treated mice. The basal cytokine release by splenocytes was not significantly different between the groups. The whey-specific IL13, IL5 and IFNγ levels in supernatant of re-stimulated splenic lymphocytes from allergic mice were significantly increased compared with sham-sensitized mice. GF-treated mice showed decreased cytokine responses upon whey restimulation. PUFA alone or combined with GF did not affect the re-stimulated cytokine responses.

Conclusion

In vitro, the combination of GF and EPA induced a Th1 cytokine response, suggesting that GF combined with PUFAs might have a preventive effect in allergy management. In vivo data suggests that combined GF and PUFAs had no additional preventive effect on measured clinical parameters and immune profile, only GF showed significant effects on preventing the drop in temperature and reducing the whey-specific response in splenocytes.

P030 Jug R 6 Is The Allergenic Vicilin Present In Walnut Kernels Responsible For IgE Cross-Reactivities To Other Tree Nuts And Seeds

Pawel Dubiela[1], Stefan Kabasser[1], Nicolas Smargiasso[2], Sabine Geiselhart[1], Merima Bublin[1], Christine Hafner[3], Gabriel Mazzucchelli[2], Karin Hoffmann-Sommergruber[1]

1. Medical University of Vienna, Vienna, Austria
2. University of Liege, Liege, Belgium
3. University Hospital St. Poelten, St. Poelten, Austria

Keywords: CRD, Jug R 6, Novel Food Allergen, Proteomic, Walnut Allergy

Introduction

Walnuts like other tree nuts are ranked high in the list of the culprit foods inducing severe allergic reactions. Jug r 2 has been identified as a major allergen in common walnut by cDNA cloning from a somatic cell line. So far studies were performed on the allergenic activity of recombinant Jug r 2, yet there is still no evidence about the physicochemical characteristics of the natural allergen. Therefore, we aimed to purify and deeply characterize natural Jug r 2 and to assess IgE cross-reactivity among vicilins from different tree nuts and seeds.

Method

Vicilin was purified from walnut kernels and characterized by highly sensitive mass spectrometry based methods. In parallel, recombinant Jug r 2 was expressed in Pichia pastoris. The entire mass of purified protein was identified by MALDI-TOF and ESI-TOF/orbitrap mass spectrometry. Optimized multi-enzymatic digestion was applied for

extensive protein characterization including post-translational modifications analysis and de novo sequencing. Secondary structure was assessed by CD spectroscopy and the IgE binding activity of vicilin was tested in ELISA and Western Blot using sera from 77 walnut allergic patients. Level of cross-reactivity between detected allergen and selected homologues was assessed by inhibition ELISA.

Results

Extensive mass spectrometry analysis of the purified vicilin provided a protein mass of 47.1–48.8 kDa and allowed identification of the protein sequence that displayed only 44% identity to Jug r 2. The newly identified vicilin was designated by IUIS committee as Jug r 6. Sequence analysis revealed typical for vicilin two cupin domains and high sequence identity with homologues from hazelnut, Cor a 11 (72%), sesame seeds, Ses i 3 (60%) and pistachio, Pis v 3 (54%). Jug r 6 is represented in the native state as a complex trimeric protein and is composed of a mixed population of alfa-helices and beta-sheets Allergen was recognized by IgE of 26% in walnut allergic patients' sera tested. In contrast to Jug r 2, Jug r 6 displayed a remarkable level of cross-reactivity when tested with homologues from hazelnut, sesame and pistachio.

Conclusion

This is the first report on the purification of walnut vicilin from kernels, designated Jug r 6. Our data also provide evidence that Jug r 6 is involved in the cross-reactivities among tree nuts and seeds.

P031 Household Exposure To Food Allergens: A Risk For Sensitization?

Izabel Alvares[1], Max Bermingham[1], Martin D Chapman[2], James Hindley[3]

1. Indoor Biotechnologies LTD, Cardiff, United Kingdom
2. Indoor Biotechnologies Inc, Charlottesville, United States
3. Indoor Biotechnologies LTD, Virginia, United Kingdom

Keywords: Dust, Household, Food, Exposure

Introduction

Rationale: Exposure to food allergens is a pre-requisite to the development of food allergy. It is not fully understood what levels of exposure to allergens or what route of exposure is most important for allergic sensitization. Food allergens present within household dust may contribute to allergic sensitization of individuals susceptible to food allergies. We sought to determine the precise levels of specific food allergens in household dust and establish their stability in the environment.

Method

Method: To determine which allergens were present, settled dust samples were collected from houses within Europe. To determine stability, a single stock dust was created by combining and homogenizing settled dust samples from houses in the UK. This stock dust was divided and stored at three temperatures; room temperature (21oC), 4oC and -20oC. Fractions of the dust were subsequently extracted at six timepoints leading up to a year and immediately stored at -20oC. Seven allergens were simultaneously quantified using a highly sensitive multiplex array for allergens from peanut (Ara h 3 and Ara h 6), milk (Bos d 5), egg (Gal d 2), hazelnut (Cor a 9), cashew (Ana o 3) and shrimp (tropomyosin).

Results

Results: Each of the allergens assessed were readily found within household dust. Major allergens from egg (Ga ld 2) and milk (Bos d 5) were found to be the most abundant, with levels as high as 275µg allergen/gram dust. The least abundant food allergen was Cor a 9. The stability analysis showed that Bos d 5 was the sole allergen to drop in levels by three-fold at room temperature, the other six allergens showing to be remarkably stable across the one-year period.

Conclusion

Conclusions: Allergens present in household dust are within the same range as those known to cause sensitization to common indoor allergens. Milk and egg are especially

prominent exposures. These findings suggest that household dust may be an important source of food allergen sensitization. With the exception of Bos d 5, the allergens remain stable in the environment over a one-year period and thus possibly still effectual after this time.

P032 Functional And Phenotypic Analysis Of Allergen-Specific B Cells In Cow's Milk Allergy And Tolerance

Pattraporn - Satitsuksanoa[1], Willem - Van De Veen[1], Kari - Nadeau[2], Mübeccel - Akdis[1]

1. Swiss Institute of Allergy and Asthma Research (SIAF), Davos Platz, Switzerland
2. Stanford University, California, United States

Keywords: Allergen-Specific B Cells, Alpha S 1 Casein, Cow's Milk, Food Allergy, IgG4

Introduction

Abstract
Background: The prevalence of food allergy is an increasing public health concern affecting millions of people worldwide. Besides their role in the production of allergen-inducing IgE antibodies, allergen-specific B cells may play a role in the induction of allergen tolerance. This study examines the role of B cells in cow's milk allergy. B cells specific for the major cow's milk allergen, αS1-casein were purified from allergic and healthy individuals for subsequent analysis of their immunoglobulins.

Method

Methods: Peripheral blood mononuclear cells (PBMC) from cow's milk allergic donors were isolated and allergen-specific B cells were identified and purified using dual-color staining with fluorescently labeled αS1-casein allergen by flow cytometry. αS1-casein specific B cells were immortalized through transduction with a retroviral vector containing GFP, BCL6, and Bcl-xL and expanded by culturing with CD40L and IL-21. Total and specific IgE, IgG and IgG subclass (IgG1and IgG4) antibodies from culture supernatants of immortalized B cells were measured by ELISA.

Results

Results: αS1-casein specific B cells and non-specific B cells were successfully purified and immortalized. Specific IgE, IgG1, and IgG4 production from culture supernatants of αS1-casein positive B cells were significantly elevated compared to αS1-casein negative cells, while total IgE, IgG1, and IgG4 levels were comparable.

Conclusion

Conclusions: This study is focused on the characterization of allergen-specific B cells in cow's milk allergen. We have successfully established a method for the purification and immortalization of αS1-casein specific B cells. This paves the way for in-depth analysis of these cells in healthy, allergic and allergen-immunotherapy-treated individuals in terms of gene expression (using RNAseq), as well as detailed analysis of the antibody repertoire and potential isolation of αS1-casein-specific antibodies that may protect against allergies through neutralization of IgE-allergen interaction.

P033 Titanium dioxide nanoparticles may prevent food allergy and anaphylaxis development

Natalia Aliakhnovich[1], Denise Heiden[2], Martina Klems[2], Dmitrij Novikov[1], Eva Untersmayr[2]

1. Department of Clinical Immunology and Allergology, Vitebsk State Medical University, Vitebsk, Belarus
2. Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Keywords: Titanium Dioxide, Food Allergy, Anaphylaxis, Interleukin 10

Introduction
Titanium dioxide (TiO2) is a widely used white food pigment and often added to pharmaceutical products and cosmetics entering the human body in particles with 30-300 nm diameter size (average intake 0,5-1,1 mg/kg bodyweight/day in adults, 1,4-3,2 mg/kg bodyweight/day in children).
Method
To analyze the intestinal and systemic effects of TiO2-NPs on food allergy and anaphylaxis development, female BALB/c mice were fed TiO2-NPs with or without pre-absorption to Bovine serum albumin (TiO2-NPs and TiO2-NPs+BSA) or BSA alone for 14 days. Thereafter, mice were sensitized to the egg allergen Ovalbumin (OVA) under concomitant acid-suppression.
Results
After pretreatment, immunization with OVA lead to increases of total IgA levels in intestinal lavages only in mice fed with pure TiO2-NPs compared to the groups fed with TiO2-NPs+BSA or pure BSA ($p<0.01$, $p<0.05$). Moreover, higher titers of OVA specific IgE and IgA antibodies were observed in intestinal lavages of sensitized animals pretreated with TiO2-NPs or with BSA compared to naïve mice ($p<0.05$), but not in animals pretreated with TiO2-NPs+BSA.
Systemic allergen challenges only in mice after pretreatment with TiO2-NPs or with BSA, induced a significant drop of body temperature 10 minutes after challenge compared to naïve mice ($p<0.05$). In line, we revealed higher levels of mMCP1 in serum. Unstimulated splenocytes of mice fed with TiO2-NPs+BSA secreted significantly more IL-10 compared to splenocytes of naive mice ($p<0.05$), while stimulation of splenocytes with ConA induced comparable levels of IL-10 in all mouse groups.
Conclusion
These data indicate that binding of TiO2-NPs to proteins can change the immunogenic characteristics of TiO2 and influence allergy and anaphylaxis development.
This work was supported by an EAACI Research fellowship award (to NA) and by Austria science fund grants KLI284 and WKP039 (to EU).

P034 Unprocessed Cow's Milk Suppresses Allergic Symptoms In A Murine Model For Food Allergy – A Potential Role For Epigenetics

Suzanne Abbring[1], Veronica Ayechu Muruzabal[1], Mara A P Diks[1], Bilal Alashkar Alhamwe[2], Fahd Alhamdan[2], Hani Harb[2], Ton Baars[3], Harald Renz[2], Holger Garn[2], Johan Garssen[1], Daniel P Potaczek[2], Betty C A M Van Esch[1]

1. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
2. Institute of Laboratory Medicine, member of the German Centre for Lung Research (DZL), Philipps-Universität Marburg, Marburg, Germany
3. Research Institute of Organic Agriculture (FiBL), Frick, Switzerland

Keywords: Epigenetics, Farming Effect, Food Allergy, Milk Processing, Raw Milk

Introduction
Epidemiological studies have shown an inverse relation between unprocessed cow's milk consumption and the development of asthma and allergies. This protective effect seemed to be abolished by milk processing. Previously, we confirmed the epidemiological findings on asthma by showing causality. In the present study, we investigated whether unprocessed cow's milk is also protective in a murine model for food allergy. Besides, we looked at possible changes in histone acetylation to investigate the involvement of epigenetic regulation.

Method
C3H/HeOuJ mice were sensitized intragastrically (i.g.) once a week for five weeks with ovalbumin (OVA) using cholera toxin (CT) as an adjuvant (d0, 7, 14, 21, 28). Prior to sensitization, mice were orally treated with unprocessed milk, processed milk or PBS (as control) for eight consecutive days (d-9 to -2). Five days after the last sensitization (d33), mice were challenged intradermally (i.d.) in the ear with OVA to determine acute allergic symptoms. On the same day, mice were challenged i.g. with OVA. Eighteen hours after the i.g. challenge mice were killed and organs were obtained for ex vivo analysis (d34). In addition, epigenetic modifications in Th1-, Th2- and regulatory T cell-related genes of splenocyte derived CD4+ T cells were analyzed after milk treatment (d-1) as well as at the end of the study (d34).

Results
OVA sensitized mice receiving unprocessed milk showed decreased allergic symptoms compared to sensitized mice receiving PBS. The acute allergic skin response and anaphylactic shock symptoms were reduced and the body temperature remained high. OVA-specific IgE levels were also decreased. These protective effects were not observed when sensitized mice received processed milk. Looking at epigenetic modifications, unprocessed milk exposure for eight days led to higher acetylation of Th1-, Th2- and regulatory T cell-related genes of CD4+ T cells compared to processed milk (d-1). At the end of the study (d34) this general immunostimulation was resolved and acetylation of Th2 genes was lower compared to processed milk.

Conclusion
Unprocessed cow's milk reduces allergic symptoms in a murine model for food allergy. This protective effect was not observed after exposure to processed milk. The general immunostimulation in the spleen after exposure to unprocessed milk could be responsible for the observed tolerance induction, suggesting that epigenetic mechanisms contribute to the allergy protective effect of unprocessed cow's milk.

Saturday, January 26

Poster Session II - Topic 5: Respiratory allergies and asthma
21:00 - 22:00

P035 Association Between Circulating And Mucosal Associated Type 2 Innate Lymphoid Cells In Patients With Asthma

Lobna Abdelaziz El-Korashi, Huda Fathy Ebian, Rehab Hosni El-Sokary, Ahmed Mohammed El-Gebaly, Nadia Mohsen El-Akabawy, Nelly Said Abdelrahman, Lamiaa Gaber Zake

Zagazig University, Zagazig, Egypt

Keywords: Innate Lymphoid Cells, Asthma

Introduction
Emerging evidence has shown that Type 2 Innate lymphoid cells (ILC2), on guard at mucosal sites, are importantly involved in the pathogenesis and development of a variety of intestinal and lung diseases, i.e., helminth infections, allergic airway inflammation, and airway hyper-responsiveness. ILC2 preferentially localize to the interface between the host and the environment (lung, intestine, skin), and this strategical localization allow them to represent a critical link between the innate and adaptive components of type 2 immunity. Although, ILC2 was studied in asthma, the activation dynamic and the role of ILC2 in the lung remains poorly characterized. Therefore, we aimed to study the correlation between activated ILC2 in the gut, peripheral blood, and sputum of patients with asthma. We also investigate the association between the ILC2 of and asthma severity.
Method
Multicolor flow cytometry was used to enumerate blood and sputum ILC2 (Lin- CD45+ CD127+) and intracellular level of IL-5 in adult patients with asthma (n=27) and apparently healthy controls (n=27). Immunohistochemistry for gut biopsies from both these asthmatics and healthy individuals was done to characterize their gut derived ILC2.
Results
ILC2 were significantly expanded in severe asthmatic patients, compared to healthy individuals and mild asthmatics (P= 0.001; 0.04 respectively). the peripheral blood ILC2, were positively correlated with asthma severity (r = 0.468, P=0.02). Moreover, the percentage of peripheral blood, sputum, gut ILC2 were positively correlated with each other.
Conclusion
Our data showed that blood, sputum and gut derived ILC2s in asthmatic patients were positively correlated with each other and also correlated with asthma severity.

P036 Association Of CD14 Rs2569190 Polymorphism With Perennial Allergic Rhinitis In The Population Of Kiev, Ukraine

Taras Baranovskyi, Taras Baranovskyi

Bogomolets National Medical University, Kiev, Ukraine

Keywords: Perennial Allergic Rhinitis,CD14 Rs2569190, 'Hygienic Hypothesis',SNP

Introduction
According to the concept of 'hygienic hypothesis', the CD14 receptor plays an important role in the balance between Th1 and Th2, which affects IgE response. Previous studies have shown that the single nucleotide polymorphism detected at position −159 in the

promoter region of the CD14 gene (rs2569190) is associated with asthma and allergic rhinitis in various ethnic populations.

Method

There was studied CD14 rs2569190 polymorphism of CD14 receptor gene in 93 patients with perennial allergic rhinitis. The control group included 90 non-atopic volunteers. Single-nucleotide polymorphism of -159C/T was detected by allele-specific PCR. Patients and volunteers were recruited at the Bogomolets National Medical University, Kiev, Ukraine and provided written informed consent for the genetic study.

Results

In the control group, the frequency distribution of genotypes (C–20(22.2%), CT–48 (53.3%), TT–22(24.5%)) was significantly different from perennial allergic rhinitis (CC–40(43.0%), CT–35(37.6%), TT–18(19.4%), $\chi 2=6.20$, $p=0.013$) phenotypes. The risk analysis for the T allele ([CC]<->[CT+TT]) showed that the frequency of the genotype CT+TT in patients with perennial allergic rhinitis (57.0%) was significantly lower (OR=0.379, CI=[0.199-0.721], $\chi 2=8.97$, $p=0.003$) compare to control group (77,8 %).

Conclusion

The CD14 rs2569190 polymorphism is associated with perennial allergic rhinitis in the Kiev population of Ukraine.

P037 Glucocorticoid Signaling In Different Asthma Phenotypes – A Potential Role For MRNA-MiRNA Interactions

Julie Weidner, Carina Malmhäll, Linda Ekerljung, Emma Winberg, Kristina Johansson, Madeleine Rådinger

Krefting Research Centre, University of Gothenburg, Gothenburg, Sweden

Keywords: MicroRNA, Asthma, Peripheral Blood Mononuclear Cells, Eosinophils, Gene Expression

Introduction

Asthma is a heterogeneous disease that affects over 300 million people world-wide. The disease is often characterized by phenotypes such as blood eosinophil numbers or allergic status and one of the most common treatments for asthma is through the use of inhaled corticosteroids (ICS). In recent years, the complexity of the disease has become apparent and there are a growing number of endotypes described. Gene expression can be regulated on many levels and one class of posttranscriptional regulatory molecules are microRNAs (miRNAs). In our study we asked whether ICS affected miRNA expression in blood cells from allergic (AA) and non-allergic asthmatic (NAA) individuals. Additionally, we aimed to determine if different asthma phenotypes exhibited alterations in the glucocorticoid signaling pathway.

Method

Twenty-six individuals (Healthy=4, AA+ICS=5, AA-ICS=6, NAA high eosinophil=6, NAA low eosinophil=5) were recruited and peripheral blood mononuclear cells (PBMCs) and eosinophils were isolated from whole blood. Total RNA was isolated from both cell populations, reverse transcribed and subjected to qPCR analysis for miRNA expression or for examination of gene expression via a glucocorticoid signaling gene array.

Results

miR-155, miR-146a, and miR-126 showed overall higher expression in PBMCs, whereas expression of miR-223, miR-135 and miR-374a were increased in eosinophils. Interestingly, gene array analysis of PBMCs derived from the NAA subjects with low eosinophil levels (≤0.2x 109 eosinophils/L) exhibited a distinct profile compared to any of the other asthmatic group. In addition, NAA and AA subjects on ICS clustered more closely than those without ICS and, moreover, certain genes appeared to be up and down regulated in an asthma specific manner.

Conclusion

Through the use of a well-defined clinical cohort, we have identified miRNA signatures, in

two distinct blood cell populations. Furthermore, we have examined expression changes in the glucocorticoid signaling pathway and found distinct differences between healthy controls and different asthmatic groups, suggesting varied levels of regulation among the diseased individuals. Future studies will focus on identifying mRNA targets for the miRNAs composing the asthmatic signatures. Together these data will lead to a more thorough, mechanistic understanding of the complex regulation of gene expression in asthmatic cells.

P038 The Role Of Sphingosine-1-Phosphate In The Pathogenesis Of Asthma

Thomas James Aidan Maguire, Grzegorz Woszczek

King's College London, London, United Kingdom

Keywords: Asthma, Sphingosine-1-Phosphate, Airway Smooth Muscle

Introduction

Sphingosine-1-phosphate (S1P) is a key signalling lipid in the immune response and is involved in immune cell trafficking between blood and tissue, and pathogenesis of inflammatory diseases, such as asthma. Several observations suggest that S1P may affect the three key phenotypic features of asthma: airway remodelling, airway hyperresponsiveness, and bronchoconstriction. Previous data have shown that after allergen challenge, S1P is found at higher levels in the bronchoalveolar lavage of asthmatics than in healthy subjects. Previous data have shown expression of three of the S1P receptors (S1PR1, S1PR2, and S1PR3) on primary Airway Smooth Muscle (ASM) cells. Additionally, gene array data has shown S1P to induce a pro-remodelling phenotype in ASM. In this project we aimed to analyse the role of S1P in mediating ASM proliferation and airway contraction.

Method

ASM cells were isolated from bronchial biopsies from healthy and asthmatic subjects and proliferation assessed via 3H-Thymidine incorporation assays. Gene expression was assessed by qPCR. Bronchioles were isolated ex vivo from human lung fragments and healthy C57BL/6 mice and wire myography used to investigate contraction in response to contractile agonists and S1P.

Results

S1P caused a significant concentration-dependent increase in human ASM proliferation. Using novel agonists and antagonists specific for the S1P receptors, we have identified that S1PR3, signalling through intracellular calcium mobilisation, acts as the major receptor in the pro-remodelling proliferative response in human airways. Via qPCR, Sphingosine Kinase 1 was shown as significantly upregulated in this response. Preliminary myography data from isolated ex vivo healthy mouse bronchioles suggests that preincubation with S1P leads to increased airway hyperresponsiveness, with increased contraction in response to carbachol and other ASM-specific agonists after S1P treatment. Investigations are currently ongoing to confirm the role of S1P in bronchoconstriction and airway hyperresponsiveness in ex vivo human airways, with preliminary data suggesting a role in both.

Conclusion

S1P plays a role in airway remodelling and smooth muscle hyperplasia, directly inducing ASM cell proliferation via the S1PR3 receptor. In healthy mice, S1P does not directly cause airway constriction, but increases airway hyperresponsiveness. Ongoing investigations will also confirm if S1P is involved in contraction and hyperresponsiveness in human airways, as preliminary data suggests.

P039 MicroRNA Expression In Cytokine Stimulated Airway Epithelial Cells And Their Involvement In Asthma Related Pathways

Elisabeth Ax[1], Zala Rojnik[2], Julie Weidner[3], Cecilia Lässer[3], Henric Olsson[2], Madeleine Rådinger[3]

1. AstraZeneca R&D Gothenburg/University of Gothenburg, Gothenburg, Sweden
2. AstraZeneca R&D Gothenburg, Gothenburg, Sweden
3. University of Gothenburg, Gothenburg, Sweden

Keywords: Asthma, Epithelial, MicroRNA, TGFb, Remodeling

Introduction

Data from large cohorts strongly supports heterogeneity in asthma with the presence of overlapping phenotypes affected by different signaling pathways. Different -omics approaches (e.g. genomics, transcriptomics, proteomics) have been used stratify asthmatic patients and to understand the main disease drivers. microRNAs (miRNAs), a class of small (~22 nt) non-coding RNAs regulating mRNA translation, have been found to be differentially expressed in asthmatic compared to healthy individuals. The aim of this study was to identify miRNAs regulated by disease-relevant cytokines in airway epithelial cells and to investigate their mechanistic role in driving pathophysiological changes.

Method

Primary human bronchial epithelial cells cultured at air-liquid interface were stimulated with different cytokines for 24h or left untreated. Small RNA species (<200 nt) were purified from cell lysates, sequenced using Next-Generation Sequencing (NGS) and the reads were mapped to miRBase. Differential expression for each miRNA was calculated by relating normalized read counts for each stimuli to that of the non-stimulated control. mRNA targets of miRNAs and pathways affected were identified using Ingenuity Pathway Analysis and mirPath v.3. Target mRNA expression was analysed using data from NGS performed on the large RNA fraction from the same experiment.

Results

Cluster analysis of the NGS data revealed groups of miRNAs differently affected by the stimuli applied. Under all stimulation conditions, effects were seen on adherens, tight and gap junctions as well as focal adhesion and TGFb signaling by pathway analysis. We then compared our results to miRNAs previously identified as differentially regulated in asthma versus healthy and found that direct TGFb1 stimulation of epithelial cells could recapitulate those findings, thus strengthening our pathway analysis. Additionally, mRNA targets most strongly affected by TGFb1 are involved in pathways related to remodeling (junction signaling, EMT and Wnt/β-catenin signaling) and the PTEN/PI3K pathway.

Conclusion

Inflammatory cytokines alter airway epithelial cell miRNA expression that most likely affect pathways related to remodeling, which is a feature of asthma. TGFb signaling, both indirectly and directly induced, could be a driver of these changes and induces miRNA expression patterns aligned with those seen in asthmatics.

P040 Clinical And Immunological Approaches Between The Obesity Asthma Phenotype

Marina Bantulà Fonts

IDIBAPS - Hospital Clínic, Barcelona, Spain

Keywords: Asthma, Obesity, Lymphocytes, Inflammation

Introduction

Epidemiologic studies have suggested that obesity increases asthma incidence and is

associated with a reduced asthma-related quality of life, more frequent exacerbations, and a decreased response to asthma medication such as corticosteroids, however, the possible mechanisms remain uncertain. We hypothesized that the poor response to glucocorticoid (GC) treatment in obese asthma patients is due to alterations in the normal functioning of the GC receptor, resulting from the metabolic syndrome and the abnormal systemic and/or pulmonary inflammatory process associated to obesity. Moreover, vitamin D deficiency has been associated with obesity and poor asthma control. Furthermore, reduced response to GC could be reversed by vitamin D intake in vivo and in vitro studies.

Method

Determine the clinical, inflammatory and functional characteristics of severe obese subjects with asthma before and after bariatric surgery. Study the in vitro suppression of PHA-induced CD4+ T cell proliferation by dexamethasone and the effects from vitamin D3 addition in CD4+ T cell cultures from obese and/or asthmatic subjects. Flow cytometry analysis of T cell populations and cytokines present in each subject.

Results

We evaluated severe (BMI ≥40 kg/m2) and moderate (BMI ≥35 kg/m2) obese asthmatics patients (OA), before bariatric surgery (n=18 [14 female]; age: 57 yr; FEV1: 77,5%; BMI: 38,5kg/m2). We also evaluated a group of non-obese asthmatics patients (A) (n=3 [2 female]; age: 45 yr; FEV1: 86%; BMI: 23,6kg/m2), a group of obese non-asthmatics patients (O) (n=6 [6 female]; age: 48 yr; FEV1: 96%; BMI: 43,6kg/m2) and a group of healthy subjects (C) (n=9 [6 female]; age: 41 yr; FEV1: 96%; BMI: 24,4kg/m2). We observed that CD4+ T cell proliferation was suppressed in vitro by dexamethasone in a dose-dependent manner in all studied groups. OA group shown a trend toward a lower GC sensitivity compared to healthy subjects (IC50 OA: 33,51 nM; IC50 C: 24,98 nM, respectively). Moreover, when we added vitamin D to GC treatment we found a significant reduction in the IC50 in the OA group (IC50 VitD OA: 21,4 nM) ($p<0,007$) and in healthy subjects (IC50 VitD C: 12,61 nM) ($p<0,007$).

Conclusion

Obese asthma patients differ from healthy subjects in pulmonary characteristics and inflammatory profile. The OA group presented GC insensitivity in vitro, this could be due to some alterations in the normal functioning of the GC receptor, and suggest a possible mechanism of the poor response to GC treatment in these patients.

P041 House Dust Mite (HDM) Allergen Induces Type 2 Inflammation In A Novel Experimental Asthma Model In Guinea Pig

Patricia Ramos-Ramírez[1], Malin Noreby[1], Jielu Liu[1], Jie Ji[2], Suado Abdillahi[2], Henric K. Olsson[2], Gunnar Nilsson[1], Sven-Erik Dahlén[1], Mikael Adner[1]

1. Karolinska Institutet, Stockholm, Sweden
2. Astra Zeneca, Mölndal, Sweden

Keywords: Bronchoconstriction, Airway Hyperresponsiveness, Eosinophils, Asthma Model, Remodeling

Introduction

Animal models have been extensively used to study the mechanisms underlying asthma as well as potential therapeutic agents. However, there is not a model that closely resembles the pathophysiology of asthma. Guinea pigs share several anatomical, physiological and pharmacological features with human airways, including bronchoconstriction induced upon allergen provocation. The aim of the study was to develop an alternative model of allergic asthma in the guinea pig using house dust mite (HDM) as a clinically relevant allergen.

Method

Guinea pigs were sensitized intranasally twice with HDM extract. Allergen challenges were performed once per week for five weeks and the allergen-induced bronchoconstriction was monitored by whole-body plethysmography giving Penh recordings. Lung function was

assessed 24h after the last challenge using forced oscillation in the flexiVent™ system. Cellular content of bronchoalveolar lavage fluid (BALF) was determined in May Grünwald-Giemsa stained cytospins and type 2 cytokines were quantified in cell-free BALF by ELISA. HDM-specific immunoglobulins were measured in serum and lung tissue was evaluated by conventional histological analysis. Control animals were sensitized and challenged with PBS.

Results

HDM-sensitized guinea pigs exhibited a significant increase in the baseline Penh values by more than 200% upon every successive challenge. Further assessing the lung mechanics with flexiVent™ revealed a marked airway reactivity, expressed as the decrease in PD200 to methacholine challenge, in HDM-sensitized guinea pigs. Besides, eosinophilic airway inflammation and serum allergen-specific IgG1 and IgG2 were found in HDM-treated guinea pigs. Type 2 cytokines IL-4 and IL-13 levels were significantly elevated in HDM-treated guinea pigs whereas IL-5 remain unchanged. Histologic examination demonstrated that HDM exposure induces large areas of infiltrating inflammatory cells, as well as an increase of the subepithelial collagen deposition, mast cells and goblet cell hyperplasia in guinea pig airways.

Conclusion

Repeated intranasal exposure to HDM induces an asthma-like model in guinea pigs where type 2 inflammation might drive the asthma features of the early allergic reaction, airway hyperresponsiveness, and remodeling.

P042 "CHARACTERISTICS OF PATIENTS ADMITTED TO EMERGENCY DEPARTMENT FOR ASTHMA ATTACK: A REAL-LIFE STUDY"

Monica Fornero

Ospedale Umberto I, Torino, Italy

Keywords: Asthma, Emergency Room

Introduction

The aim of the study was to examine the characteristics of adult patients admitted to ED of the general hospital of a 90,000 inhabitants town of South Italy, with a diagnosis of acute asthma attack, focusing on previous diagnosis of asthma and current asthma therapy.

Method

After aquiring a written signed consent a structured questionnaire, assessing previous asthma diagnosis and management, was administered to all patients admitted to ED for acute asthma in one year period.

Data on oxygen saturation, heart and respiratory rate, severity code admission at ED, and hospitalization or ED discharge, had been obtained by chart review.

Results

201 patients (126 male), mean age 50.3 years (range 12-65), had been enrolled. Only 118 patients (58.7%) had received a previous diagnosis of asthma (DA), the others were confirmed asthmatic patients after a functional respirstory test and a pneumologist/allergist consultance . In DA patients, the diagnosis had been made 17.5 ± 5.88 years before, and 35.6% of them had a specialist examination in the last 12 months. Allergic rhinitis was reported by 54% of DA and 6% of patients without previous diagnosis of asthma (UA) ($p < 0.001$). Self-medication before the ED access, consisting in short-acting beta-2 agonist (90%) and oral corticosteroids (1%), had been used by 53.3% of DA patients, even if none of them had a written asthma action plan (WAAP). Almost all DA patients (112/118) were on regular therapy, consisting in inhaled corticosteroids (ICS) in 61% of patients, associated with LABA in 85% of them. Previous ED access during the last 12 months was reported by 16.7% of DA patients. The hospitalization rate was 39% (78/201) for the whole study population, significantly higher in DA compared to UA patients (64 and 36% respectively, p=0,017). Multivariate analysis showed that significant risk factors for hospitalization were the oxygen saturation lower than 94% breathing

ambient air (OR 9.91, p<0.001), the inability to complete a sentence (OR 9.42, p<0.001) and the age of the patients (OR 1.02, p= 0.049).

Conclusion

Despite guidelines recommendation about asthma diagnosis and treatment, up to 40% of patients presenting to the ED received the diagnosis of asthma for the first time, and only 61% of DA patients were receiving regular ICS treatment. Moreover, it is disappointing that none of the patients had a WAAP. This could explain why only 53% of the patients used self-administered medication before their admission at ED.

P043 Protocol For Randomized, Outcome Assessor Blinded, Clinical Study: The ATOM Study: Asthma Severity In Women: The Influence Of Training And Menopause

Erik Sören Halvard Hansen[1], Morten Hostrup[2], Hanne Rasmusen[3], Ylva Hellsten[2], Vibeke Backer[1]

1. Respiratory Research Unit, Bispebjerg Hospital, Copenhagen, Denmark
2. Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark
3. Department of Cardiology, Bispebjerg Hospital, Copenhagen, Denmark

Keywords: Late-Onset Asthma, Physical Exercise, ACQ

Introduction

Late-onset asthma in women is characterized by poor disease control and reduced quality of life despite intensive treatment with inhaled steroid and beta2-agonist. The condition is further worsened at menopause due to the loss of estrogen leading to increased asthma exacerbation frequency, increased airway inflammation and decreased lung function. Exercise training may increase disease control in asthma patients, but to what extent the same effect is seen in postmenopausal women with late-onset asthma is unknown. These patients represent a phenotype that is characterized by low eosinophilic airway inflammation, severe symptoms, moderate obesity and poor response to conventional medicine. Thus, our hypothesis is that regular physical exercise is especially associated with an improvement in asthma control in this phenotype. The aim of this project is to test this hypothesis and to assess whether an improvement is associated with reduced local and systemic inflammation.

Method

40 postmenopausal women with late-onset asthma are recruited via the outpatient clinic at the Respiratory Department at Bispebjerg Hospital and through advertisement. The participants are randomized 1:1 into two groups. One group performs supervised exercise training (spinning) three times per week for 12 weeks while the other group is a control group. Before and after the intervention asthma control, local and systemic inflammation, heart function and body composition is examined.

Results

Analysis will be performed to detect changes within and between the groups before and after intervention. Primary outcome is change in ACQ (Asthma Control Questionnaire). Local and systemic inflammation is measured by changes in bronchial challenge to methacholine, blood samples and analysis of IL-4/6/8/17 and TNF-alpha in sputum and serum. Furthermore, secondary outcomes include change in heart function measured by stress-echocardiography and change in body composition measured by Dual-energy X-ray absorptiometry (DEXA).

Conclusion

There are to date no prospective studies that can support recommendations containing asthma rehabilitation with supervised regular physical activity for postmenopausal women. Thus, this study will provide novel understanding of the impact of regular physical exercise on both objective and subjective parameters in postmenopausal women suffering from asthma.

Saturday, January 26

Poster Session II - Topic 6: Allergy treatment and immunomodulation
21:00 - 22:00

P044 Safety Of Subcutaneous Allergen Immunotherapy In Seniors- 2 Year Observational Study

Piotr Lacwik[1], Malgorzata Bochenska- Marciniak[2], Piotr Kuna[3], Maciej Kupczyk[1]

1. Medical University of Lodz, Lodz, Poland
2. Department of Internal Medicine, Lodz, Poland
3. Asthma and Allergy, Lodz, Poland

Keywords: SCIT, Adverse Events, Allergen Immunotherapy

Introduction
Allergen immunotherapy (AIT) has been proven to be an effective treatment of allergic diseases in numerous studies. However, its use in the aged population remains limited and questionable, due to common comorbidities and limited evidence of efficacy and safety of AIT in aging population.
The aim of presented study was to assess the safety of AIT in patients over 55 years of age undergoing subcutaneous immunotherapy (SCIT) and analyze the potential risk factors of adverse reactions in this population, compared to younger adults.
Method
We followed subcutaneous immunotherapy in a group of 1302 patients treated in the outpatient clinic of Medical University of Lodz, of whom 163 were aged 55 and older (118 between the age of 55-60, 31 aged 61-65 and 14 patients above the age of 65) . We recorded detailed information of each administration and corresponding adverse reactions over the period of 2 years. We compiled results of our observations with hospital records to compile a database, which we then analyzed using statistical software.
Results
568 patients (43,6%) experienced at least one adverse reaction, local or systemic, after SCIT. We observed no significant difference in AE occurrence between adult (under 55 years of age) and senior group (44,1% and 30,5% respectively). Further analysis showed that while both groups had similar per-patient incidence of local reactions (41,9% vs 40,5%), we observed noticeably fewer systemic events in seniors (10,4% vs 14,7 %, p= 0,047). Interestingly, while less common, systemic AEs appeared to be more severe in the aged population, with as many as 4,9% of the group having experienced WAO grade 2 reactions, compared to 2,4% in young adult group. No grade 3 or 4 reactions were observed in the course of the study.
While older patients had significantly more comorbidities, multivariate statistical analysis of potential risk factors did not reveal any difference between groups, with both adults and seniors being more likely to experience AEs during immunotherapy with house dust mite compared to other allergens, as well as under treatment with native allergen extracts compared to allergoids.
Conclusion
While numerous comorbidities and concomitant medications may promote more intense adverse events in patients of 55 years and above, we observed no severe reactions in this aged population during a 2 year observation period. Our results suggest that allergen immunotherapy in the elderly is safe and well tolerated.

P046 Therapeutic Effects Of Mesenchymal Stem Cells In An Animal Model Of Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis

In Young Oh, Tae-Bum Kim

Asan Medical Center, Seoul, South Korea

Keywords: Stevens-Johnson Syndrome; Toxic Epidermal Necrolysis; Drug Adverse Reactions; Mesenchymal Stem Cells; Stem Cell Therapy; NOG Mouse; Humanized Mouse; SJS/TEN Animal Models

Introduction

Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are very rare, but extremely severe diseases affecting the skin and mucous membranes, with high mortality rates and the potential for permanent sequelae. The aims of this study were to develop a mouse model of SJS/TEN and verify the therapeutic effects of mesenchymal stem cells (MSCs) in this model.

Method

For this animal model, immunocompromised NOD/Shi-scid IL-2Rγnull (NOG) mice were used. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of a patient with SJS (F, 45 y) and injected intravenously (1.5×106 cells/ea) into mice, which were then administered the drug Lamotrigine. MSCs (2.0×106 cells/ea) derived from umbilical cord blood were transferred into the mice in the therapeutic group.

Results

The eyeballs of the negative control mice [no treatment (#1; PBMC-drug-MSCs-), PBMC-only injected (#2; PBMC+drug-MSCs-), and drug-only treated (#3; PBMC-drug+MSCs-)] were normal for the assessed eye elements. However, the disease model mice administered PBMCs and drug without MSCs (#6; PBMC+drug+MSCs-) displayed damage to the shape of the cornea, limbus, nuclear layers, and eyelids. Eyes from mice receiving PBMCs, drug, and MSCs (#4 and 5; PBMC+drug+MSCs+) were comparable to those of the negative control groups (#1, #2, and #3) due to the therapeutic effects of the MSCs.

Conclusion

This is the first study demonstrating that intravenous injection of MSCs is a potential therapeutic candidate for SJS/TEN, based on the recovery of circumocular structures after treatment. A possible mechanism for SJS/TEN was strongly suggested through this research. This is an important landmark study supporting the need for further MSC studies focusing on treatments for SJS/TEN caused by different drugs.

P047 The Role Of Allergen-Specific IgG Antibodies In The Induction Of Clinical Tolerance For The Birch Pollen-Related Apple Allergy

Gabriela Sánchez Acosta[1], Tamar Kinaciyan[2], Christian Möbs[3], Wolfgang Pfützner[3], Barbara Bohle[1]

1. Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria
2. Department of Dermatology, Medical University of Vienna, Vienna, Austria
3. Clinical & Experimental Allergology, Department of Dermatology and Allergology, Philipps University Marburg, Marburg, Germany

Keywords: Birch Pollen-Related Apple Allergy, Sublingual Immunotherapy, IgG Antibodies

Introduction

Birch-pollen-related apple allergy [BPRAA] is one of the most prevalent food allergies in adults and results from immunological cross-reactivity between the major birch-pollen allergen Betv1 and its apple-homolog Mald1. Interestingly, birch pollen-immunotherapy

has no convincing benefits on BPRAA. We recently showed in a double-blind placebo-controlled pilot study that 16 weeks of sublingual immunotherapy with recombinant [r] Mald1 [rMald1-SLIT] significantly improved BPRAA, whereas rBetv1-SLIT did not. To investigate the immune mechanisms underlying the induction of clinical tolerance to apple we characterized the levels, blocking capacity, and primary specificity of SLIT-induced Mald1-specific IgG antibodies [Abs].

Method

Serum levels of Mald1-specific IgG subclasses were measured by ELISA and ImmunoCAP, respectively. The presence of IgE-blocking Abs in post-SLIT sera was evaluated as their ability to inhibit rMald1-induced activation of basophils from apple-allergic donors. IgG1 and IgG4 Abs were depleted from post-SLIT sera samples to investigate their contribution to IgE blocking. The primary specificity of Mald1-specific IgG4 Abs was assessed by pre-incubating post-SLIT samples with titrated concentrations of either rBetv1 or rMald1 in a competition ELISA.

Results

Mald1-specific IgG1, IgG2 and IgG4 significantly increased during rMald1- and rBetv1-SLIT. Production of Mald1-specific IgG3 was only induced by rBetv1-SLIT. Mald1-induced basophil activation was only inhibited by post-rMald1-SLIT sera, and our preliminary data suggest that this blocking potential was mediated by both IgG4 and IgG1 Abs. Pre-incubation of post-rMald1-SLIT sera with rMald1 but not with Betv1 completely abrogated IgG4-binding to rMald1. Pre-incubation of post-Betv1-SLIT sera with rMald1 and rBetv1 completely abrogated IgG4-binding to rMald1, suggesting that these Abs bind to common cross-reactive epitopes on both allergens.

Conclusion

Both Mald1- and Betv1-SLIT induced Mald1-specific IgG Abs. However, only post-Mald1-SLIT sera prevented Mald1-induced mediator release and contained IgG4 Abs primarily specific for Mald1. We conclude that Mald1-SLIT induces highly specific Mald1-specific Abs with blocking activity, mediating clinical tolerance to apples.

P048 Mapping The Antibody Specificity In Birch-Related Soy Allergic Patients Before And After Allergen Immunotherapy

Lisbeth Ramirez Caballero

Fraunhofer IZI, Leipzig, Germany

Keywords: Peptide Phage Display. Allergen Immunotherapy, Birch-Related Food Allergy,

Introduction

Patients allergic to birch pollen are often sensitized to a variety of foods, mainly due to the homology between Bet v 1, the major allergen in birch pollen, and other allergens contained in food such as Gly m 4 (soybean). Treatment with Bet v 1 was expected to improve the quality of life of patients with birch pollen-related soy allergy during the clinical trial BASALIT (Birch Associated Soy Allergy and ImmunoTherapy, EudraCT-Nr.: 2009-011737-27). Two groups of patients were treated with either a recombinant hypoallergenic variant of the major birch allergen Bet v 1 or placebo. Although no significant differences were observed in the patient's improvement of quality of life, a deeper analysis of the specificity of the antibodies elicited after treatment might give an explanation of the different outcomes observed during the clinical study.

Method

Serum collected from BASALIT patients were used in two rounds of peptide phage display experiments involving a highly diverse, 16mer random library with almost even amino acid distribution. DNA encoding peptides from the selected clones were sequenced using illumina MiSeq®. The B-cell epitope profiles before and after immunotherapy were determined in silico by comparing the allergen's 4mer motifs statistical values of the phagemid pools and the naïve library. Epitope profiles are currently being used to

complement the information provided by the primary endpoints of the clinical study, in order to re-evaluate the effects of allergen immunotherapy in BASALIT patients.

Results

Preliminary results comprise the epitope profiles of Bet v 1 and Gly m 4 before and after allergen immunotherapy in BASALIT patients. Significant changes were detected in the specificity of antibodies elicited after treatment; however its relationship with the success/failure of the treatment with recombinant Bet v 1 is still unclear.

Conclusion

Personalized IgE and IgG4 epitope profiles during the course of allergen-immunotherapy might lead to the discovery of diagnostic and/or therapeutic peptides and would also give a broader insight into B-cell mediated response to allergen immunotherapy.

P049 Tolerance Induction By Prophylactic Epicutaneous Allergen-Specific Immunotherapy In A Preclinical Model Of Hymenoptera Venom-Sensitized Mice

Mathias Schuppe[1], Christopher Kiselmann[2], Dorota Dobler[3], Anja Wacker[4], Oliver Schmidt[4], Frank Runkel[5], Thomas Schmidts[3], Wolfgang Pfützner[6], Christian Möbs[6]

1. Philipps University Marburg, Marburg, Germany
2. University of Applied Sciences Mittelhessen, Gießen, Germany
3. Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Gießen, Germany
4. Engelhard Arzneimittel GmbH & Co. KG, Niederdorfelden, Germany
5. Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Faculty of Biology and Chemistry, Justus-Liebig-Universität Gießen, Gießen, Germany
6. Clinical & Experimental Allergology, Department of Dermatology and Allergology, Philipps-Universität Marburg, Marburg, Germany

Keywords: Wasp, Ves V 5, Epicutaneous Immunotherapy, Mouse

Introduction

Allergy to Hymenoptera venom (HV) is the second most common cause of IgE-mediated anaphylaxis. While subcutaneous HV-specific immunotherapy (HV-IT) shows high efficacy in inducing allergen tolerance, it is associated with potential severe systemic reactions. Utilizing an HV-allergic mouse model, we investigated whether epicutaneous HV-IT represents a safe and effective therapeutic alternative. Mice sensitized to one of the major allergens of either honeybee venom, Api m 1, or wasp venom, Ves v 5, were treated with different topically applied concentrations of the respective allergen and both clinical outcome and immunological changes were assessed.

Method

Balb/c mice were topically treated for 4 weeks with different concentrations of Api m 1 (0, 0.25, 0.625, and 1mg/ml) or Ves v 5 (0 and 1mg/ml), solved in either PBS or microemulsion (ME). Subsequently, mice were sensitized intraperitoneally (i.p.) by 3 separate injections of 5µg allergen and then challenged by an i.p. injection of 100µg Api m 1 or 150µg Ves v 5, respectively. Tolerance was assessed by measurement of rectal temperature. Allergen-specific IgE and IgG serum concentrations were determined by ELISA, and, T cell subsets from peripheral blood samples or isolated from lymph nodes and spleen one day after challenging were analyzed by either flow cytometry or ELISpot analysis.

Results

Mice receiving HV-IT with allergen doses solved in PBS showed a maximum rectal temperature drop of up to 5°C. In contrast, prophylactic treatment with allergen in ME, which was well-tolerated, led to a marked reduction in temperature drop and a substantially faster recovery in a dose-dependent manner. This was associated with increased production of allergen-specific IgG antibodies. Of note, no significant changes in

frequencies of allergen-specific IL-5-, IL-10- and IFN-γ-secreting T cells as well as Foxp3+ regulatory T cells were noticed.

Conclusion

Epicutaneous HV-IT shows high efficacy preventing anaphylaxis in mice sensitized with either Api m 1 or Ves v 5. Tolerance induction was dependent on both the dose and formulation of allergen, and most likely due to the induction of allergen-specific IgG antibodies, while T cellular effects seem to be of less importance . Thus, epicutaneous IT might present a promising alternative to establish allergen tolerance in patients suffering from HV-allergy.

P050 Assessment Of The Potential Anti-Inflammatory Effect Of Phytocannabinoids In An In Vitro Mouse Inflammatory Model System

Noémi Miltner[1], Johanna Mihály[1], Raphael Mechoulam[2], Tamás Bíró[1]

1. University of Debrecen, Department of Immunology, Faculty of Medicine, Debrecen, Hungary
2. Phytecs Ltd., Los Angeles, United States

Keywords: Inflammation, Phytocannabinoids, Terpene, Mouse Model

Introduction

Cannabidiol (CBD) is the most abundant non-psychotropic phytocannabinoid present in the plant Cannabis sativa. The sesquiterpene beta-caryophyllene (BCP) is a major essential oil of many different spice and food plants like rosemary and it can be found also in Cannabis sativa. Our group previously demonstrated that BCP, CBD and its fluorinated CBD derivatives (F-CBDs) exert anti-inflammatory effects in human dermatitis models. In our current experiments, we aimed at assessing the potential anti-inflammatory effect of BCP, CBD and F-CBDs in a murine macrophage inflammation model.

Method

The effect of BCP, CBD and semi-synthetic F-CBDs (HUF-101, HUF-103, HU-559a) on cell viability of RAW 264.7 murine macrophages and RAW-Blue reporter cells was investigated by colorimetric MTT assay. Gene expression of pro-inflammatory cytokines was assessed by RT-qPCR while secreted embryonic alkaline phosphatase (SEAP) activity measurement was performed by Quanti-Blue assay on RAW-Blue reporter cells.

Results

The 10 μM concentration of HUF-101 reduced the viability of RAW 264.7 cells after 24h, while the viability of RAW-Blue cells was reduced when 30 μM CBD and 300 μM BCP was applied for 24h, however long term (72 h) cell viability was not influenced. BCP and CBD decreased mRNA expression levels of Il-1a, Il-1b pro-inflammatory cytokines in an LPS-induced in vitro inflammatory mouse model however the F-CBDs exhibited significantly higher efficacy than the non-fluorinated, plant-derived CBDs.

SEAP activity was reduced by all applied concentrations of CBD but not BCP on RAW-Blue reporter cells.

Conclusion

Our study provides the first evidence that BCP and CBD exerted anti-inflammatory actions on RAW 264.7 mouse macrophages while fluorinated semi-synthetic phytocannabinoids proved to be more effective than the non-fluorinated, plant-derived CBDs in an in vitro pro-inflammatory LPS-model system.

Subject to further testing these data invite further pre-clinical and clinical studies to exploit the therapeutic potential of certain compounds in various inflammatory diseases.

Sunday, January 27

Oral Abstract Presentation V – Innate immunity
09:20 - 11:00

O017 House Dust Mite Drives Pro-Inflammatory Eicosanoid Reprogramming And Macrophage Effector Functions

Fiona Henkel[1], Antonie Friedl[1], Mark Haid[2], Carsten Schmidt-Weber[2], Jerzy Adamski[2], Julia Esser - Von Bieren[1]

1. Zentrum Allergie und Umwelt, München, Germany
2. Helmholtz Zentrum München, München, Germany

Keywords: House Dust Mite, Eicosanoids, LC-MS/MS, Type 2 Inflammation

Introduction
Eicosanoid lipid mediators play key roles in type 2 immune responses, e.g. in allergy and asthma. Macrophages represent major producers of eicosanoids and they are key effector cells of type 2 immunity. We aimed to comprehensively track eicosanoid profiles during type 2 immune responses to house dust mite (HDM) or helminth infection and to identify mechanisms and functions of eicosanoid reprogramming in human macrophages.
Method
We established an LC-MS/MS workflow for the quantification of 52 oxylipins to track mediator reprogramming in human monocyte derived macrophages (MDM) during exposure to HDM or during nematode infection in mice. Expression of eicosanoid enzymes was studied by qPCR and western blot while cytokine production was assessed via multiplex assays.
Results
Differentiation of macrophages with GM-CSF and TGFβ1 resulted in a phenotype ("aMDM") with features of airway macrophages such as high expression of 5-lipoxygenase (5-LOX). Exposure of aMDM to HDM suppressed 5-LOX expression and product formation, while triggering prostanoid (thromboxane and prostaglandin D2 and E2) production. This eicosanoid reprogramming was p38-dependent, but Dectin-2-independent. HDM also induced pro-inflammatory cytokine production, but reduced granulocyte recruitment by aMDM. Nematode infection in mice induced similar eicosanoid profiles as HDM, characterized by high levels of prostanoids, but suppressed 5-LOX metabolism.
Conclusion
Our findings show that type 2 immune responses are characterized by a fundamental reprogramming of the lipid mediator metabolism with macrophages representing particularly plastic responder cells. Targeting mediator reprogramming in airway macrophages may represent a viable approach to regulate pathogenic lipid mediator profiles in allergy or asthma.

O018 Induction Of In Vitro And In Vivo Cross-Tolerance In Birch Pollen Allergic Patients With Associated Food Allergy By Human Tolerogenic IL-10 Modulated Dendritic Cells

Patricia Vanessa Rostan[1], Edith Graulich[1], Verena Katharina Raker[1], Andrea Wangorsch[2], Robert Ose[3], Iris Bellinghausen[4], Stephan Scheurer[2], Kerstin Steinbrink[1]

1. University University Medical Center of the Johannes Gutenberg-University, Department of Dermatology, Division for Experimental and Translational Research, Mainz, Germany
2. Paul-Ehrlich-Institut, Molecular Allergology, Langen, Germany

3. University University Medical Center of the Johannes Gutenberg-University, Department of Dermatology, Mainz, Germany
4. University University Medical Center of the Johannes Gutenberg-University, Department of Dermatology, Mainz, Germany

Keywords: Pollen-Associated Food Allergy, Dendritic Cell, Regulatory T Cell, Tolerance Induction,

Introduction
Type I allergies, including pollen-associated food allergies, affect the quality of life of an increasing number of patients. Due to structural homologies between the associated allergens, 70 % of patients allergic to birch pollen (Bet v 1 (Bet)) develop a secondary allergy towards at least one food allergen (e.g. hazelnut [Cor a 1] (Cor)). Standard specific immunotherapies for type I allergies have severe side effects and mostly do not influence the secondary food allergies. Thus, there is a high need for developing novel therapies. We previously showed that human IL-10 treated tolerogenic dendritic cells (IL-10 DC) induce anergic regulatory T cells (iTreg) with strong suppressive activity.
Method
We investigated IL-10 DC from allergic patients with birch pollen and cross-reactive hazelnut allergies in regard to their potential to induce allergen-specific and cross-tolerance in vitro and in vivo. We generated unspecific and Bet-specific iTreg by coculture of CD4+ T cells from allergic patients with syngenic unloaded and Bet-loaded IL-10 DC, respectively. For analysis of specific T cell proliferation and suppressive activity, iTreg were either restimulated in vitro with Bet- or Cor-loaded mature DC (mDC) or employed in in vitro suppressor assays together with responder T cells and allergen-loaded mDC as antigen-presenting cells. To verify their suppressive capacity in vivo, Bet-specific iTreg were also applied in a humanized mouse model of allergy using immunodeficient mice reconstituted with human PBMC from allergic patients.
Results
In contrast to unspecific iTreg being anergic during primary culture and restimulation, Bet-specific iTreg displayed the anergic phenotype only during primary culture, but did strongly proliferate after restimulation with Bet-loaded mDC. Nevertheless, Bet-specific iTreg were able to suppress Bet- and Cor-induced proliferation of syngenic responder T cells in vitro, demonstrating their capacity to induce cross-tolerance in type I allergies. Intriguingly, using a humanized mouse model, we confirmed the inhibition of birch-mediated allergic immune reactions by Bet-specific iTreg in vivo.
Conclusion
Phase 1 trials demonstrated that administration of autologous tolerogenic DC in patients with various diseases is extremely safe without severe adverse effects. Based on our study, we consider human allergen-specific tolerogenic IL-10 DC as potential candidates for (cross-) tolerance-inducing cellular therapies in pollen-and pollen-associated food allergies.

O019 Neutrophils Promote Allergic Inflammation By Presenting Allergen To Specific CD4+ T-Cells

Dominika Polak, Nazanin Samadi, Caterina Vizzardelli, Gabriela Sanchez-Acosta, Sandra Rosskopf, Peter Steinberger, Barbara Bohle

Medical University of Vienna, Vienna, Austria

Keywords: Neutrophils, T-Cells, Late-Phase Allergy, Antigen Presenting Cells

Introduction
Recently, we provided evidence that neutrophils may serve as antigen-presenting cells (APC) in allergic late phase reactions (LPR). However, despite upregulating HLA-DR,

cytokine-activated neutrophils did not express significant levels of CD80 and CD86, both primary ligands for CD28. T-cell receptor (TCR) signalling in the absence of CD28-costimulation has been demonstrated to result in clonal anergy. Anergic T-cells poorly respond to specific TCR stimulation even in the presence of costimulation. Here we investigated whether neutrophils induced clonal anergy in allergen-specific CD4+ T-cells.

Method

Allergen-specific T-cell cultures were expanded from the peripheral blood of allergic individuals and stimulated in two steps: First, synthetic 12mer peptides containing the respective T-cell epitopes without APC or allergen plus autologous PBMC or allergen plus cytokine-activated neutrophils were added for 48 h. Then cells were washed, rested for 72 h, and re-stimulated with allergen and autologous PBMC. After 48 h proliferative responses were assessed. Cytokine-activated neutrophils were tested for expression of various costimulatory molecules by flow cytometry. Neutralizing anti-CD58, anti-CD28 Ab or isotype controls were added to proliferation tests. The T-cell-stimulatory activity of neutrophils was investigated in non-obese diabetic severe combined immunodeficient γc-/- (NSG) mice engrafted with PBMC from allergic donors.

Results

Stimulation of T-cells with 12mer peptides containing their T-cell epitopes induced anergy. T-cells stimulated with allergen-pulsed neutrophils showed similar responses to specific re-challenge as compared to initial stimulation with allergen-pulsed PBMC. Neutrophils constitutively expressed CD58 and the addition of anti-CD58 antibodies resulted in markedly reduced proliferative responses of T-cells to specific stimulation by neutrophils. The injection of cytokine-activated neutrophils into engrafted NSG mice prior to intranasal allergen challenge significantly exacerbated allergen-induced airway inflammation.

Conclusion

Neutrophils do not induce anergy in allergen-specific effector T-cells because they involve CD58 as costimulatory molecule. Consequently, their antigen-presenting capacity rather promotes than suppresses T-cell-mediated inflammation in allergic diseases.

O020 Reprogramming Of Lipid Mediator Metabolism Determines Macrophage „training" During Type 2 Immune Responses

Antonie Friedl[1], Dominique Thomas[2], Aurélien Trompette[3], Marta De Los Reyes Jiménez[1], Pascal Haimerl[1], Benjamin Marsland[4], Julia Esser-Von Bieren[1]

1. Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany
2. pharmazentrum frankfurt/ZAFES, Institute of Clinical Pharmacology, Goethe University Frankfurt, Frankfurt, Germany
3. Faculty of Biology and Medicine, University of Lausanne, Service de Pneumologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
4. Department of Immunology and Pathology, Monash University, Clayton, Australia

Keywords: Macrophage, Allergy, Eicosanoids, Type 2 Immunity

Introduction

Macrophages represent important airway janitors but also matter in pulmonary immunity. During type 1 immune responses (e.g. bacterial infections), macrophages can be epigenetically reprogrammed into a state of 'trained innate immunity' so that in case of a second contact, their response can be either enhanced or tolerized. Arachidonic acid (AA) derived eicosanoid lipid mediators can be dysregulated in asthma and allergy. However, how AA metabolism and inflammatory capacity of macrophages are reprogrammed during type 2 immune responses remains unknown.

Method

Bone marrow cells from mice sensitized or not to Dermatophagoides (house dust mite, HDM) were differentiated to bone marrow-derived macrophages (BMDM) and monocytes from human volunteers to alveolar-like monocyte-derived macrophages (aMDM). For

training experiments, aMDM or BMDM were exposed to HDM on the day of isolation and after 1 week. Supernatants were analyzed by LC-MS/MS (eicosanoids), multiplex cytokine assays or ELISA (cytokines). BMDM from WT or PTGS2-/- pulsed for 24h with a helminth extract (HpbE) were transferred intranasaly to HDM-sensitized mice during 4 consecutive challenge days. BALF and lung histology samples were collected 18h after the last challenge.

Results

BMDM from HDM-challenged mice produced more PGE2 and PGD2 and expressed higher levels of the PGE2-producing terminal synthase mPGES1 than control BMDM. In vitro previously HDM-exposed, trained aMDM produced more PGE2, TXB2, LTB4 and 5-HETE as well as IL-6, TNFα and IL-8 than control macrophages. Western blotting showed upregulation of cyclooxygenase-2 (COX2) in response to HDM in trained and control macrophages but mPGES1 was more abundant in previously HDM-exposed macrophages. Intranasal transfer of HpbE-pulsed BMDM during HDM challenge diminished airway inflammation and eosinophilia while the effect was lost with HpbE-pulsed PTGS2-/- BMDM.

Conclusion

Allergic airway inflammation altered the ability of macrophages to produce eicosanoids upon in vitro re-exposure to allergen. The enhanced capacity of allergen-trained macrophages to produce eicosanoids and cytokines could contribute to chronic airway inflammation. On the other hand, macrophages pre-treated with immunomodulatory helminth products reduced allergic inflammation in a COX2 dependent manner. Macrophages and their AA-metabolism are thus interesting therapeutic targets in type 2-inflammatory diseases.