Friday 26 January 2018
Oral abstract presentations
09:20 - 11:00 Lymphocytes

O01 The Role Of ILC2s And TH Cells In Allergen-Induced Bone Marrow Eosinophilia

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Keywords: HDM, IL-33, ILC2, ST2, Rag1 KO

Introduction
Eosinophilia is a hallmark of allergic airway inflammation. Eosinophils develop from progenitor cells in the bone marrow under the influence of interleukin (IL)-5. We have recently identified that type 2 innate lymphoid cells (ILC2s), but not T helper (TH) cells, support eosinophil development by producing IL-5 locally in the bone marrow in an in vivo model of IL-33-driven eosinophilia. IL-33 deficient mice have demonstrated impaired airway eosinophilia in response to house dust mite (HDM) allergen. However, the importance of IL-33 in the bone marrow has not been investigated in allergen-induced eosinophilic inflammation. Therefore, in the current study, we wanted to test the hypothesis that HDM-induced eosinophil development requires IL-33-responsive effector cells such as ILC2s and TH cells locally in the bone marrow.

Method
Wild type (WT) mice were sensitized and exposed to HDM by intranasal administration. WT and Rag1 knockout (KO) mice received intranasal doses of recombinant IL-33. Flow cytometry was used to analyze mature eosinophils (CD45+SSChiCD34-IL5RαloCCR3hi), progenitors (CD45+SSCloCD34+), ILC2s (CD45+SSCloLin-IL2Rα+IL7Rα+) and TH cells (CD45+SSCloCD3+CD4+) in the bone marrow compartment. Expression of the IL-33 receptor (ST2) was evaluated on ILC2s and TH cells.

Results
Preliminary data from Rag1 KO mice support our previous finding that ILC2s, but not TH cells, drive IL-33-induced eosinophil development in the bone marrow. Rag1 KO mice that lack T and B cells, demonstrated increased eosinophil numbers in bone marrow and airways in response to IL-33 challenge similar to WT mice. Elevated numbers of progenitors and mature eosinophils were found in the bone marrow of HDM-challenged WT mice, which was accompanied by eosinophil infiltration of the airways. In contrast to IL-33-challenged WT mice where TH cells decreased in the bone marrow, HDM-challenge resulted in increased ST2+ TH cells. Furthermore, while ST2 increased on ILC2s in IL-33-challenged mice, the ST2 expression on ILC2s was downregulated in bone marrow of HDM-challenged mice.

Conclusion
Allergen-induced eosinophilic inflammation involves IL-33-responsive TH cells that may support eosinophil production locally in the bone marrow. However, IL-33-responsive ILC2s support bone marrow eosinophilia in response to IL-33, suggesting distinct mechanism in allergen-dependent and allergen-independent eosinophilia.

O02 In Vitro Generated T Follicular Helper-Like Cells Reveal Distinct Signalling Pathways Required For B Helper Cell Function

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Keywords: TFH, Antibody Immunity, In Vitro Culture, Human Research.

Introduction

T follicular helper cells (TFH) are critical for antibody mediated immunity. TFH regulate the quantity, quality and type of antibody generated. TFH are primarily found within secondary lymphoid organs, where they interact with germinal centre B cells, guiding their maturation into antibody secreting plasma cells or long lasting memory B cells. The signals that regulate the differentiation and function of TFH have been extensively studied in murine models, but are less clear in humans.

Method

We have developed a 5 day differentiation protocol, which uses naïve CD4 T cells isolated from the peripheral blood of healthy volunteers to generate TFH-like cells. Following differentiation, phenotypic changes were observed by flow cytometry and qPCR. These cells were used in an autologous T:B cell co-culture system, the outcome of which was characterised by flow cytometry and IgG supernatant ELISA.

Results

Here we have found that a specific combination of cytokine and co-stimulatory signals is sufficient to generate TFH-like cells (in vitro TFH). These cells were phenotypically similar to tonsillar TFH, showing both key surface protein (CXCR5, PD1, and ICOS) and transcription factor (Bcl6) upregulation. These in vitro TFH also demonstrated functional capacity, being able to promote antibody secretion in vitro. As with in vivo TFH, in vitro TFH required constant TCR stimulation for phenotype maintenance. Further analysis revealed that cytokine families acting primarily via the STAT, Smad or Myd88 pathways controlled distinct elements of TFH differentiation and acted in concert to promote full functionality.

Conclusion

These results, for the first time, demonstrate the signalling requirements necessary to generate functional human TFH-like cells in vitro. This provides insight into their differentiation in vivo and should provide a useful tool in the exploration of human TFH maintenance and function.

O03 Role Of Tim-3/Gal-9 Axis In The Regulation Of T-Cell Homeostasis In Drug Induced Maculopapular Exanthema.

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Keywords: Tim-3, Gal-9, Homeostasis

Introduction

The involvement of lymphocytes with a Th1-phenotype has been reported in maculopapular exanthema (EMP) induced by drugs. Nevertheless, the involvement of other lymphocyte subtypes like Th17-cells, found in other skin diseases such as psoriasis, has not been studied. Moreover, it has been described the interaction of the receptor Tim-3 and its ligand Gal-9 as an important regulatory pathway, involved in the homeostasis of both cellular subtypes, inducing their apoptosis. The main purpose of the study is to analyse the role of the Tim-3/Gal-9 axis in the development of drug induced EMP.

Method

Peripheral blood cells (PBMC) were obtained from 18 allergic patients during the acute and remission phase of the reaction, and from 10 tolerant subjects. Flow cytometry were carried out to determine the presence of Th1 (CXCR3+), Th17 (RORgt+IL17+) CD4-cells and T-reg cells (CD4+CD25+IL10+). The expression of Tim-3 was assessed in the different cell subpopulations, as well as the expression of Gal-9 in T-reg cells.

Results

Higher levels of Th1-CD4-cells were observed in EMP compared to controls during the acute phase (p=0.032) and after resolution (p=0.039). However, any difference was found in the frequencies of Th17-cells between patients and controls. Lower percentages of T-reg cells were found during the acute phase and after resolution (p=0.0002 for both) compared to controls. The expression of Tim-3 in Th1-CD4-cells was decreased in patients during the acute phase (p<0.0001), but also after resolution (p=0.0025). Interestingly, the expression of Tim-3 in Th17-cells was similar in patients and controls. The expression of Gal-9 in T-reg cells were also decreased in patients during the acute phase (p<0.0115) and after resolution (p<0.0001).

Conclusion

Th1 are the main effector cells involved during the development of EMP. The low expression of Tim-3 and the decreased frequency of T-reg cells in these patients, as well as the low expression of Gal-9 could be the responsible of the impaired regulation of effector cells in EMP. This regulatory axis could be a target to develop future strategies to control or reduce the effects of these type of adverse reactions to drugs.

O04 Single Cell Analysis Of The Effect Of Dexamethasone On The Transcriptome Of Ex Vivo Isolated T Helper 2 Cells

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Keywords: Asthma, Novel Transcripts, Single Cell RNA-Seq

Introduction

The effect of glucocorticoids, a key treatment in asthma, on the transcriptome of bulk Th2 cell populations is well characterised by the use of gene expression arrays and RNA-seq. However, these bulk populations can be superficially homogeneous and contain a diverse range of cells with differing transcriptomes. Single cell analysis, while significantly more labour-intensive to analyze, has the opportunity to identify novel transcripts that are not the features of arrays and has a more accurate quantification of absolute expression levels and fold change. The advantages over bulk RNA-seq include evaluation of population heterogeneity and differential responsiveness to exogenous stimuli both of which is masked by bulk cell data.

Method

The Fluidigm C1 was used to capture individual cells of ex vivo isolated IL-13 positive Th2 cells that were treated with or without 10-7 M dexamethasone (Dex) in the presence of IL-2 for 6 hours. Dex-treated cells were cultured with a fluorescently conjugated CD45 antibody to differentiate them from the non-treated cells. Following isolation, RNA was prepared from individual cells followed by cDNA synthesis, linker ligation and amplification. Libraries were sequenced using a paired-end protocol on a HiSeq3000 sequencer. Analysis was conducted by aligning the libraries to the human genome using STAR alignment and HT-seq. Low-quality cells were then removed from the analysis. We compared the data generated with that of similar experiments analyzed using the Illumina HT12v4 arrays.

Results

Consistent with array data there was an increase in the treated cells of glucocorticoid-responsive genes such as TXNIP and GILZ. However, the transcriptional profile was not uniform across all cells with both the treated and non-treated cells showing intra-population heterogeneity. Single-cell RNA-seq data also revealed novel transcripts such as at the locus of the obligate Th2 cell master transcription factor GATA3. There was also evidence for preferential transcriptional initiation start site usage at the TSC22D3 locus that encodes the transcriptional regulator GILZ.

Conclusion

Single cell RNA-seq reveals the complexity of RNA transcription within a given cell, encompassing

alternative start site usage, splicing and termination. The second major asset of the technique is the identification of cellular heterogeneity within previously considered homogeneous cell populations.

Friday 26 January 2018

Oral abstract presentations

17:50 - 19:30 Innate immunity and epithelial cells

O05 Effect Of The Carbohydrate A10 (Ca10) From Ehrlich Tumor Cells During The Differentiation Of Human Monocyte-Derived Dendritic Cells

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Keywords: Carbohydrate A10, Ehrlich Tumor, Dendritic Cells

Introduction

Alterations in cell surface glycosylation pattern is a common feature of tumor cells that might be related to immune evasion and malignancy. The carbohydrate A10 (Ca10) is located in the surface of murine Ehrlich tumor (ET) cells and also in certain human adenocarcinomas, however, its immunomodulatory role remains fully elusive. The aim of this work is to study the effect of Ca10 on the in vitro differentiation of human dendritic cells (DCs) from monocytes, as well as its capacity to modulate the phenotype and function of DCs including their capacity to induce T cell responses.

Method

Flow cytometry, ELISA, real-time quantitative PCR assays were performed to assess the effect of Ca10 in the generation and maturation of human monocyte-derived DCs (hmoDCs) as well as the phenotype and function of Ca10-activated hmoDCs. Allogeneic co-cultures of hmoDCs and naïve CD4+ T cells, CFSE-dilution assays, ELISA blocking and pharmacological inhibition experiments were also performed. The in vitro generation of FOXP3+ regulatory T (Treg) cells was monitored by flow cytometry and their functional properties assayed by conventional suppression assays. ET cells were intramuscular inoculated in the left groin of mice and the in vivo generation of FOXP3+ Treg cells was analysed in spleen and inguinal lymph nodes by flow cytometry. Ca10 levels in serum from immunized mice was measured by ELISA.

Results

The presence of Ca10 during differentiation of hmoDCs from monocytes increase the expression of PD-L1, ICOSL and CD14 compare to hmoDCs generated by conventional methods. HmoDCs generated in the presence of Ca10 produce lower levels of cytokines than conventional hmoDCs after LPS stimulation. Ca10 induces PD-L1 expression, pro-inflammatory cytokines and IL-10 production, and the expression of tolerogenic molecules at the mRNA level in conventional hmoDCs. Ca10-activated DCs generate functional CD4+CD25highCD127-FOXP3+ Treg cells by mechanisms depending on Syk-coupled CLRs, MAPKs and NF-κB. In vivo data showed that the percentage of CD4+CD25highFOXP3+ Treg cells increases in spleen and inguinal lymph nodes from mice with ET.

Conclusion

Our results suggest that Ca10 might well favour the induction of tumor tolerance by generating Treg cells. These findings might contribute to develop novel cancer immunotherapy strategies in future.

O06 Identification And Quantification Of Functional Cannabinoid Receptor 1 In Human Blood And Tonsil Immune System Cells

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Keywords: Cannabinoid Receptor 1, Immune System Cells, Dendritic Cells

Introduction

The endocannabinoid system (ECS) is a complex signalling network involving a large number of physiological processes. The ECS consists of the endocannabinoid ligands, the enzymes related to the synthesis and degradation and the cannabinoid receptors (CBRs). Immune system cells express cannabinoid receptor 1 (CB1), but its functional role remains poorly understood due to the lack of suitable tools. In humans, the mRNA expression levels of CB1 are upregulated in tonsils and peripheral blood of allergic patients. The aim of this work is the visualization and quantification of CB1 at the protein level in human peripheral blood and tonsils and the study of the functional properties of this receptor in innate immune system cells.

Method

ECS expression was analysed by real-time quantitative PCR. CB1-specific fluorescent chemical probe was used to visualize and quantify CB1 expression in monocytes, human monocyte-derived macrophages, human monocyte-derived DCs and total DCs from peripheral blood and tonsils by flow cytometry and confocal microscopy. Cytokine production was analysed by ELISA.

Results

We showed that monocytes, human monocyte-derived macrophages, human monocyte-derived DCs and total DCs from peripheral blood express CB1, CB2, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). We developed and validated a novel chemical probe specific for CB1 to identify CB1-expressing immune system cells from blood and tonsils by flow cytometry and confocal microscopy. We demonstrated that monocytes and in vitro generated macrophages and DCs expressed significant levels of functional CB1 by using the validated CB1-specific chemical probe. In addition, our data showed that the cannabinoid receptor agonist WIN55212-2 reduced the production of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β in LPS-activated monocytes, human monocyte-derived macrophages, human monocyte-derived DCs and total DCs from peripheral blood. Interestingly, our results indicated that WIN55212-2 induced tolerogenic DCs with the capacity to generate functional CD4+CD25+CD127-FOXP3+ Treg cells.

Conclusion

The fluorescent chemical probe can be used to visualize and quantify the expression of functional CB1, which has immunomodulatory effects on human DCs and other innate immune system cells.

O07 BAFF-BAFFR Signaling Regulate NLRP3 Inflammasome Activation In LPS Stimulated Monocytes

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Keywords: BAFF, Monocytes, Inflammasome, NLRP3, Inflammation

Introduction

B-cell activation factor (BAFF) and proliferation inducing ligand (APRIL), two members of tumor necrosis factor superfamily are playing significant role in normal B cell development and function. In addition, they promote survival and proliferation of malignant B and acute myeloid leukemia blasts. However, to date, the effects of both ligands on normal myeloid cells are unknown. Therefore, in present study we aimed to understand the effects of BAFF and APRIL on function of monocytes/macrophages. Notably, both described ligands interact with membrane receptors, namely TACI (Transmembrane activator and CAML interactor) and BCMA (B-cell maturation antigen), while BAFF-R (B-cell activating factor receptor) is specific to BAFF only.

Method

In this study, we used peripheral blood monocytes isolated from healthy donors. By using, functional assays, flow cytometry, ELISA, qPCR, and Wester Blot we analyzed immune regulatory role of BAFF and APRIL in LPS stimulated monocytes.

Results

First, we confirmed expression of TACI, BCMA and BAFF-R receptors on peripheral blood monocytes by qPCR and flow cytometry. Next, we stimulated monocytes from normal donors with LPS in presence of recombinant human BAFF or recombinant human APRIL. We found that BAFF, but not APRIL, regulates production and release of IL-1β by monocytes. Finally, by using blocking antibodies, we found that BAFF-R, but not TACI and BCMA-mediated signaling decreased inflammasome activation and IL-1β processing in monocytes.

Conclusion

Altogether, we showed novel evidence that BAFF-BAFFR signaling may play a role in regulation of innate immune responses related to human monocytes/macrophages.

O08 Inhibition Of IgE Production By CpG-ODN Requires MyD88 Expression On Dendritic Cells

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Keywords: Allergy, IgE, Lung Inflammation, MyD88, CD11c

Introduction

Asthma is a chronic respiratory disease characterized by airway inflammation and high IgE production. We have shown in the ovalbumin (OVA)-model of asthma that the administration of a toll like receptor (TLR) agonists during allergic sensitization blocked the development of allergic asthma and IgE production. CpG, a TLR9 agonist, acts directly on different immune cells including Dendritic cells (DCs) and B cells. We investigated the role of MyD88 expression on DCs, using a CD11c-Cre MyD88 Floxed mice, which are deficient in MyD88 signaling in CD11c DCs (CD11cMyD88-KO), in the inhibition of allergic responses. Our results showed that CpG could not inhibit allergic responses including IgE production and lung inflammation in CD11cMyD88-KO mice. Using dendritic cells differentiated from bone marrow (BMDCs) of WT mice we were able to re-establish the inhibitory effect of CpG in mice deficient of MyD88 in CD11c cells.

Method

C57BL/6(WT), MyD88-deficient (KO), RAG-KO or CD11cMyD88-KO mice were sensitized on days 0 and 7 subcutaneously (sc.) with 4ug of OVA into 1.6mg of alum (OVA/Alum) or OVA/Alum plus 10ug CpG 2395(OVA/Alum/CpG). For some experiments, 1x107 BM-DCs of WT were stimulated with 100µg/mL of OVA (DC-OVA) or DC-OVA plus 1ug/mL of CpG(DC-OVACpG) and were used for sensitization on day 0 and

7 sc.. On days 14 and 21, mice were intranasally challenged with OVA (10ug). Samples were collected on day 22.

Results

In WT animals the Ova/Alum/CpG group showed a significant reduction in lung allergic inflammation ($p<0.01$), mucus production ($p<0.01$) and the inhibition of Total and Ova-specific IgE ($P<0.05$) production when compared to OVA/Alum group.

RAG mice reconstituted adaptive lymphocytes from MyD88-KO(RAG-MyD-KO) sensitized with OVA/Alum/CpG also showed a reduction in lung inflammation($p<0.01$), inhibition of Total and Ova-specific IgE($P<0.05$) when compared with RAG-MyD-KO sensitized with OVA/Alum.

This inhibition was abolished in MyD88-KO and CD11cMyD88-KO mice sensitized with OVA/Alum/CpG when compared with OVA/Alum group.

BMDCs pulsed with OVA and CpG(DC-OVACpG) injected into WT and CD11cMyD88-KO mice prevented lung inflammation($p<0.01$) and IgE production($p<0.01$) when compared with DC-OVA into WT and CD11cMyD88-KO group.

Conclusion

In conclusion, the addition of CpG during sensitization inhibited IgE production and lung inflammation through MyD88 signalling in CD11c cells, indicating that targeting CD11c DCs is a promising strategy to improve the effectiveness of anti-allergic formulations.

Friday 26 January 2018

21:00 - 22:00: Poster Session I

Topic 1 - Basic Immunology

P02 Thymic T Cell Populations Are Deregulated In Mice After Mild In Utero Cigarette Smoke Exposure

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Keywords: Thymus, T Cells, CD4, CD8, Smoking

Introduction

Epidemiological studies demonstrate that in utero cigarette smoke exposure negatively affects lung development, thereby increasing risks for asthma and COPD (Svanes, Thorax 2010). Smoking in pregnant women is frequently underreported, therefore mild smoking might not be reported. We hypothesize that mild maternal smoking alters immune development in offspring.

Objective: A) to develop a murine model of mild maternal smoking as defined by normal body weight and lung function in offspring and B) to assess immune cells in thymus and lung.

Method

Female C57BL/6 mice were exposed to mainstream smoke 4 days before mating and during pregnancy until birth for 1 hour per day; equals 6 cigarettes (research cigarettes 3R4F); inExpose exposure system (SCIREQ, Canada). T cells were analyzed by flow cytometry in thymus and lung. Lung function was assessed by FlexiVent system (SCIREQ, Canada).

Results

Thymic CD3high T cell populations and CD4+ T cells were increased in exposed offspring; CD8+ T cells were not affected; double-positive, double-negative (CD4/CD8) T cells and Treg (male) showed a decline in exposed offspring compared to air controls. In the lung, CD4+ T cells were increased whereas CD8+ T cells were decreased in both sexes.

Conclusion

Our data reveal altered T cell composition in the thymus and lung in a mild maternal smoking model, despite normal body weight and lung function. This could be a first indication of disturbed T cell development in in utero smoke-exposed offspring. These observations could lead to altered peripheral immune responses and a higher asthma risk observed in in utero cigarette smoke-exposed children. Detailed analysis of the altered T cell composition is required.

P03 Functional Characterization Of TRP Channels In Bone Marrow-Derived Dendritic Cells

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Keywords: TRP Channels, TRPV4, Calcium Signalling, Dendritic Cells

Introduction

Several dendritic cell (DCs) stages, such as differentiation, maturation and migration, are strongly modulated by changes in intracellular Ca2+ concentration. These changes are promoted by activation of Ca2+-release activated channels, ryanodine and purinergic receptors that are activated downstream of signalling pathways initiated by membrane receptors (G-protein coupled receptors) or by damage-associated signals (ATP). Recently, transient receptor potential (TRP) channels have been described to be expressed in immune cells, including DCs. However, the roles of these cation-permeable channels in these cells remain obscure. In this study, we determined the expression of TRP channels in mouse bone marrow-derived dendritic cells (BMDCs).

Method

BMDCs were generated from WT and Trpv4 KO mice and were used to identify TRP channel expression via qPCR. We assessed the functional expression of TRPV2 and TRPV4 using calcium imaging. An immunofluorescent staining was performed to confirm the presence of TRPV4 in the plasma membrane of DCs. We used flow cytometry to check the purity of the BMDC cell population

Results

We found that TRPM2, TRPM4, TRPM7, TRPV2 and TRPV4 are expressed in the CD11c+ BMDCs, and confirmed the functional expression of TRPV2 and TRPV4. Furthermore, we show that TRPV4 is dispensable for the differentiation and the LPS-induced maturation of CD11c+ BMDCs, and that activation of this channel induces an immediate transient expression of CCL-11, a chemotactic protein for eosinophils.

Conclusion

Since TRPV4 is a polymodal sensor, activated by mechanical and thermal stimuli, our findings suggest that TRPV4 activity may condition the activation state of DCs, as well as regulate the infiltration of eosinophils in the absence of pathogenic insults.

P04 Aluminium Hydroxide Impairs Tolerogenic Properties Imprinted By Allergoids Conjugated To Nonoxidized Mannan In Dendritic Cells

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Keywords: Immunotherapy, Vaccine, Dendritic Cell, Alum Adjuvant

Introduction

Allergen immunotherapy (AIT) is the only curative treatment for allergy, but it faces problems related to

efficacy, security, duration and patient compliance. Recent studies demonstrated that glutaraldehyde-polymerized grass pollen allergoids coupled to nonoxidized mannan (PM) represent novel suitable preparations to formulate improved vaccines for AIT. Aluminium hydroxide (alum) is the most widely used adjuvant in human vaccines but its way of action is not fully understood. The main aim of this work is to study the immunological mechanisms by which alum condition the capacity of PM to generate human dendritic cells (DCs) able to promote healthy immune responses to allergens.

Method

The expression of surface markers and cytokine signature were determined by flow cytometry or ELISA, respectively. Allogeneic cocultures of PM-activated human monocyte-derived DCs (hmoDCs) and naïve CD4+ T cells in the presence or absence of alum were performed to analyse T cells polarization. Forkhead box P3 (FOXP3) regulatory T cells were quantified. Blocking and pharmacological inhibition experiments were performed in hmoDCs.

Results

The production of IL-10 and IL-6 by PM-activated hmoDCs is significantly reduced in the presence of alum. In contrast, alum significantly increases the production of IL-1β, IL-23 and TNF-α in PM-treated hmoDCs. PD-L1 expression in PM-treated hmoDCs was reduced in the presence of alum, which was accompanied by decreased numbers of induced CD4+CD25highCD127- forkhead box P3 (FOXP3)+ Treg cells. Accordingly, alum significantly decreases IL-10-producing T cells generated by PM-treated hmoDCs, while increasing IL-5- and IFN-γ-producing T cells. Blocking experiments suggest that Syk, Akt and mTOR might contribute to the underlying immunological mechanisms modified by alum in PM-activated hmoDCs. In vivo immunizations of BALB/c mice showed that alum significantly reduces the numbers of FOXP3+ Treg induced by PM and increases proliferation and cytokine production in splenocytes.

Conclusion

We provide novel insights into the influence of aluminium hydroxide in the immunomodulatory properties imprinted by allergoids conjugated to nonoxidized mannan in dendritic cells, which might have important implications for future development of novel AIT protocols.

P05 Cytokine-Induced Endogenous Production Of PGD2 Is Essential For ILC2 Activation

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Introduction

Group 2 innate lymphoid cells (ILC2) play key role in the initiation and maintenance of type 2 immune response. The PGD2-CRTH2 receptor axis is a profound mechanism to induce cytokine production and migration of ILC2. We aimed to examine whether ILC2 are capable of prostaglandin production and to determine the importance of this process in the ILC2 function.

Method
The effects of the COX-1/2 inhibitor flurbiprofen and the CRTH2 antagonist cay10471 were determined on human ILC2 receptor expression, cytokine production and gene expression by flow cytometry, ELISA and quantitative RT-PCR, respectively. Concentration of lipid mediators was measured by LC-MS/MS.
Results
We showed that ILC2 constitutively express HPGDS, and upregulate COX-2 upon cytokine stimulation. Consequently, ILC2 produce metabolites of the PGD2 pathway. We revealed that endogenously produced PGD2 is essential in the cytokines-induced ILC2 activation, as blocking COX-1/2 enzyme or the CRTH2 receptor abolished ILC2 function.
Conclusion
We show that PGD2 produced by ILC2 is, in an autocrine manner, essential in the innate cytokines-induced ILC2 activation. Hence, CRTH2 antagonists represent a promising therapeutic tool for allergic diseases by controlling ILC2 function.

P06 The Impact Of Caffeine And Phosphodiesterase Inhibitors On The Activation And Cytokine Production Of Neonatal T Lymphocytes

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Keywords: Caffeine, Calcium Influx, CD4 Cell, Phosphodiesterase Inhibitor, Neonatal T Cell
Introduction
Caffeine and selective phosphodiesterase (PDE) inhibitors are widely used in the clinical management of preterm and term neonates. However, data on how these compounds interact with the neonatal adaptive immune system is scarce. We hypothesized that these compounds do not have immunosuppressive effects on neonatal T cells. Therefore, we aimed to describe the effects of caffeine, milrinone and sildenafil on the activation and cytokine production of T cells from umbilical cord blood (UCB) compared to adult peripheral blood (APB).
Method
We isolated mononuclear cells from 10 APB and 6 UCB samples. We assessed intracellular cytokine production of stimulated CD4 cells and parameters of calcium influx (AUC and maximal cytoplasmic calcium level) in lymphocytes upon phytohemagglutinin (PHA) activation following treatment with caffeine, milrinone, sildenafil, dbcAMP or a specific A2A receptor antagonist, ZM241385 using flow cytometry.
Results
ZM241385, but none of the other compounds increased parameters of calcium influx in APB samples. On the contrary, all compounds increased calcium influx in UCB. This effect was more pronounced in the case of caffeine and dbcAMP compared to milrinone, sildenafil or ZM241385. In line with data from the literature, intracellular levels of IFN-g in CD4 cells were lower in UCB compared to APB. Intracellular levels of the studied cytokines were unaffected by the applied compounds in both APB and UCB samples.
Conclusion
Our results demonstrate that neonatal T cells show sensitivity to caffeine and selective PDE inhibitors in contrast with adult T cells. Caffeine increases short-term activation in neonatal lymphocytes to a larger extent than milrinone or sildenafil. This effect appears to be mediated primarily via increased cAMP levels rather than A2A receptor inhibition. However, cytokine production of neonatal CD4 cells remains relatively unaffected. Overall, the application of caffeine, sildenafil or milrinone does not appear to have immunosuppressive effects on neonatal T cells based on our findings.

P07 Utilisation Of PIPE Cloning For Rapid Generation Of Human IgG1 And IgE Antibodies To The Major Birch Pollen Allergen Bet V 1

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Introduction

Advanced molecular cloning techniques are useful to provide monoclonal antibodies for research. Fast generation of expression vectors can be done by Polymerase Incomplete Primer Extension (PIPE) cloning, allowing restriction-site independent cloning of any DNA sequence. In case of immunoglobulins, heavy and light chains can be exchanged rapidly and further modified with site-directed mutagenesis (Ilieva et al. 2017). We here used PIPE cloning for rapid generation of IgG1 and IgE antibodies targeted against major birch pollen allergen Bet v 1.

Method

PIPE cloning was used to amplify sequences of the expression vector (pVitro1) containing kappa constant region sequences of IgG and IgE as well as variable region sequences from anti-Bet-v-1 antibody M0418 (Levin et al. 2014), respectively. Resulting PCR products were digested with DpnI and the corresponding sequences were pooled and afterwards transformed into E. coli DH10B. Clones were validated using colony PCR and sequencing, and antibodies produced using Expi293 expression system.

Results

Agarose gel electrophoresis of PCR products showed sharp bands matching the suspected sizes at ~380 – 4100 bp. Success rates for the exchange of both variable regions were 77 % and 22 % for IgG1 and IgE, respectively, and high antibody yields were obtained from expression in Expi293F cells.

Conclusion

In this study, anti-Bet-v-1 antibodies were successfully produced using PIPE cloning, a time-saving alternative to classical cloning techniques. The obtained antibodies are suitable for elucidation of the divergent functional properties of the subclasses IgG1 and IgE in type I allergy.

Supported by the Austrian Science Fund FWF, MCCA W1248-B30 and SFB F4606-B28.

P08 Poly (IC) Affects DC Populations In A Murine Smoke Model To Study COPD Exacerbation

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Keywords: COPD, Exacerbation, Animal Model, Biomarker

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide. The main risk factor is cigarette smoking. Exacerbations, episodes of acute worsening of clinical symptoms, can occur in Patients with COPD and are mostly triggered by viral infections. This leads to permanent loss of lung function and death. Identifying affect cells and molecules in early stages of exacerbations could predict imminent exacerbations and thus allow the development of novel treatments which would prevent the development of symptoms.

The aim of the project is to investigate the inflammatory response of poly (IC) (polyinosinic-polycytidylic acid) (mimicking a viral infection) in a murine smoke model to study COPD exacerbation.

Method

Female C57Bl6/J mice were exposed daily to either cigarette smoke (CS) (24 cigarettes for 24 days) or room air as a control. On day 24, mice were treated intranasally with 0.1 µg, 1 µg, 10 µg poly (IC) in PBS or PBS alone one hour after the last CS exposure. Thereafter, immune relevant cell types in BALF (bronchoalveolar lavage fluid) and lungs were analyzed via flow cytometry 24 hours after the application.

Results

A significant increase of plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs) and its subpopulations were detected after application of poly (IC) between the CS-exposed group and the air group. Poly (IC) has no effect on other investigated cell types compared to PBS or the air and CS-exposed groups. IFN-g, VEGF, IL-6, IL-1b and TNF-a have an enormous effect in the "exacerbation group" or only response in this.

Conclusion

The inflammatory response of cDC and pDC in smoke-exposed mice with poly (IC) application differ significantly in comparison to the other groups. Furthermore, a strong effect on dendritic cell-related cytokines was found. These results could help to understand viral-induced COPD exacerbations in detail and therefore could give a hint in revealing biomarkers for the detection of an imminent exacerbation.

P09 Dissecting T Helper Cell-Associated Inflammation And Neutrophil Subsets In Alpha 1 Antitrypsin Deficiency

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Keywords: Th17, Inflammation, AAT Deficiency, Neutrophils

Introduction

Patients with alpha 1 antitrypsin deficiency (AATD) are predisposed to early onset chronic obstructive pulmonary disease (COPD). Neutrophils are strongly implicated in the pathogenesis of COPD in AATD. AAT is crucial to block neutrophil protease function, but is also increasingly proposed to have anti-inflammatory effects. Previous data from our lab indicates patients lacking AAT have increased inflammatory markers, manifest as increased "pathogenic" Th17 cells in the peripheral blood of AATD patients. Furthermore, evidence for subsets of neutrophils with distinct phenotypes has recently emerged. With many examples of co-operation between Th17 cells and neutrophils, we have investigated neutrophil subtypes in AATD.

Method

Whole blood and peripheral blood mononuclear cells (PBMCs) were isolated from AATD patients and age-matched healthy controls. Neutrophil subtypes were assessed using flow cytometry and further phenotyped using markers of maturity and activation. To investigate their effect on cytokine production a specific neutrophil subtype, low density neutrophils (LDNs), were depleted from the PBMC population using magnetic isolation and PBMCs cultured for 24 hours. PBMCs were then assessed for cytokine expression using flow cytometry.

Results

AATD patients have a significantly higher percentage of LDNs in the PBMC population compared to healthy controls ($p<0.0001$), defined as CD66b+ cells following density centrifugation. Further phenotyping indicates the majority of these cells as immature (CD33+) and inactivated (CD62L+). Depletion of the small population of LDNs from healthy PBMCs increases expression of IL-10 in CD4+ T cells. However, depletion from AATD PBMCs has no effect on IL-10, indicating that LDNs in AATD patients are not suppressive, at least with regards to this anti-inflammatory cytokine.

Conclusion

A recently described neutrophil subtype, the LDN, is significantly increased in AATD patients compared to healthy controls. We have shown that these are immature, non-activated neutrophils within the PBMC

population. In cancer, these cells have been proposed to have a suppressive effect on the immune system; however, we have shown that this is unlikely in the case of AATD.

Friday 26 January 2018

21:00 - 22:00: Poster Session I

Topic 2 - Immune Mechanisms

P10 Bacterial-Derived Histamine Influences Lung Inflammatory Responses

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Keywords: Asthma, Histamine, Gut Microbiota, M.Morganii

Introduction

Microbiome-host interactions are believed to significantly influence host immunologic homeostatic mechanisms and microbial dysbiosis has been associated with many inflammatory diseases, including asthma. The potential influence of gut bacteria on asthma development is not only related to their relative abundance or their taxonomic affiliation, but also to the metabolites that they produce. Histamine is one such bacterial metabolite with important immunomodulatory effects.

The aims of this study were to quantify histamine secreting bacteria within the gut of asthma patients and healthy volunteers, to identify the bacterial species responsible for histamine secretion and to investigate the influence of bacterial-derived histamine in murine models.

Method

(In the results part)

Results

Following PCR screening of fecal samples from 148 asthma patients and healthy volunteers, we observed that the bacterial histidine decarboxylase (HDC) gene copy number was significantly higher in asthma patients compared to healthy volunteers. Fecal samples were cultured on multiple growth media, under multiple different growth conditions in the presence of histidine to isolate histamine-secreting microbes. Escherichia coli, Lactobacillus vaginalis and Morganella morganii (M. morganii) were identified as the species capable of high levels of histamine secretion within the gut of asthma patients. Histamine from these strains activated the human histamine 1 receptor, as demonstrated using a reporter cell line. Finally, we cloned the HDC gene from M. morganii to an Escherichia coli (E. coli) strain. Oral administration of the HDC-tranfected E. coli to mice reduced eosinophil recruitment to the lung following OVA challenge, while administration of the HDC- wild-type strain did not influence inflammatory cell recruitment to the lung. The protective effect of the HDC transfected E coli was lost in HDC-deficient mice.

Conclusion

In conclusion, bacterial-derived histamine may have clinical relevance as increased levels of these bacteria are present within the gut microbiota of asthma patients. In addition, murine models demonstrate that bacterial-derived histamine can influence inflammatory responses within the lung. This study suggests that the analysis of composition and metabolic activity of asthma patients' microbiome could assist more accurate patient phenotyping, while novel therapeutics directly targeting microbiome activities may be considered as complementary to existing approaches.

P11 Anti-Inflammatory Effects Of Novel Semi-Synthetic Phytocannabinoids In Human In Vitro Inflammatory Keratinocyte Model Systems

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Keywords: Anti-Inflammatory, Phytocannabinoids, Keratinocyte, Model

Introduction

Keratinocytes were already shown to produce anti-microbial peptides and pro-inflammatory cytokines, which are important in regulating cutaneous immune processes. Thus, acting on keratinocytes augurs to be a highly efficient and targeted way to alleviate cutaneous inflammation. Recently, a series of fluorinated derivatives of cannabidiol have been synthesized, which is the major non-psychoactive component of the plant Cannabis sativa. The goal of the current study was to assess the potential cutaneous anti-inflammatory actions of these compounds (F-CBDs) in previously optimized in vitro epidermal keratinocyte models, whick closely mimic certain in vivo epidermal irritation/inflammattion signals.

Method

The viability of immortalized (HaCaT, HPV-KER) human epidermal keratinocytes was investigated by MTT-assay. Gene expression changes for detected by qRT-PCR, while protein release was determined by ELISA.

Results

CBD and F-CBD did not decrease the long term viability of HaCaT cells, while the viability of HPV-KER was impaired after 72 hr treatments in high concentrations.

Expressions of certain pro-inflammatory cytokines, e.g. interleukin (IL)-1α, IL-1β, IL-6 and IL-8, were significantly down-regulated upon the administration of CBD and F-CBDs in most models. Importantly, all F-CBDs exhibited significantly higher efficacies in comparison to the non-fluorinated counterpart CBD in the in vitro human epidermal keratinocyte models.

Conclusion

Our study provides the first evidence that F-CBDs exert more potent anti-inflammatory actions than CBD on human epidermal keratinocytes. Subject to further testing these data invite further pre-clinical and clinical studies to exploit the therapeutic potential of certain F-CBDs in a various inflammatory skin conditions.

P12 Upper Airways Mucosal Innate Immune Response In Presence Of St. Aureus Or H. Parainfluenzae Colonization In Children With Bacterial Acute Otitis Media And Acute Rhinosinusitis

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Keywords: Innate Immunity, Acute Otitis Media, Acute Rhinosinusitis, St. Aureus, H. Parainfluenzae

Introduction

Studies of recent years have proved that microbiome is involved in modulation of local immune response on the host mucosal surfaces. Bacterial-immune interactions in respiratory tract are linked to frequency and severity of respiratory tract infections (RTIs). The study objectives were to investigate mucosal innate immune response in upper airways in relation to microbial colonization in children with complicated upper RTIs.

Method

The study enrolled a total of 214 children (6.0 (3.7; 12.0) years) with upper RTIs complicated with bacterial acute otitis media (AOM) or acute rhinosinusitis (ARS), including 128 children with low frequency of complicated upper respiratory infections (group I) and 86 children with high (>4 episodes per year which is considered to be a recurrent course) one (group II). We performed nasopharyngeal or middle ear exudates cultures as well as mucosal immunology study: lysozyme (Lys), lactoferrin (La), human cathelicidin hCap-18/LL-37 levels in oropharyngeal discharge measured in early and late disease, and 2 weeks after recovery. The controls were 36 children with uncomplicated viral upper RTIs.

Results

In both groups the immune response to bacterial infection was registered as a rapid synchronous increase of hCap-18/LL-37 and La levels which peaked at the onset the disease and significantly exceeded the response to viral RTIs. Lys performed more slowly and the increase was maximal in the late disease. Though, group II demonstrated lower levels and slower pace of increase of all studied innate protective factors during the disease episode; and showed a higher rate of cultures negative for typical pathogens of AOM/ARS (H. influenzae, Str. pneumoniae etc.), along with higher rate of St. aureus and H. parainfluenzae colonization. Predominantly the latter agents were linked to low levels of mucosal antimicrobial peptides in both groups. However, at the onset of AOM/ARS, H. parainfluenzae infection was linked to high levels of La in group I and St. aureus was linked to high Lys and hCap-18/LL-37 concentrations in group II (See Table). It's noteworthy that significant subsequent decline of La, Lys and hCap-18/LL-37 respectively was registered in these patients in further reference points of the study, maximally pronounced in group II after recovery.

Conclusion

St. aureus and H. parainfluenzae colonization is associated with altered mucosal natural immune response in upper airways in children with bacterial AOM/ARS, especially in case of recurrent form of the disease.

Antimicrobial peptide / protein	Group 1 (episodic bacterial RTIs), N=104*		p**	Group 2 (recurrent bacterial RTIs), N=56*		p**
	H. parainfluenzae-positive (N=12)	*H. parainfluenzae*-negative (N=92)		*St. aureus*-positive (N=28)	*St. aureus*-negative (N=28)	
Lactoferrin, ug/ml	80.07 (64.11; 96.03)	64.10 (48.34; 76.40)	0.042	55.56 (45.66; 69.18)	56.39 (38.33; 67.52)	0.492
Lysozyme, pg/ml	20.21 (13.92; 26.49)	40.00 (31.87; 47.21)	0.023	29.27 (19.34; 33.45)	17.85 (11.59; 21.96)	0.003
Human cathelicidin hCap-18/LL-37, ng/ml	68.56 (50.65; 86.47)	63.57 (27.89; 98.70)	0.451	45.97 (35.26; 82.49)	32.63 (19.56; 62.02)	0.045

Notes: 1. * Microbiological success rate in the group;
2. ** – Mann-Whitney U-test.

P13 Butyrate And Fructo-Oligosaccharides Support Oral Immunotherapy By Suppressing Basophil And Mast Cell Activation And Induce Possible Epigenetic Changes In Mast Cell Progenitors

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Keywords: Food Allergy, Oral Immunotherapy, Butyrate, Non-Digestible Oligosaccharides, Mast Cells

Introduction

In previous work, we showed that a fructo-oligosaccharide (FOS) supplemented diet enhanced oral immunotherapy (OIT) efficacy in a mouse model for cow's milk allergy, which led to reduced mast cell activation upon allergen challenge. Fermentation of FOS by intestinal bacteria leads to production of short-chain fatty acids (SCFA, i.e. butyrate). Increased levels of butyrate were found in the caecum of OIT+FOS mice. The exact contribution of FOS and/or butyrate in dampening the allergic response is however unknown. Objective: Investigating the effect of combining OIT with FOS or butyrate supplementation on the development and IgE-mediated activation of mast cells and basophils.

Method

C3H/HeOuJ mice were sensitized to the cow's milk protein whey and subjected to OIT with or without FOS or butyrate supplementation. Bone marrow was collected during and after OIT and cultured with IL-3 and SCF into bone marrow-derived mast cells (BMMCs). c-Kit and FcεRI expression on BMMCs was analyzed using flow cytometry and IgE-mediated degranulation was determined by measuring β-hexosaminidase. After OIT, whole blood samples were used to perform a Basophil Activation Test (BAT) and intradermal (i.d.) and intragastric (i.g.) challenges were conducted to measure the acute allergic skin response (delta ear swelling) and mucosal mast cell degranulation (mMCP-1 in serum). After challenge, caecum content was collected to measure SCFA levels.

Results

BMMCs developed from bone marrow of FOS exposed mice showed reduced expression of c-Kit and FcεRI and IgE-mediated activation was also reduced. Allergen-induced basophil activation was reduced in OIT+butyrate blood samples compared to OIT samples. These findings were in accordance with the observed reduction in the acute allergic skin response and the reduction in mast cell degranulation in OIT+FOS and OIT+butyrate mice compared to sensitized controls. A significant increase in butyrate in the caecum content was observed in OIT+FOS mice compared to sensitized controls and OIT mice.

Conclusion

FOS and butyrate either or not combined with OIT have profound inhibitory effects on allergic effector cells like mast cells and basophils. These inhibitory effects may partly be explained by the induction of epigenetic changes, because in vitro development of mast cells from bone marrow progenitors is affected. Further research is needed to investigate if this approach may improve treatment strategies for food allergies in the future.

P14 The Effects Of Real World Particulate Matter From London On The Viability And Cytokine Production Of Monocyte Derived Dendritic Cells.

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Keywords: Air Pollution, Dendritic Cells, Asthma

Introduction

Epidemiological studies have shown that there is an association between exposure to high concentrations of particulate matter (PM) and exacerbation of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). A central mechanism for this adverse response has been attributed to the pro-inflammatory milieu produced by PM stimulated dendritic cell (DC) via cytokine secretion. We examined the impact of PM, collected in 2013 from heavily trafficked and urban background locations in

London on the viability and cytokine secretion profile of human monocyte derived DCs (MDDCs).
Method
A set of annual PM2.5 and PM10 samples were collected in 2013 and extracted from filters from Marylebone Road (London roadside) and North Kensington (London background). SRM-1648a, from the National Institute of Standards and Technology was used as a reference control for the urban PM. The effects on viability of peripheral blood human MDDCs by these PM at various concentrations were evaluated using Pierce™ LDH Cytotoxicity Assay. Cytometric bead array (CBA) was employed to measure concentration of cytokines within the supernatants collected from PM stimulated DC with a at 10µg/ml of PM concentration.
Results
PM2.5 and PM10 from both locations does not affect the viability of MDDCs compared to the control. SRM-1648a, London roadside PM10 and background PM10 significantly increased the secretion of cytokines IL-6, IL-8 and IL-23p40 by MDDC, whilst no significant difference was found between the PM and the control for IL-1α, IL-1β and IL-12p70. The increase in secretion of TNFα by MDDC was exclusive to London background PM10 and SRM-1648a.
Conclusion
Particulate matter collected from selected London locations does not present cytotoxicity to MDDCs at the concentrations used. PM-fraction dependent responses were observed with PM2.5 and PM10, the secretion of pro-inflammatory cytokines was largely restricted to the coarse mode PM10, with little evidence of a PM2.5 effect. In summary, these results suggest that MDDCs exposed to real world PM10 were able to contribute in establishing a pro-inflammatory milieu that is consistent in affecting the phenotype and differentiation of the adaptive immune system in the lungs.

P15 MicroRNA-Regulation Of Antiviral Responses In Asthmatic Airways

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Keywords: Primary Macrophage, Poly(I:C), Virus, Asthma

Introduction
Respiratory viruses are one of the most common triggers of asthma exacerbations, however, knowledge of the underlying molecular mechanisms leading to the pathological response is limited. Macrophages are abundant in the respiratory tract. They play a central role in early host defense against viruses but may also promote excessive inflammation and tissue remodeling. In this study, we tested the hypothesis that specific microRNAs (miRNAs) regulate antiviral responses mediated by airway macrophages which contribute to asthma pathology.
Method
Bronchial lavage was obtained from well-characterized asthmatic (n=20) and healthy control (n=10) subjects. Airway macrophages were enriched by adherence to cell culture plates. Cells were rested for 48h followed by stimulation with poly(I:C) for 4h to mimic an acute viral infection. miRNA expression in stimulated and unstimulated control macrophages were assessed by miRNA microarray. Additionally, inflammatory mediators in cell-free conditioned media were analyzed by multiplex immunoassay.
Results
Our preliminary results demonstrate that primary airway macrophages secrete pro-inflammatory mediators in response to acute poly(I:C)-stimulation. Importantly, macrophages from asthmatic subjects, but not healthy subjects, produced asthma-related mediators such as the IL-6-family member Oncostatin M and the inflammatory marker S100A12/EN-RAGE that have previously been implicated in airway

remodeling and hyperreactivity in asthma and allergic inflammation. Furthermore, uniquely expressed miRNAs were found after poly(I:C)-stimulation of macrophages from asthmatics compared to healthy subjects. Analysis of miRNAs present in both groups generated a limited number of miRNAs with opposing expression pattern upon poly(I:C)-stimulation of asthmatic versus healthy cells.

Conclusion

Primary macrophages obtained from asthmatic airways respond to virus-stimulation with altered miRNA expression and release of inflammatory mediators related to asthmatic disease, demonstrating that they are part of the immunopathology underlying virus-triggered responses in asthma.

P16 Immunomodulatory In Vitro Potential Of Vitamin D3 To Reduce TNF-a-Producing CD16-Positive Monocytes In Asthmatic Patients.

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Keywords: Vitamin D3, Immunomodulation, Steroids, Asthma

Introduction

Asthma is an inflammatory disorders associated inter alia with enrichment of highly pro-inflammatory subpopulations of monocytes expressing CD16. We demonstrated previously that CD16-positive monocytes could be reduced significantly in asthmatic patients in the course of glucocorticoid (GC) treatment. Here we intended to investigate potential of vitamin D3 (VIT.D3) to modulate GC-mediated effects exerted on CD16-positive monocyte subsets.

Method

We recruited 47 healthy volunteers and 9 asthmatic patients in order to isolate peripheral blood mononuclear cells (PBMCs) cultured for next 24 hours in the presence of methylprednisolone (MP) and/or VIT.D3 to evaluate their effects on monocyte subpopulations. Stimulations with LPS for 6/24-hours were performed to assess VIT.D3 effects on TNF-α and IL-10 production in monocytes. GC receptor and pan-caspase inhibitors were used to investigate possible mechanism of VIT.D3 action on studied cells. Flow cytometry was used in the reseach to evaluate changes in monocyte subpopulations frequencies and cytokines production.

Results

We found that 24-hour stimulation with VIT.D3 caused significant reduction of CD16-positive monocytes, and moreover, allowed for 5-fold reduction of GC dose maintaining their anti-inflammatory activity. Stimulation with VIT.D3 was shown to exert bipolar effects on monocytes leading to decrase in pro-inflammatory TNF-α production, with concomitant drop in anti-inflammatory IL-10 within monocytes. Experiments with GC receptor and pan-caspase inhibitors revealed potentially different mechanism of VIT.D3 action on each monocyte subsets expressing CD16.

Conclusion

We demonstrated novel immunomodulatory properties of VIT.D3 in reference to TNF-α-producing CD16-positive monocytes. Furthermore, collected data suggest possible application of in GC-based treatment approach to allow for significantly reduction of steroid dose maintaining anti-inflammatory potential of these drugs.

P17 Children with pre-school wheeze have airway inflammation driven by bacteria and viruses during periods of clinical stability

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Introduction

Wheeze is a common symptom in young children. Wheezing episodes commonly result from acute respiratory infection and necessitate emergency treatment. Preventative treatments effective in asthma have little benefit in preschool wheeze (PSW), and a limited pathophysiological understanding has inhibited treatment development. We examined the airway immune environment in children with PSW during stable disease by phenotyping the leukocyte composition of bronchoalveolar lavage (BAL) and examined the association of bacteria and viruses on immune status.

Method

Children undergoing clinically indicated bronchoscopy to investigate recurrent respiratory symptoms were recruited. The PSW group consisted of children aged between 1 and 5 years with confirmed, severe, recurrent wheeze. The control group consisted of children aged between 1 and 16 years with respiratory symptoms that did not include wheeze. The control group was split into those with a negative BAL bacterial culture and negative BAL viral PCR (control negative, (CNeg)); and those who had a positive bacterial culture and/or viral PCR (control positive, (CPos)).

BAL leukocyte populations were delineated by expression of cell surface markers; macrophages (CD206+), monocytes (CD14+), eosinophils (Siglec 8+), neutrophils (CD15+), B-cells (CD19+), cytotoxic T-cells (CD3+, CD8+), T-helper cells (CD3+, CD4+), NK-cells (CD56+) and NKT-cells (CD3+, CD56+).

Results

Comparison was first made between PSW (n=20) and the CNeg groups (n=7). BAL leukocyte proportions in the CNeg group were comparable with published values in healthy children. Bacteria and/or viruses were detected 17/20 (85%) of PSW BAL. There was higher airway inflammation in the PSW group compared to the CNeg group, with increased proportions of neutrophils, monocytes, B-cells and NK-cells, and a lower proportion of macrophages. Comparison between the PSW and CPos groups (n=16) revealed a similar inflammatory pattern.

Combining all groups (n=43) the proportion and number of neutrophils, B-cells and NK-cells in the BAL correlated positively with burden of infection (sum of virus and bacteria species) and the proportion of macrophages correlated negatively.

Conclusion

During stable disease children with PSW demonstrate abnormal BAL leukocyte proportions likely driven by the presence of bacteria and viruses. Correcting these abnormalities during periods of clinical stability may inhibit exacerbations of wheeze.

Friday 26 January 2018

21:00 - 22:00: Poster Session I

Topic 3 - Allergic Immune Response

P18 Novel Biomarkers For Immunophenotyping Atopic Dermatitis In Children

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Introduction

Atopic Dermatitis (AD) is a complex inflammatory skin disease with marked clinical heterogeneity. It results from a poorly understood interplay between genetics, elements of the epidermal barrier, environment and the immune system. It is thought that a number of environmental factors, such as microbial exposure and dietary habits may significantly influence the immunological mechanisms that lead to AD

development. The aim of this study was to investigate immune signatures that may be associated with AD in South African children from genetically similar backgrounds but from two different environments, i.e. rural versus urban Cape Town.

Method

We obtained plasma from 160 age-matched children (n=31 urban AD cases; n=37 urban controls; n=48 rural AD cases; n=44 rural controls) and quantified a panel of 43 different cytokines, chemokines, angiogenesis and growth factors using the Mesoscale MSD multiplex platform in order to investigate possibly diverse biological pathways that may account for AD heterogeneity.

Results

Plasma levels of TARC, MCP-4, MIP-1beta, IL-8, TNF-alpha, IL-1beta, IL-2, IL-4, IL-12p70, IL-13 and IL-16 were significantly elevated ($p<0.05$) in children with AD compared to controls. Within the AD patients, a subgroup was identified, which co-expressed the highest levels of plasma IL-4, IL-2 IL-12p70 and IL-1beta. In contrast to previous reports on AD in Asian populations, South African AD patients had significantly ($p<0.05$) reduced plasma levels of IL-17A. In addition, IL-27 levels were also significantly reduced with a strong trend ($p<0.10$) for reduced levels of IL-21, IL-23, IL-31 and TNF-alpha. Furthermore, several environment- but not AD-related differences were observed. IL-22, IL-15, Flt-1, bFGF and PIGF were elevated in urban groups compared with their rural counterparts, while IL-6, IL-8, TNFalpha, IL-1alpha, IL-21, MCP-1, MIP-1alpha, MIP-1beta, Tie-2, soluble ICAM-1 and VCAM-1 were elevated in children from rural communities.

Conclusion

These results indicate that population-specific and environment-related immune signatures are evident in South African children with AD. Interestingly, we also observed distinct non-AD related immune signatures in rural and urban groups that may reflect different environmental exposures, and early childhood immune polarization in this ethno-genetically homogenous study population. The discovery of biomarkers that identify population-specific immunophenotypes will facilitate the effective use of targeted therapies.

P19 Discrete Circulating MicroRNA Profiles Are Observed In Non-Allergic And Allergic Asthmatics With High Blood Eosinophilia

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Keywords: MicroRNA, Asthma, Serum, QPCR

Introduction

Nearly 300 million people world-wide are affected with asthma, a heterogeneous disease with a variety of different phenotypes. Asthmatics are currently classified based on their inflammatory cell profiles, such as eosinophils or neutrophils, or by their atopic status. microRNAs (miRs) are ~18-24 nucleotide, non-coding RNAs involved in post-transcriptional gene regulation, usually through the downregulation of targeted messenger RNA (mRNA). Several studies have aimed to explore the possibility of miRs as biomarkers for diseases, such as cancers. In our study, we examined six different miRs in serum from non-allergic and allergic asthmatics and healthy control subjects. The examined miRs candidates have previously been shown to be involved in eosinophil development and other immune responses.

Method

Serum from healthy individuals as well as age matched non-allergic asthmatics (NAA) and allergic asthmatics (AA) were utilized. Additionally, the NAAs and AAs subjects had high eosinophila (≥0.4 x 109cells/L) compared to healthy controls (≤0.1 x 109cells/L). Asthmatic subjects were included irrespective of inhaled corticosteroid usage. RNA was extracted from serum, reverse transcribed and subjected to qPCR analysis. Six candidate miRs, miR-126, -145, -146a, -155, -223, and -374, were investigated for differences in expression between subjects.

Results
Two miRNAs examined, miR-155 and miR-146a, were significantly upregulated in AAs as compared to NAA or healthy subjects. Additionally, a trend in increased expression was apparent in NAA in three (miR-223, -126, and -374) of the six miRNAs. Furthermore, this change in the NAA miRs appeared to correlate with the use of inhaled corticosteroids.
Conclusion
Using six miRs found in the literature to be involved in eosinophila or immune responses, we were able to detect expression changes in the serum of healthy and asthmatic individuals. Moreover, were able to distinguish between healthy individuals, AAs, and NAAs on inhaled corticosteroids, leading to the possibility that these miRs may be valuable future biomarkers for asthma.

P20 Time-Series Assessment Of Nasal Microbiome In Allergic Rhinitis Patients And Healthy Subjects Under Natural Pollen Exposure

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Introduction
The incidence of allergic airway diseases has been increasing over the last decades. This has been partly attributed to higher or longer exposure to airborne pollen, which is a major cause for respiratory symptoms in sensitized patients. Imbalances and changes in mucosal microbiota parallel allergic disease outcome, the effect cause question being unsolved. We recently showed that also pollen harbor a specific microbiome. Since pollen derived mediators and adjuvant factors, as well as pollen-associated microbiota, challenge the nasal epithelium, it stands to reason that pollen-associated microbiota also play a role in the pathophysiology of allergic rhinitis.
Our aim is to describe the changes in the nasal microbiome of healthy and allergic rhinitis (AR) patients, as well as identify key microbes, both host- and pollen-derived, that are associated with AR under pollen exposure.
Method
Over the course of one year, monthly or bi-weekly (within birch pollen season) swabs of the middle nasal meatus of eight AR patients and eight non-atopic volunteers were taken. From those, 16S rRNA hypervariable regions V1-V3 and V7-V9 were sequenced using the Illumina MiSeq sequencing platform to study the microbial composition. During the birch pollen season, we additionally collected 60 birch pollen samples from characterized sampling sites in Augsburg, Germany for 16S sequencing of pollen-associated microbes.
Results
We observed that the individual nasal microbiome of non-allergic individuals did not change during or outside of the birch pollen season, whereas the alpha diversity of samples from AR patients increased especially during the pollen season. Differences were also observed not only in diversity, but also in the richness of the microbiome of healthy and sensitized patients, with some families being predominantly present in healthy subjects but not in allergic patients and vice versa.
Conclusion
In healthy subjects, the nasal microbiome is comparatively stable independent of pollen exposure, whereas the microbiome of sensitized patients changes during the pollen season. Comparison and characterization of nasal and pollen specific microbiomes in a multi-omic approach will enable us to fathom macroenvironment and microenvironment interactions in allergy.

P21 Combining Dietary ScGOS:LcFOS And N-3 PUFA Did Not Lead To Additional Preventive Effects In Cow's Milk Allergy In Female Mice

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Keywords: Cows Milk Allergy, Behaviour, Prebiotic, N-3 PUFA

Introduction

Cow's milk allergy (CMA) subjects are not only affected by the immune and gastrointestinal consequences of CMA but might also be affected by CMA associated behavioural changes. The dietary components short-chain galacto- and long chain fructo-oligosaccharides (scGOS:lcFOS) and omega-3 poly unsaturated fatty acids (n-3 PUFA) have immune- and neuronal regulatory capacities and have been demonstrated to reduce the allergic symptoms in a murine model of CMA, however, it is unknown if an additional effect occur when these dietary components are combined. The objective of this study was to evaluate the preventive effect of a combination of scGOS:lcFOS and n-3 PUFA on CMA development and to evaluate the effects on a possible CMA mediated behavioural impairment.

Method

3-4 weeks old C3H/HeOuJ female mice received a control or a supplemented diet with 1% (9:1) scGOS:lcFOS, 6% n-3 PUFA or a combination of 1% scGOS:lcFOS and 6% n-3 PUFA (n=12-15) from day -14. For 5 weeks (day 0-28) the mice were weekly sensitized to 20 mg cow's milk whey protein in PBS with 20 ug cholera toxin (CT) or CT only (control). Anxiety-like, explorative and social behaviour were assessed by marble burying (day 22), open field (day 29) and social interaction (day 35) tests, respectively. Clinical parameters were measured after intradermal and oral challenge. After the mice were killed (day 43) mesenteric lymph nodes (MLN), spleen and lamina propria (LP) were isolated to measure the levels of Th1 and Th2 subsets. Serum was collected to determine levels of mouse mast cell protease 1 (mMCP-1) and antigen specific immunoglobulins.

Results

The scGOS:lcFOS diet or n-3 PUFA diet reduced the acute allergic skin response significantly. The combination diet showed no significant reduction of the acute allergic skin response. All the tested diets caused no significant reduction in CMA-induced mMCP-1 and immunoglobulins IgE, IgG1 and IgG2a serum levels. Th1 and Th2 subsets in spleen, MLN and LP were neither affected by CMA nor by the dietary supplementations. In female mice CMA had no significant impact on social-, anxiety-like- and explorative behaviour.

Conclusion

A dietary supplementation with scGOS:lcFOS or n-3 PUFA in a preventive setting reduce the acute allergic skin response in CMA mice. No additional effect was observed when these components were combined. CMA appeared to have no influence on Th1 and Th2 subsets in spleen and gut-associated lymphoid organs and also no impact on behaviour in female mice.

P23 Towards The Development Of Predictive And Diagnostic Assays For Drug Hypersensitivity

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Keywords: Predictive Assay T Cell Priming

Introduction

Whilst there are diagnostic assays such as the lymphocyte transformation test, more predictive approaches to drug hypersensitivity reactions are needed. Understanding and predicting reactions would be beneficial to patients and the drug development process. Drug hypersensitivity is multi-factorial, including: HLA associations, immune checkpoint regulation, and the precursor frequency of T cells. The challenge has been to develop assays, to incorporate these aspects.

The aim is to develop medium throughput assays to (1) predict the number of individuals and (2) the number of T cells that respond to a drug. To do this, we have established an HLA-typed cell bank containing PBMC form 1000 donors and two assays named the T-MWA (multiple well assay) and the T-MDA (multiple donor assay).

Method

An optimised in vitro T cell priming assay was performed in a single 96-well plate, where healthy volunteer naïve T cells and autologous DCs were cultured for two weeks with SMX-NO, Dapsone-NO, Bandrowski's Base or piperacillin (T-MDA; 6 wells per donor, T-MWA; 48 wells). Co-cultures were then re-challenged and assessed for antigen specific T cell proliferation and cytokine secretion; using thymidine incorporation and IFN-y ELISA respectively.

Results

SMX-NO specific T cell responses were detected by T-MDA in 5/5 donors in a single 96-well plate format. More detailed assessment of allergenicity using the T-MWA allowed for the detection of T cell responses to model drug and chemical antigens SMX-NO (55-70% response) and BB (60-100% response) respectively; but also, antigens that induced a lower frequency of specific T cells in the population, such as piperacillin (10-20% response) and Dapsone-NO (<10% response).

Conclusion

The T-MDA and T-MWA have been developed to enable (a) the priming of healthy volunteer naïve T cells to drug and chemical antigens, and (b) assess the comparative allergenicity of multiple compounds in a single 96-well plate format. In the future, the assays will enable the development of a traffic light system that indicates low, moderate, and high responses to antigens and outline the potential that novel compounds will cause hypersensitivity reactions when administered widely to humans.

P24 Suppression Of Food Allergic Symptoms By Unprocessed Cow's Milk Is Retained After Skimming But Abolished After Heating The Milk

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Keywords: Farming Effect, Food Allergy, Milk Processing, Mouse Models, Raw Milk

Introduction

In line with epidemiological studies, we have previously shown that raw, unprocessed, cow's milk prevents the development of asthma in a murine HDM-induced allergic asthma model. Heat sensitive milk components, possibly together with the fat content of the milk seemed to be responsible for the observed effects. In the present study, we investigated if unprocessed cow's milk is also protective in a murine model

for food allergy. Besides, we studied pasteurized and skimmed milk to investigate the effect of heating and the effect of the fat content of the milk.

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. Non-sensitized mice received CT alone. Prior to sensitization, mice were orally treated with unprocessed milk, pasteurized milk, skimmed milk or PBS (as control) for eight consecutive days. Five days after the last sensitization, mice were challenged intradermally (i.d.) in the ear with OVA to determine the acute allergic skin response. 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.

Results

Although no significant differences were observed in OVA-IgE levels between groups, OVA sensitized mice receiving unprocessed cow's milk showed a decreased acute allergic skin response compared to sensitized mice receiving PBS. This effect was retained after skimming, but abolished after pasteurization of the milk. Concentrations of the Th2 cytokines, IL-5 and IL-13, tended to increase in the pasteurized milk group compared to the unprocessed milk group in OVA stimulated splenocyte cultures. These cytokine levels remained low in the skimmed milk group. The percentage of CD103+ DCs was elevated in the MLN of the unprocessed and skimmed milk groups compared to the sensitized control group. However, no effects were observed on regulatory T cells.

Conclusion

Unprocessed cow's milk reduces allergic symptoms in a murine model for food allergy. The effects are abolished after heating the milk but retained after skimming, suggesting that the fat content of the milk does not contribute to the observed effects. Future research with milk fractions separated on size might give more insight in the specific heat-sensitive components involved.

P25 Innate Immune Responses To Microbial And Allergenic Compounds In Nasal Epithelial Cells

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Keywords: Nasal Epithelial Cells, Allergy

Introduction

Background: The epithelial cell-derived cytokine milieu has been discussed as a "master switch" in the development of allergic disease.

Aim of the study: To understand the role of innate immune response in nasal epithelial cells during allergic inflammation. For this aim we created a minimal invasive method to isolate human nasal epithelial cells (HNECs) from well clinically and immunologically characterized patients, to establish fast and efficient culture, to characterize the cells in a translational approach.

Method

Methods: HNECs from healthy volunteers and from allergic rhinitis (AR) patients were compared with respect to their growth, barrier integrity, pattern recognition receptor expression and immune responses to allergens and an array of pathogen-associated molecular patterns (PAMPs), such as TLRs and inflammasome.

Results

Results: Cells from nasal scrapings were clearly identified as nasal epithelial cells by staining of pan-Cytokeratin, Cytokeratin-14 and Tubulin. Additionally, Mucin 5AC staining revealed the presence of goblet

cells while staining of tight-junction protein Claudin-1, Occludin and ZO-1 displayed the ability of HNECs to form a tight barrier. Cells of atopic donors grew more slowly than cells of non-atopic donors. Baseline expression of CCL2, CCL5, CCL20, IP-10 and GM-CSF was lower in atopic donor´s cells as compared to cells of non-atopic donors. PolyI:C, Pam3Cys, flagellin and Nigericin were potent activators of CCL2, CCL5, CCL20, IP-10, GM-CSF and IL-8 release, with PolyI:C being the most potent stimulus. In atopic donor´s cells, PolyI:C-, flagellin- and Pam3Cys stimulation resulted in up-regulation of CCL5 and down-regulation of GM-CSF and IL-8 whereas Nigericin induced IL-1α, CCL20 and IP-10 expression. Stimulation of HNECs with aqueous pollen extracts (birch, timothy grass, ragweed) resulted in a weaker overall chemokine response. Release of IL-1β, CCL2 and CCL20 was detected in both, cells of atopic and non-atopic donors, however, non-atopic donor´s cells showed a higher pollen-induced chemokine release.

Conclusion

Conclusion: HNECs of allergic and non-allergic donors respond differentially to PAMPs and pollen derived mediators. This might highlight a role of the nasal epithelium as “master switch” in the pathophysiology of allergic airway disease.

P26 Metabolic Characterization Of Severe Allergic Phenotype To Profilin Linked To Respiratory Allergy

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Keywords: Respiratory Allergy, Food Allergy, Metabolomics, Profilin, Phenotype

Introduction

Respiratory allergy is a prominent disease which prevalence has dramatically increased in last decades. This is a heterogeneous disease driven by diverse immunological and inflammatory mechanisms, which to date are not fully understood. Previous results have reported that profilin, a minor allergen, was able to cause severe mediated reactions in oral provocations to patients sensitized to grass pollen. This fact has been linked to the high level of exposition to grass pollen allergen. The application of the -omics sciences, such as metabolomics towards respiratory allergy research might represent a potential tool to define and characterize the metabolic severe allergic phenotype. In this way, it can help to understand the molecular mechanisms involved and could represent a strategy to personalize medical treatment to sensitized patients. Metabolomics employs high throughput analytical techniques such as mass spectrometry (MS), to obtain a representative profile of metabolites present in a biological sample. Significant metabolites may represent potential biomarkers which could define different phenotypes.

Method

The aim of this study was to obtain and compare the metabolic profiles of non-allergic subjects and patients with respiratory allergy linked to food allergy using multiplatform techniques; gas chromatography coupled to MS (GC-MS) and liquid chromatography–MS (LC-MS).

Results

Plasma samples came from 4 hospitals in Spain. These were analysed using GC-MS and LC-MS techniques. Participants were subjected to an oral provocation using profilin. The first step was to make a multivariate prediction model using the extreme groups; non-allergic subjects and allergic patients that showed clearly systemic reactions based on the provocation test. The mathematical algorithm was used to classify the rest of the allergic patients into three different groups; mild, moderate and severe. Statistical significant

differences were found in both analytical platforms. Significant metabolites were identified using MS/MS experiments for LC-MS technique. Afterwards, metabolic changes were related to energy and bile acid metabolism, and changes in amino acids, sphingolipids, phospholipids and fatty acids were observed.

Conclusion

The present work demonstrates a new approach to study a complex disease such as the respiratory allergy. Further evaluation of selected metabolites is need. However, this might result in a new way to stratify patients and predict severe allergic reactions to profilin.

Saturday 27 January 2018

Oral abstract presentations

09:20 - 11:00: Food allergy: allergens and treatment

O09 SUBLINGUAL IMMUNOTHERAPY WITH RECOMBINANT MAL D 1 INDUCES IGE-BLOCKING ANTIBODIES

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Keywords: Birch Pollen-Related Apple Allergy, Sublingual Immunotherapy, Allergen-Specific IgG4 Antibodies,

Introduction

Birch pollen-related apple allergy (BPRAA) affects up to 70% of birch pollen-allergic individuals and results from immunological cross-reactivity between the major birch pollen allergen Betv1 and the structurally related major apple allergen Mald1. Still, the effects of allergen-specific immunotherapy (AIT) with birch pollen on BPRAA are controversial. We conducted a single center, double-blinded placebo-controlled pilot study including 60 Betv1-sensitized patients with BPRAA randomized to sublingual immunotherapy (SLIT) with recombinant (r) Mald1 (n=20), rBetv1 (n=20) or placebo (n=20). Serum samples were collected before and after the treatment period of 16 weeks. Notably, only rMald1-treated patients significantly improved BPRAA. To study the immune effects underlying this finding, we characterized SLIT-induced rMald1-specific IgG4 antibodies (Abs) in more detail, as the induction of this subclass indicates the activation of tolerance-inducing mechanisms.

Method

rMald1-specific IgG4 Abs in pre- and post-SLIT sera were quantified using ImmunoCAP. Their binding to rMald1 was assessed after pre-incubation of the post-SLIT sera with rBetv1 or rMald1 in competition ELISA. The IgE-blocking activity was characterized by their ability to reduce IgE-Mald1-complex formation in ELIFAB assays and Mald1-induced activation of basophils.

Results

Mald1-specific Abs increased significantly during SLIT with Mald1 and Betv1, respectively. IgG4-binding to Mald1 of post-Betv1-SLIT sera was completely abrogated after pre-incubation with rBetv1 and rMald1 indicating that all epitopes of Mald1 recognized by these Abs were also present on Betv1. In contrast, only Mald1 and not Betv1 completely abrogated IgG4-binding to rMald1 of post-rMald1-SLIT sera. Moreover, post-rMald1-SLIT sera inhibited both the formation of Mald1-IgE complexes and Mald1-induced basophil degranulation whereas post-rBetv1-SLIT sera showed a significantly limited capacity to block IgE-binding to rMald1.

Conclusion

Collectively, our data indicate marked differences between rMald1-specific IgG4 Abs induced by SLIT with rMald1 or rBetv1. Betv1-SLIT induced in the first line Betv1-specific, cross-reactive Abs whereas rMald1-SLIT-induced Abs were primarily Mald1-specific and displayed blocking capacity. These immunological findings accorded with the clinical improvement of BPRAA after rMald1-SLIT and indicate that functional blocking Abs are only induced by treatment with the food allergen.

O10 Contribution Of Conformational And Linear IgE Epitopes To Ara H 2-Specific IgE-Binding - In Vivo And In Vitro Studies

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Keywords: Ara H 2, IgE Epitopes, IgE-Binding, In Vivo,In Vitro

Introduction

Ara h 2 is the most important peanut allergen. Ara h 2 with reduced IgE-binding capacity is a promising candidate for specific immunotherapy of peanut allergy. Little is known about the role of conformational and linear IgE epitopes of Ara h 2. We aimed to define the patient-specific epitope profiles of Ara h 2-specific IgE.

Method

An Ara h 2 mutant (mt) lacking surface-exposed loops that contain most linear epitopes and the wild-type protein (wt) were expressed in the baculovirus insect cell system. Purified allergens as well as the natural protein (n) were reduced and alkylated (red/alk). Physicochemical characteristics were determined using MALDI MS, CD spectroscopy and N-terminal sequencing. IgE-binding was tested by direct and inhibition ELISA using sera of 54 Ara h 2 allergic children and adults. Female C3H/HeJ mice were sensitized orally with peanut extract and challenged intraperitoneally with the various proteins to determine their anaphylactogenic potencies.

Results

mt, wt and nAra h 2 showed the predicted masses and alpha helical structures. Complete reduction and alkylation and the ensuing destruction of the secondary structure was verified. IgE ELISAs revealed a significantly lower IgE-binding to the respective red/alk proteins ($p<0.001$), confirming the importance of conformational epitopes. Mt (no linear epitopes) revealed up to 70% ($p<0.001$) reduced IgE-binding in comparison with n and wtAra h 2, confirming the relative importance of linear epitopes. Results were confirmed with inhibition ELISA. A spearman rank correlation test revealed a weak but significant correlation between the recognition of linear epitopes and the level of Ara h 2 specific IgE ($r=0.305$, $p=0.025$) and a significant negative correlation between the recognition of conformational epitopes and the level of Ara h 2 specific IgE ($r=-0.5$, $p=0.0001$). In the anaphylaxis model, mice reacted with a temperature drop upon challenge with n, wt and mt proteins but not with the red/alk proteins, indicating that primary sensitization is targeted against conformational epitopes.

Conclusion

The obtained results indicate that conformational and linear epitopes are both important for Ara h 2 specific IgE-binding. Relative contributions of both types of epitopes to IgE-binding are patient-specific.
Supported by the Austrian Science Fund doctoral program W1248-B30.

O11 Investigation Of Immunological And Clinical Reactivities Of Parvalbumins, The Major Fish Allergens

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Keywords: Fish Allergy, Parvalbumins, Fish Allergy Diagnostics, Basophil Activation Test

Introduction

Fish allergy affects up to 2% of the world population and often causes severe life-threatening reactions such as systemic anaphylaxis. Major fish allergens parvalbumins (PVs) occur in two evolutionary sublineages, α and β, with bony fish β PVs being generally allergenic. Great diversity among fish PVs presents a challenge for establishing diagnostic methods and for analyzing the cross-reactivity among different fish species. The calcium-binding regions of PVs play a critical role in their IgE reactivity. Recently, a hypoallergenic derivative of carp parvalbumin with mutated calcium-binding sites has been developed for specific immunotherapy of fish allergy.

Method

We investigated the immunological and clinical reactivity of 11 PVs from different organisms (bony fish β PVs; cartilaginous fish α PVs; PVs from frog and chicken; mutant PV from carp) with 15 fish allergic subjects. Specific IgE was quantified by ELISA. Functional and clinical reactivities were analysed by basophil activation test (BAT) and skin prick test (SPT), respectively.

Results

β PVs showed higher allergenic activity in all three diagnostic tests (ELISA, SPT and BAT) compared to α PVs. PV-specific IgE, commonly used as a diagnostic marker for fish allergy, correlated poorly with the IgE cross-linking and degranulation capacities observed in BAT. Mutant carp PV showed a reduced IgE reactivity and capacity to activate basophils when compared with natural β PVs. Only 20% of the patients reacted to the mutant PV as compared to 60-90% reacting to natural β PVs in ELISA and BAT.

Conclusion

We demonstrated that BAT has the potential to confirm true fish allergy, predict cross-reactivity of PVs, and can possibly be used as an additional diagnostic tool in patients with inconclusive SPT and/or ELISA tests. Furthermore, this study highlights the possible importance of conformational IgE epitopes of PVs for inducing allergic reactions and confirms the effectiveness of the hypoallergenic PV mutant.
Supported by doctoral program W 1248-B30 (MCCA), the COST Action FA1402 (ImPARAS) and the MESR, Luxembourg.

O12 Genetic Biomarkers In Pollen-Mediated Food Reactions Useful For The Characterization Of Severe Allergic Profile.

Leticia Mera[1], Paloma Fernández[1], Domenico Rosace[1], Isabel Adoración Martín[2], María Teresa Belver[3], Tania Ramos[3], Victoria Múgica[3], María Del Carmen Domínguez[4], Ángel Vega[4], Guadalupe Marco[5], Manuel De Pedro[5], Leticia Sánchez[5], María De Las Mercedes Arnás[6], Marcela Santaolalla[6], Montserrat Fernández-Rivas[5], María Isabel Alvarado[4], Carlos Blanco[3], María Marta Escribese[2], Domingo Barber[1]

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Introduction

The prevalence of allergic diseases and their severity have been increased worldwide, so it is necessary to improve the classification of allergic patients in order to give them an effective treatment. In some areas of Spain with high levels of grass pollen, allergic patients may lead to sensitization to minor allergens, such as profilin, and surprisingly, some of them develop severe profilin-mediated food reactions. Our objective here was to identify genetic biomarkers useful to improve the classification and treatment of patients with a higher probability of developing severe reactions.

Method

22 patients were classified in 4 groups, according to their reactions to pure profilin provocation: non-allergic (n=6), mild (n=5), moderate (n=6) and severe (n=7). RNA extraction was performed on ficoll-isolated PBMCs using the RNeasy® Mini Kit (Qiagen) and its quality was assessed with Experion RNA StdSens analysis kit (Bio-Rad). The gene expression profile of all the samples was analyzed using GeneChip® WT PLUS Reagent Kit (Thermo Fisher Scientific) and two specific softwares: Affymetrix® Expression Console™ and Affymetrix® Transcriptome Analysis Console (TAC). The microarray results were validated by quantitative real-time PCR (qPCR) and a Gene Set Enrichment Analysis (GSEA) was also performed

Results

A total of 596 transcripts were identified as being significantly different between all experimental groups. The biggest differences were observed between mild and severe groups and moderate and severe groups. In order to characterize the severe profile, we carried out a GSEA. The results show that most representative genes sets were related to coagulation, complement, DNA repair, fatty acid metabolism and some interleukins pathways. Moreover, these pathways were significantly increased in mild and moderate phenotypes.

Conclusion

In the course of our study we found out that severe patients have a different transcriptomic profile compared to mild and moderate patients and there are transcripts involved in different pathways that could be potential biomarker as they increase / decrease in relation to the degree of allergic inflammation. These findings might lead to improve the diagnosis of this type of allergic patients and their treatment.

Saturday 27 January 2018

Oral abstract presentations

17:50 - 19:30: Microbiome

O13 Protective Bacterial-Derived Signals Modify The Respiratory Response To Viral Infection

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Introduction

Excessive immune responses to viral infection result in asthma and COPD exacerbations and acute respiratory distress syndrome (ARDS). Recently it was suggested by us and others that signals derived from the commensal microbiota may protect against respiratory viral infection and subsequent inflammatory sequelae.The aims of this study are to determine the effect of commensal bacteria and their components on influenza (IFV) viral replication and on associated respiratory anti-viral immune responses.

Method

Female BALB⁄c mice were infected with 100 PFU of IFV strain PR8 (H1N1) and monitored for morbidity and mortality over 10 days. Subgroups of mice received Bifidobacterium longum, its isolated cell wall components or placebo intranasally at two hours prior to IFV infection and treatments were repeated intranasally on days 1, 3 and 5 post-infection. One group was not treated prior to infection, but treatment started 1 day post-infection. Lung tissue, broncho alveolar lavage fluid and serum samples were obtained at days 3, 5, and 10. Viral titre, total and differential inflammatory cell counts, cytokines, chemokines, interferons and markers of lung barrier damage (lactate dehydrogenase, albumin) were quantified.

Results

The number of animals surviving to the end of the experiment was significantly higher in the treated group compared to the placebo group.This was associated with a significantly reduced viral titre and a potent induction of cytokines, chemokines and type III interferon at day 3 in treated animals. However, by day 5 post-infection, placebo-treated animals displayed the highest levels of cytokines, chemokines and interferons, which did not protect against the further development of infection. Markers of barrier damage were also elevated at day 5 for placebo-treated animals, but not for bacterial-treated animals. The intact bacterium and its isolated cell wall fraction were both protective, although the cell wall fraction was more effective. Administration of the cell wall fraction 24 hours after viral infection was equally effective in reducing viral titre and associated lung inflammation.

Conclusion

This study suggests that regulated, appropriate and efficient early induction of lung immune responses by certain bacterial components results in improved anti-viral defence and protection against lung damage. This study also supports the concept that the composition and activity of the respiratory microbiota may influence viral immune responses in asthma and COPD patients.

O14 Oral Exposure To Beta-Lactoglobulin-Derived Peptides And A Specific Mixture Of Fructo-Oligosaccharides And Bifidobacterium Breve M-16V Facilitates A Tolerance-Prone Immune Environment And Whey Allergy Prevention In Mice

Atanaska I Kostadinova[1], Alba Pablos-Tanarro[2], Mara A. Diks[3], Betty C. Van Esch[1], Johan Garssen[1], Leon M. Knippels[1], Linette E. Willemsen[3]

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Keywords: Beta-Lactoglobulin, Cow's Milk Allergy, Dendritic Cells, Oral Tolerance, Peptides

Introduction

Cow's milk allergy (CMA) prevails in infants and brings increased risk of developing other allergic diseases. Oral administration of specific beta-lactoglobulin (BLG)-derived peptides (PepMix) and a specific blend of short- and long-chain fructo-oligosaccharides and Bifidobacterium breve M-16V (FF/Bb) was found to

partially prevent CMA development in mice. In this study, we aimed to expand the knowledge on the preventive potential and the underlying mechanisms of this approach.

Method

Three-week-old female C3H/HeOuJ mice were orally exposed to PepMix ± FF/Bb prior to a 5-week oral sensitization with whole whey and cholera toxin as an adjuvant. The acute allergic skin response was determined after an intradermal challenge with whole whey protein. Following an oral challenge with whey, regulatory T cells (Tregs) in the small intestine lamina propria (SI-LP) and mRNA expression of immune markers in the Peyer's patches (PP) were investigated. The early impact of PepMix and FF/Bb interventions on the immune system during the oral tolerance (OT) induction phase was investigated after the last OT administration.

Results

Pre-exposing mice to PepMix+FF/Bb partially prevented the acute allergic skin response compared to PBS and increased Tregs and activated T cells in the SI-LP compared to sham-sensitized mice. It also increased the mRNA expression of Tbet over GATA3 in the PP of whey-sensitized mice. Directly upon the 6-day OT phase, FF/Bb intervention enhanced caecal content levels of propionic and butyric acid in PepMix-fed mice and the former was positively correlated with Foxp3+ cell numbers in the colon. In the PP of PepMix+FF/Bb exposed mice, IL-22 mRNA expression increased and IL-10 followed the same tendency, while the Foxp3 expression was increased over GATA3 and RorγT. In the colon, the Tbet mRNA expression increased over GATA3, while IL-22 decreased. In addition, the Foxp3+/GATA3+ and regulatory/effector T cell ratios in the MLN and the CD11b+/CD11b- conventional DC ratio in the SI-LP were increased.

Conclusion

In conclusion, the FF/Bb diet facilitates the capacity of the specific BLG-peptides to partially prevent the allergic response after sensitization to whole whey protein, possibly by creating a tolerance-prone environment during the OT phase. Such a dietary intervention might contribute to tailoring successful strategies for CMA prevention.

O15 Diet-Induced Obesity Potentiates IgE-Mediated Food Allergic Reponses

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Keywords: IgE-Mediated Food Allergy Type 2 Immunity Obesity

Introduction

The past few decades have witnessed an increase in the prevalence of allergic diseases and number of obese individuals in tandem. Epidemiological studies suggest that obese children are more likely to have IgE mediated food allergies. However, the mechanisms by which diet-induced obesity may modulate susceptibility to IgE mediated food allergies remain unknown. Employing an experimental model of IgE mediated food allergy, we sought to test alterations in food allergen sensitization, immunological responses and gut barrier integrity in food allergic mice fed either a control diet or a diet rich in sugars and fat.

Method

Mice were fed a Western diet for 12 weeks prior to epicutaneous food allergen sensitisation on an atopic dermatitis like skin lesion followed by intra-gastric allergen challenge. Allergic manifestations in response to oral food allergen challenge were assessed by a clinical allergy score. Total- and allergen specific IgE levels were measured by ELISA. Accumulation of mast cells in the small intestine was quantified by flow cytometry or on tissue sections by means of chloroacetate esterase staining. In ongoing studies, we are currently analysing the impact of a Western diet on gut barrier integrity and allergen uptake.

Results
While no changes in food allergen sensitization through an atopic dermatitis-like skin lesion were observed between the two groups, mice fed a Western diet presented with increased serum IgE levels and mucosal mast cell infiltration, two hallmarks of intestinal food allergy, that manifested in an overall increased clinical allergy score compared to food allergic mice on a control diet.
Conclusion
Consumption of a Western diet promoting obesity potentiates the magnitude of IgE-mediated food allergic responses in an experimental model of food allergy.

O16 Exposure To Soil In The Living Environment Modifies Gut Microbiota And Enforces Immune Tolerance In A Mouse Model
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Keywords: Microbiota, Allergy, Biodiversity, Mouse Asthma Model
Introduction
Sufficient exposure to natural environments, in particular soil and its microbes, has been suggested to be protective against allergies. The research performed so far provides only some clues to understanding the connection between immune tolerance and microbial colonization from the environment. Here we aim at gaining more direct evidence of the “environment-microbiota-health axis” by studying the colonization of gut and lung microbiota in mice, after exposure to soil, and by examining the immune status in both a steady state situation and during allergic inflammation.
Method
The small intestinal, faecal and lung microbiota of mice kept on a clean bedding or in contact with soil was analysed, and the data were combined with immune parameters of the mice. Both healthy mice and those exposed to the murine asthma model were used in the study.
Results
We observed marked differences in the small intestinal and faecal microbiota composition between mice housed on a clean bedding or in contact with soil, with a higher proportion of Bacteroidetes relative to Firmicutes in the soil group. The housing environment also influenced mouse intestinal gene expression as shown by up-regulated expression of the immunoregulatory proteins IL-10, Foxp3 and CD86 in the soil group. Importantly, using the murine asthma model we found that exposure to soil polarizes the immune system towards Th1 and a higher level of anti-inflammatory signalling, alleviating Th2 type allergic responses. The inflammatory status of the mice had a marked influence on the composition of the gut microbiota, suggesting bi-directional communication along the gut-lung axis.
Conclusion
Our results provide direct evidence of the role of environmentally acquired microbes in protecting against Th2 driven inflammation, which relates to allergic diseases.

Saturday 27 January 2018
21:00 - 22:00: Poster Session II
Topic 4 - Allergens and allergic inflammation

P27 House Dust Mite Exposure Primes Asthmatic Bronchial Epithelium For Inflammasome Activation After Rhinovirus Infection

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Keywords: Inflammasome, Asthma, House Dust Mite, Bronchial Epithelium, Pattern Recognition Receptors

Introduction

Inflammasomes are important biological complexes participating in the pathophysiology of several inflammatory disorders. There is limited knowledge of the role of inflammasome activation in human asthmatic bronchial epithelium. We aimed to understand the involvement of inflammasome activation during exposures to house dust mite (HDM) and human rhinovirus (HRV16) in the pathogenesis of asthma and upon viral infections.

Method

Primary human bronchial epithelial cells (HBE), bronchoalveolar lavage fluid (BAL) samples and bronchial biopsies from healthy and asthmatic patients at baseline and/or after infection with HRV16 were analysed using next generation sequencing, multiplex immunoassays and confocal microscopy. Air-liquid interphase cultures of HBE treated with HDM and HRV16, mouse models of HDM-induced allergic airway inflammation and poly-IC-induced lung inflammation mimicking viral-induced lung inflammation were used to examine inflammasome activation and expression of inflammasome-related molecules.

Results

We found striking changes in the expression of inflammasome-related genes in HBE after HRV16 infection in healthy and asthmatic subjects. Changes in inflammasome- and virus-related pathways and their functions were accompanied with full activation of inflammasome, represented by formation of ASC specks, increased secretion of IL-1ß, which was blocked by caspase-1 inhibitor. Notably, release of mature IL-1ß was limited to the apical surface of polarized cells, which corresponded with ex vivo apical expression of ASC in human lung biopsies and increased IL-1ß secretion into BAL of asthmatic patients. Upregulation of DDX58 (RIG-I) gene expression upon HRV16 infection and HDM exposure in HBE, altogether with ex vivo apical expression of RIG-I protein in epithelium in human lung biopsies highlight that RIG-I is a candidate for inflammasome sensor of viral infections in HBE. Additionally, increased expression of several inflammasome-related genes such as Aim2, Casp1, Asc and Il1b confirms inflammasome signature of lungs from mouse models of HDM-induced asthma and poly-IC-induced lung inflammation with specific upregulation of viral sensors namely Ddx58 and Mda5 only in poly-IC model mimicking viral-induced lung inflammation.

Conclusion

These data demonstrate house dust mite priming enhances inflammasome activation and apical accumulation of inflammasome-related molecules in asthmatic bronchial epithelium after rhinovirus infection.

P28 Immunoglobulin Class Switching Der P 1-Specific B Cells In Immune Tolerance During 2-Year House Dust Mite-Specific Immunotherapy

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Keywords: Immunoglobulin, B Cells, Allergen-Specific, Immunotherapy,

Introduction

B cell tolerance during allergen-specific immunotherapy (AIT) is little known especially in long-term followed allergen-specific B cells. We investigated Der p 1-specific B cell responses during 2-years house dust mite (HDM) AIT and compared between clinically responder and non-responder patients.

Method

Peripheral blood mononuclear cells obtained from 25 patients before, after 10 weeks, 30 weeks and >2 years of AIT were analyzed. Der p 1-specific B cells were detected by double fluorescence-labeled allergen method and flow cytometry. The characteristics of IgA, IgG1 and IgG4-switched Der p 1-specific B cells were investigated and correlated to clinical response to AIT. Clinical response was observed by visual analogue scale (VAS).

Results

Sixteen of 25 patients accomplished the study. Eleven patients showed a successful clinical response to AIT as determined by decreased VAS from 79.5±9 to 13.6±1.0, whereas it did not show any significant change from 83±16.4 to 62±10.9 in five non-responder patients. Skin prick test reactivity to HDM decreased in responders from 7.0±1.3 mm to 2.7±0.5 mm and in non-responders from 7.6±1.1 mm to 5.6±0.5 mm. After 2 years, increased frequencies of Der p -1 specific B cells were observed in responder patients (0.049±0.012% to 0.165±0.030%) and non-responder patients (0.060±0.012% to 0.093±0.032%). Increased the frequency of Der p 1-specific IgG4+ B cells were shown in responder patients (0.0009±0.0006% to 0.0270±0.0065%) and non-responder patients (0.0002±0.0002% to 0.0086±0.024%). Frequency of Der p 1-specific IgA+ B cells increased in responder patients (0.007±0.003% to 0.019±0.003%) but did not change in non-responder patients (0.010±0.004% to 0.010±0.002%) after 2 years of AIT. There was no difference observed in Der p 1-specific IgG1 B cells during AIT. Only Der p 1-specific IgG4+ B cells revealed significantly higher levels compared to non-responder patients after 2 years (P = 0.01). The frequency of IL-10+, IL-1RA+ and IL-10+IL-1RA+ Breg cells was higher in responder compared to non-responder patients after 2 years. Increased frequency of Der p 1-specific IgG4+ B cells and IL-10-producing Breg cells significantly correlated with improved clinical symptoms over the course of AIT.

Conclusion

AIT induces allergic-specific B cells immune tolerance in the responder patients with increased IgA and IgG4 expressing Der p 1-specific B cells particularly after 2 years of AIT.

P29 Novel Bet V 1 – Mannan Neoglycoconjgates Activate Complement And Induce Potent Specific Immune Responses After Epicutaneous Immunization.

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Keywords: Neoglycoconjugates, Epicutaneous Immunotherapy, Bet V 1

Introduction

Today, the only curative approach to treat allergic diseases is allergen-specific immunotherapy, however it is lacking efficacy and non-invasive methods of effective antigen delivery. In recent clinical trials, epicutaneous immunization via intact skin reduced allergic symptoms in patients, but required high amounts of purified allergen to achieve a therapeutic effect. One promising approach to increase efficacy is to target dendritic cells (DCs) via C-type lectin receptors (CLR) using allergen-neoglycoconjugates. Here, we show that coupling of mannan from saccharomyces cerevisiae to the major birch pollen allergen Bet v 1 results in high molecular weight conjugates with superior complement activation and in vivo immunogenicity.

Method

Bet v 1 - mannan conjugates were generated using mild periodate oxidation of polysaccharide and coupling to allergen via reductive amination. We assessed conjugates immunogenicity in vitro in bone marrow-derived dendritic cells (BMDCs) and complement activation in human serum was showed by western-blotting of cleaved fragment C3a. In vivo immunogenicity was evaluated in BALB/c mice after epicutaneous immunization via laser-generated micropores.

Results

We report that Bet v 1 - mannan drives dendritic cell activation and enhances antigen uptake in comparison to unconjugated Bet v 1. Neoglycoconjugates induced strong activation of a complement pathway in a dose-dependent manner, whereas soluble mannan showed only modest cleavage of C3. While soluble Bet v 1 was non immunogenic after epicutaneous or intradermal immunization, Bet v 1 – mannan induced potent humoral and cellular immune responses.

Conclusion

We demonstrated that Bet-mannan activated dendritic cells and facilitated antigen uptake via C-type lectin receptors. Moreover, Bet v 1 - mannan conjugates activated complement pathway in vitro in human plasma, which may contribute to its superior immunogenicity observed in mice. Due to its ability to induce strong immune responses Bet-mannan may be an attractive candidate for allergen-specific immunotherapy.

P30 Glycosylated Nanostructures Including Pru P 3-Epitopes Induce Tolerance To Peach In Murine Model

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Keywords: Immunotherapy, Peach, Pru P 3, Murine Model

Introduction

Food-specific immunotherapy is a promising treatment for LTP-syndrome. Immunotherapy using T-cell peptides from Pru p 3 together with CpG, as an adjuvant, have shown to induce protection from anaphylaxis in a murine model of peach allergy, which persists after ending treatment. Functionalization with multivalent mannose ligands can enhance dendritic cell (DC) targeting and a Th1/Treg response. We propose a novel sublingual immunotherapy (SLIT) to peach with mono- and tetravalent systems that combines a Pru p 3 T-cell peptide with mannose dendrimers.

Method

LTP-peach anaphylactic mice were treated sublingually with different concentrations of D1ManPrup3 or

D4ManPrup3. Mice were challenged intraperitoneally with Pru p 3 one/four weeks after SLIT. Tolerance was assessed by changes in body temperature, determination of Pru p 3-sIgE and -sIgG1 by ELISA and ELISpot and lymphocyte proliferative response, Treg cell (CD4+CD25highFoxP3+) and DC (CD11c+CD103+) percentages and cytokine production by flow cytometry.

Results

Only mice receiving monovalent dendrimers D1ManPrup3 at 2nmol were protected from anaphylaxis, with no change in body temperature and a significant decrease of Pru p 3-sIgE and -sIgG1 antibodies and secreting cells compared to non-treated. Moreover, a significant decrease of Pru p 3-specific CD4+ T-cells and an increase of CD4+CD25highFoxP3+cells were found, alongside shifts to a regulatory pattern (IL10+/IFNγ+) in CD4+ T-cells and CD11c+CD103+ DC. These changes were maintained for four weeks after stopping treatment.

Conclusion

The monomeric glycodendrimer, D1ManPrup3, represents a promising new sIT approach that does not require additional adjuvant. It is easily synthesized, induces protection from anaphylaxis and persists in suppressing clinical symptoms after ending treatment.

P31 Expression And Characterization Of Recombinant Wild-Type-Like Versions Of The Major Parietaria Allergens, Par J 1 And Par J 2.

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Keywords: Recombinant Allergens, Par J 1, Par J 2, Parietaria

Introduction

Parietaria judaica is one the most common pollen allergen sources in the Mediterranean area with a long period of pollination from February to November and therefore can be considered a perennial allergen. Par j 1 and Par j 2, the major Parietaria allergens, belong to the family of lipid-transfer proteins and are recognized by more than 80% of Parietaria-allergic patients.

Method

We expressed and purified recombinant Par j 1 and Par j 2 molecules which mimic the structural and immunological features of the natural allergens. Recombinant allergens were expressed in baculovirus-infected insect cells (BvPar j 1 and BvPar j 2) as well as in Escherichia coli (EcPar j 2) and purified by affinity chromatography.

All recombinants were characterized by SDS-PAGE under reducing and non-reducing conditions, the molecular masses determined by MALDI MS matched the masses calculated according to the amino acid sequences of all proteins and the data was confirmed by gel filtration.

Results

The analysis by circular dichroism (CD) showed that BvPar j 1 and BvPar j 2 assumed mainly α-helical structures whereas EcPar j 2 contained mainly unordered species.

Comparison of folded BvPar j 2 and unfolded EcPar j 2 showed a significant prevalence in IgE –reactivity of a folded protein in ELISA experiment using sera from 27 Parietaria allergic patients from Mediterranean region.

Rat basophil leukemia cells were used in order to compare the allergenic activity of various concentrations of BvPar j 1, BvPar j 2 and EcPar j 2 in terms of their capability to induce mediator release. EcPar j 2 and BvPar j 1 revealed weaker allergenic activity in low doses comparing to BvPar j 2. BvPar j 2 also showed a significantly stronger capacity to inhibit Parietaria judaica extract comparing to BvPar j 1 in inhibition ELISA.

Conclusion

Our results thus show that the eukaryotic expression of Par j 1 and Par j 2 in insect cells yielded folded

recombinant proteins with superior IgE reactivity over E. coli expressed Par j 2. BvPar j 1 and BvPar j 2 can now be used to study the three-dimensional structure of the allergens and for IgE-based diagnostic testing for identifying Parietaria allergic patients.

P32 Biomarkers Of 12 SQ House Dust Mite Sublingual Immunotherapy (SLIT)-Tablet Treatment After Nasal Allergen Challenge

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Keywords: HDM SLIT, Nasal Allergen Challenge

Introduction

Allergy immunotherapy alters the underlying immunologic mechanisms that cause allergic rhinitis (AR). This phase 1b trial examined the ability of SQ house dust mite (HDM) sublingual immunotherapy tablet (12 SQ-HDM dose) to alter serum biomarkers and responses to HDM nasal allergen challenges (NAC).

Method

In this exploratory double-blind, multicenter, randomized trial (NCT01852825), 23 subjects (aged 18-55 years) with HDM AR with/without mild, controlled asthma received daily 12 SQ-HDM or placebo for 12 weeks. The primary end point was change in HDM-specific IgG4 and IgE blocking factor. NAC were conducted at 2 weeks pre-treatment (baseline) and weeks 8 and 12. Nasosorption for IL-5 levels was performed at 1h pre-NAC, 15 minutes post-NAC, and 6.5h post-NAC. Nasal curettage for mucosal gene expression was conducted at baseline and 6.5h post-NAC. Visual Analog Scales of nasal symptoms were recorded by subjects in relation to NAC.

Results

HDM-specific IgG4 and IgE blocking factor significantly increased with 12 SQ-HDM versus placebo at weeks 8 and 12. No significant differences in nasosorption IL-5 or eosinophil mucosal gene expression were observed between 12 SQ-HDM and placebo. Nasal symptoms up to 1 hour post-NAC significantly decreased from baseline to week 12 for 12 SQ-HDM versus placebo. There was no effect on later (2-6 hours post-NAC) symptoms.

Conclusion

Treatment with 12 SQ-HDM induced HDM-specific IgG4 and IgE blocking factor by week 8, increasing at week 12. Nasal symptoms in the early phase after NAC significantly improved. However, there was no effect on NAC-induced late phase symptoms, mucosal IL-5 and eosinophil-associated gene expression.

P33 Jug R 6 But Not Jug R 2 Is The Allergenic Vicilin Present In Walnut Kernels Responsible For IgE Cross-Reactivities To Other Tree Nuts And Seeds

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Keywords: Allergen; Component Resolved Diagnosis; Food Allergy; Jug R 6, Mass Spectrometry; Vicilin; 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 the 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. The 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 the identification of the protein sequence that displayed only 44% identity to Jug r 2. The newly identified vicilin was designated by the IUIS committee as Jug r 6. Sequence analysis revealed two cupin domains typical for vicilin 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 alpha-helices and beta-sheets. The allergen was recognized by serum derived IgE antibodies from 26% of walnut allergic patients. 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 in part responsible for cross-reactivities among tree nuts and seeds.

P34 Monitoring Allergen-Specific Immunotherapy In Vivo And In Vitro: A Three-Year Follow-Up Of Patients Sensitized To Grass Pollen, House Dust Mites And Insects

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Keywords: Immunotherapy, Immune Response, Basophile Degranulation

Introduction

Allergen immunotherapy (AIT) is the only causative treatment of allergic diseases with strong evidence of efficacy and safety. The need of biomarkers assessing the long-term efficacy is pivotal and an aim of many allergy and clinical immunology studies. Herein we evaluate the impact of three-year Subcutaneous Immunotherapy (SCIT) on selected markers of immunological response of atopic patients sensitised to grass pollen, house dust mites and insects.

Method

Forty-eight patients with mean age of 31 years (range 9-64 years), 16 women and 32 men, were followed-up for 36 months. Grass pollen SCIT was applied to 17 patients, house dust mite SCIT to 17 patients and venom immunotherapy to 9 patients. The levels of allergen-specific IgE (gx1, d1, i1, i3) and allergen-specific

IgG4 (Gg6, Gd1, Gi1), allergen-induced basophile degranulation and the value of skin-prick test (SPT) were evaluated before the immunotherapy and after 6, 12, 24 and 36 months of SCIT. Flow cytometric determination of cytokines (IL-2, IL-4, IL-5, IL-10, TNF-a and IFN-g) was also performed. The symptom-medication score (SMS) and visual analog scale (VAS) were used to assess the patients' perspective.

Results

Significant increase of specific immunoglobulins and decrease of SMS, VAS and nonspecific basophil activation (negative control) were noted ever since the sixth month of SCIT. Strong correlations between IgE and IgG4 and between the value of basophil degranulation were observed. The dynamic of specific immunoglobulins after 36 months of SCIT was different – the IgG4 level was significantly increased while the quantity of IgE was decreased and correlated with the percentage of degranulated basophils. The value of SPT, SMS and VAS were notably diminished on the 36th month of follow-up. The SPT results also showed a correspondence with the basophile test. Investigation of the cytokines confirmed a change in the Th1/Th2 profile of the treated patients, however not so cogently as the other in vitro methods.

Conclusion

The allergen-specific immunotherapy is a complex process regarding modification of the immune response. The course of decreasing levels of IgE, basophil activation, SPT, SMS and VAS simultaneously with the increasing IgG4 quantity in patients after three years of SCIT is markers of its efficacy. BAT could be a biomarker of clinical response and basophil modulation can result in a better clinical control but still needs standardization.

Saturday 27 January 2018

21:00 - 22:00: Poster Session II

Topic 5 - Adaptive immunity

P35 Lung-Regulatory T Cells Expressing PPAR-Gamma: A Source Of IL-10 During Allergic Inflammation?

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Keywords: Tregs, PPAR-Gamma, IL-10, Lung, Allergic Inflammation

Introduction

Regulatory T cells (Tregs) play a critical role in immune response control and Foxp3 is the master transcription factor to their development and function. According to their origin, Foxp3 Tregs include subpopulations of Helios+ and Helios- cells, and recently, several markers have been also associated with their specialized functions. Particularly, adipose tissue-Tregs express the peroxisome-proliferator-activator receptor (PPAR-gamma) and produce high levels of IL-10. So far, the existence of PPAR-gamma Tregs in other inflamed tissues, as well as their origin, has not been described, therefore we investigated whether these cells might drive the regulation of lung allergic inflammation by local IL-10 production.

Method

Male BALB/c mice were sensitized twice by intraperitoneal ovalbumin (OVA) injection. Lung inflammation was induced by intranasal OVA challenges on five consecutive days. Control mice were challenged with PBS, and samples were collected 24 hours after the final challenge. Tregs, based on their Foxp3 and Helios expression, and their PPAR-gamma expression were evaluated in lung cells by flow cytometry.

Results
PPAR-gamma was expressed in lung-Tregs and its expression was significantly reduced in OVA-challenged mice (5% ±0.524 control vs 2.5% ±0.516 OVA mice, n=6; P<0.05). Approximately 60% of PPAR-gamma Tregs expressed Helios in both control and OVA mice, indicating their thymic origin. IL-10 production was 3-fold upregulated in PPAR-gamma Tregs in response to allergen. PPAR-gamma expression in both Helios+ and Helios- Tregs was significantly reduced in allergic mice compared to control (P<0.01). Helios+ PPAR-gamma Tregs produced more IL-10 than Helios- PPAR-gamma Tregs in both, allergic (21% vs 12%, respectively, P<0.05) and control (19% vs 3%, respectively; P<0.01) groups. Interestingly, allergic mice showed a significant increase in the IL-10 production by Helios- PPAR-gamma Tregs compared to control (P<0.01), whereas no differences were found in Helios+ PPAR-gamma Tregs.
Conclusion
These findings for the first time demonstrate that PPAR-gamma Tregs exist in healthy lung and becomes downregulated upon allergic inflammation. Lung inflammation increases IL-10 production by Helios- PPAR-gamma Tregs, which might play an important role in their ability to control asthmatic/allergic inflammation.

P36 Assessing Local And Systemic Humoral Immune Responses Under Real-Life Pollen Exposure Using Panel Study Cohorts
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Keywords: Allergy, Pollen Exposure, Immune Response, Immunglobulins, Panel Study
Introduction
Pollen is a major are one of the main causes of allergic airway diseases worldwide. However, the kinetics of the immune response under prolonged exposure is still unclear. Moreover, although symptoms correlate with pollen, there is no information about relevant exposure thresholds. Even less is known about potential effects of pollen exposure on non-allergic individuals. Aim of the study was to longitudinally assess effects of pollen exposure on the humoral immune response and on respiratory symptoms.
Method
Airborne pollen concentrations were monitored daily using Hirst-type traps and an automated pollen sampler. Symptoms were monitored daily by a symptom score app in two cohorts (n=8 each): (i) a pollen-sensitised allergic rhinitis (AR) cohort and (ii) a cohort of healthy volunteers. Immune monitoring occurred over the course of one year and included the determination of immunoglobulins and proinflammatory cytokines/chemokines in nasal secretions and serum, flow-cytometric characterisation of nasal immune cell infiltrates and determination of ILC2 numbers in peripheral blood. All data were analysed using multi-variate and time-series analyses to check for relationships between symptoms, immune mediators and airborne pollen concentrations.

Results

Nasal total and Bet v 1-specific IgE levels were significantly higher in allergic rhinitis patients than in non-allergic subjects. In non-allergic subjects, total nasal immunoglobulins did not change significantly during pollen exposure. In allergic patients, nasal IgA, IgG1 and IgG4 levels were significantly lower in the pollen season as compared to off-season levels. Flow cytometry of nasal curettages indicated an influx of neutrophils and eosinophils, but no monocytes, during pollen season. Most strikingly, both allergic and non-allergic subjects exhibited symptoms, which in all cases correlated with airborne pollen concentrations. Specifically, in non-allergic subjects, nasal symptoms were the most prevalent, whereas in pollen allergic patients nasal as well as pulmonary symptoms dominated. There was a consistent positive correlation of symptoms and airborne pollen concentrations of the previous day and this delay effect lasted up to approximately two weeks in both cohorts.

Conclusion

Nasal IgA and IgGs might be play an important role in the control of the local allergic immune response. Airborne pollen influences allergic patients as well as non-allergic subjects.

P38 A Jurkat Based NF?B-EGFP INKT Reporter Cell Line To Evaluate The Interaction Of Food-Derived Lipids With INKT Cell Receptors

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Keywords: Reporter Cell Line, INKT Cells, Jurkat Cells, TCR Cloning, Lipids

Introduction

In contrast to conventional T lymphocytes invariant natural killer T cells (iNKTs) recognize lipid-based antigens presented by the class I MHC homolog CD1d. Their TCR receptor consists of an invariant α-chain paired with a restricted repertoire of β-chains. It is known that recognition of iNKT antigens mostly occurs via the α-chain. Structural studies showed that the β-chain is responsible for the contact with the CD1d molecule and does not get into direct contact with antigens. However, the β-chain can indirectly impact on the binding affinity. Upon activation, iNKTs can significantly affect immune responses by promoting the secretion of Th1, Th2 or Th17 immune regulatory cytokine patterns. So far, iNKTs have been identified as important players in different types of immune responses. However, the role of iNKTs in a food allergic reaction is not well understood.

Method

An iNKT reporter system was engineered by introducing the human iNKT receptor into a human leukemic Jurkat T cell line carrying an NF-kB-driven fluorescent transcriptional reporter construct (Jkt-iNKT). Appropriate antigen presenting cells (APC) were generated by expression of human CD1d in the murine thymoma cell BW (BW-CD1d). Reporter induction (NF-kB-driven eGFP-expression) was measured by flow cytometry. The specificity and sensitivity of our system was compared with the murine DN32 hybridoma iNKT cell line (IL-2 ELISA as a readout system). Food-derived lipid extracts and separated lipid fractions were obtained by Folch extraction and preparative thin layer chromatography (TLC), respectively. Lipid samples were screened applying a plate bound assay with recombinant CD1d as well as co-culture assays with Jkt-iNKT and BW-CD1d cell lines.

Results

Jurkat cells stably expressing the human iNKT TCR receptor (Jkt-iNKT) were generated and shown to specifically react with iNKT antigens presented in the context of CD1d. Detection limit for well-known iNKT cells antigens (α-GalCer and OCH) were similar for Jkt-iNKT and DN32 cell lines. Different food-derived lipid fractions from hazelnut, walnut, sunflower, and buckwheat were separated and analyzed by TLC.

Conclusion

Our iNKT cell reporter cell line proved to be a useful tool (regarding readout and feasibility) to study the activation capacity of lipid molecules to activate human iNKT receptors. In addition, our reporter system is remarkably faster and cheaper as compared to assays based on murine hybridoma iNKT cell lines (which require cytokine measurement by ELISA).

P39 Characterisation Of An Angiogenesis-Promoting B Cell Subset

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Keywords: Angiogenesis, B Cells, IgG4

Introduction

Angiogenesis is the process of the new blood vessel formation, which is necessary for wound healing, growth, organogenesis, embryonic and fetal development, chronic inflammation and tumor formation. The role of B cells in angiogenesis has not been studied in detail.

Method

Human B cells were immortalized by transduction with BCL6 and BCL-XL, allowing long-term in vitro expansion of B cells. Angiogenesis-related genes and proteins were studied by qPCR, Bio-Plex System and ELISA. The formation of capillary-like structures by human umbilical vein endothelial cells (HUVECs) on basement membrane extract (Matrigel) was assessed in vitro to determine whether the factors released by B cell clones may promote angiogenesis.

Results

Based on the NGS data, we identified two clusters of B cell clones differentially expressing angiogenesis-related cytokines. Cluster 1 consists of IgG4+ B cell clones characterized by high expression of angiogenesis-related genes and cluster 2 – IgG1+ and IgG4+ clones with low expression of angiogenesis-related genes. Differential expression of angiogenesis-related cytokine genes was confirmed in qPCR and at protein level. Higher angiogenesis-related cytokine release was detected in clones from cluster 1 as compared to cluster 2. Tube formation was supported by the factors present in the supernatants from angiogenic B cell clones and significantly higher compared to the rest of the clones.

Conclusion

Our study revealed the potential role of B cells in angiogenesis. Further research will focus on characterization of angiogenesis-promoting B cells.

P40 The Effect Of Human Rhinovirus On T Regulatory Cells And Immune Tolerance

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Keywords: T Regulatory Cells, Rhinovirus, Immune Tolerance

Introduction

Respiratory infections with human rhinoviruses (HRV) are strongly associated with asthma exacerbations and pose a severe health risk for allergic and asthmatic individuals. How HRV infections and chronic allergic diseases are exactly 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 T regulatory cells.

Method

Tregs were were isolated from blood of healthy or allergic donors and sorted according to their flow cytometric profile CD4+CD3+CD25+CD127- and were stimulated with HRV16 with multiplicity of infection of 10 (MOI10). Viral load was measured with (+) strand and the copy number by qPCR. Additionally, Tregs were sorted from PBMCs before infection, and three days after infection from healthy and asthmatic individuals, who were experimentally infected with HRV16, and analyzed with next generation sequencing.

Results

It was demonstrated for the first time that rhinovirus can be detected inside Tregs after infection and that in both healthy and asthmatics donors the copy number of HRV16 increases in the first 8 hours. In our in vivo study, we found that Tregs from asthmatic individuals and healthy individuals have quite a large amount of differentially expressed genes. In asthmatics, some genes related to Treg function, such as FOXP3 and IKZF3 and IKZF4 are downregulated. Furthermore, some molecules related to interferon-alpha/beta signaling, such as interferon-alpha/beta receptor subunit 1/2 and NFκB are downregulated in asthmatics. Upon viral infection in both asthmatics and healthy individuals an antiviral response is induced in Tregs, including upregulation of MX1, STAT1, IRF7/9, OAS3. In healthy individuals there is an additional upregulation of FOS and JUN, transcription factors of the AP-1 pathways, and the suppressor molecule SOCS3, which was not altered in asthmatics. Furthermore, in healthy individuals CCL5 was downregulated, while unchanged in asthmatics.

Conclusion

We demonstrate that Tregs are affected upon rhinovirus infection in vivo. Tregs from healthy and asthmatic individuals showed similar anti-viral response, but also have genes that respond differently to viral stimulation which might affect Treg functions or viral clearance. These findings may play a role in impairing Treg functions during rhinovirus infections and contribute to asthma exacerbations.

P41 Characterization Of Drug-Modified Peptides That Act As T-Cell Antigens

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Keywords: Hypersensitivity, HLA, Immunopeptidomics, Hapten, DILI

Introduction

The β-lactams flu (flucloxacillin) and AC (amoxicillin-clavulanate) can cause iDILI (idiosyncratic drug-induced liver injury). Previous studies have identified a genetic association with flu and HLA-B*57:01, and have identified flu-specific CD8+ T-cells in patients with iDILI. AC-iDILI has a strong associations with the HLA-DRB1*15:01-DQB1*06:02 haplotype. Both reactions are delayed in nature, suggesting that a drug-derived antigenic determinant is formed that interacts with the HLA molecule to drive the adverse event. This study aimed to identify flu-modified naturally-processed peptides that might act as T-cell antigens

and began to investigate the functional mechanism of penicillin modified peptides by designing amoxicillin-modified HLA-binding peptides to activate T-cell clones in iDILI patients.

Method

C1R-B*57:01, B-lymphoblast cells transfected with HLA-B*57:01, were incubated with flu for 48h. MHC molecules and naturally-processed antigenic peptides were eluted from the cells and fractionated using HPLC. Amox (amoxicillin) modified designer peptides containing HLA-DRB1*15:01 binding motifs were generated. Peptides were characterised by mass spectrometry. PBMCs were isolated from patients with AC-iDILI and bulks were generated to designer peptides. Serial dilution was used to expand T-cell clones. Responsive T-cell clones were assessed using 3H-thymidine incorporation and ELISpot assays.

Results

Of the 4,400 peptides eluted from flu-treated C1R-B*57:01 cells, 2 peptides were fully annotated to show flu-lysine covalent binding, with 9 other partially annotated peptides indicating modifications. Moreover, HLA-B*57:01 was directly modified with flu. Amox modified peptides were successfully purified, and were shown to activate T-cell clones which do not cross react with the unmodified peptide, free drug or positional derivatives where the drug-lysine is placed on a different location on the peptide backbone.

Conclusion

Formation of covalently modified drug-protein adducts is thought to be the molecular initiating event in immunological drug reactions. However, our data are the first to fully characterize the nature of the drug-derived antigens presented in the context of a HLA molecule to the immune system. We have shown that designer peptides can be used to activate drug modified peptide-specific T-cell clones. In future studies we will synthesize and characterize the flu-modified peptides that activate patient T-cells, which will pave the way for improved allergy diagnosis.

P42 Effects Of Prophylactic Treatment Of Recombinant Banana Lectin On TNBS-Induced Colitis In BALB/C Mice

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Keywords: Banana Lectin, Prophylaxis, Colitis, BALB/C

Introduction

Recombinant banana lectin (rBanLec), which structurally and functionally highly resemble to its naturally occurring counterpart,is recognized as modulator of local immune response in the murine colon.The aim of this study was to investigate prophylactic effect of rBanLec on murine model of colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS).

Method

Experimental colitis was induced in BALB/c mice by a single intrarectal (i.r.) administration of 2.5 mg of TNBS / 50% EtOH. Mice were treated with rBanLec (i.r. 100 µL rBanLec/PBS; rBanLec concentration: 0.1µg/mL for rBL0.1 group, 1µg/mL for rBL1 group, and 10µg/mL for rBL10 group) 48h prior to induction of colitis. Colitic nontreated BALB/c mice were used as a referent. The effect of rBanLec pretreatment was assessed at the peak of pathology. Mice were monitored forweight loss. Collected colonic samples were evaluated for neutrophil infiltration (H&E staining), myeloperoxidase (MPO) activity, NO production, cytokine profile (IL-12, TNF-α, IL-10 and TGF-β) and expression of Th-marker key transcription factors (T-bet for Th1, GATA-3 for Th2, RORγt for Th17).

Results

The significant reduction in disease severity was marked for rBL0.1 ($P<0.005$). That was in line with reduced leukocyte infiltrations in colon of rBL0.1 mice. Confocal microscopy also showed reduced number of CD11b+ cells in colon tissue of rBL0.1 mice. All inflammation-related parameters (MPO activity, NO production, IL-12 and TNF-α levels, and T-bet and ROR-t expression) were lower in colonic samples collected from rBL0.1 mice in comparison to referent ones. Unexpectedly, the levels of IL-10 and TGF-β were also reduced. However, the ratio TNF-α / IL-10 calculated for individual samples were in positive correlation with the observed intensity of disease.

Conclusion

Obtained results show that rBanLec stimulation, in a negative dose-dependant manner, modulates local immune milieu in colon in a way to diminish deleterious potential of subsequently induced inflammatory response.

Saturday 27 January 2018

21:00 - 22:00: Poster Session II

Topic 6 - Translational immunology

P43 Role Of Neutrophils In The Development Of A Type 2 Immune Response During Nematode Infection

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Keywords: Neutrophils, Helminth Infection, Nippostrongylus, IL-17, Type 2 Immunity

Introduction

Immune responses during helminth infections are characterized by the induction of type 2 cytokines such as IL-4, IL-5 and IL-13 and eosinophilia. However, the early phase of infection with the lung-migrating nematode Nippostrongylus brasiliensis is dominated by innate IL-17 production and neutrophilia. Our lab has shown that chitinase-like proteins (CLPs) such as BRP-39, YM1 and YM2, are major drivers of this early response. CLPs are some of the most abundant proteins during type 2 mediated pathology, with roles in helminth infections, allergy and wound healing. However, little is known about the link between CLP activation and the development of type 2 immunity. In this project, we are investigating the importance of CLP-induced IL-17 and neutrophils in the development of the subsequent type 2 immune response.

Method

C57BL/6 and BALB/c mice were treated with anti-Ly6G to deplete neutrophils prior to infection with N. brasiliensis. Additionally, IL-17−/− mice were infected with N. brasiliensis. Flow cytometry was performed on bronchoalveolar lavage and lung cells. Immune pathology was furthermore assessed using histology and gene expression analysis.

Results

Neutrophil depletion during the early stages of infection with N. brasiliensis lead to impaired lung eosinophilia and decreased IL-4+ CD4+ T cell numbers in both C57BL/6 and BALB/c mice. Group 2 innate lymphoid cells, macrophages as well as IL-5+ and IL-13+ CD4+ T cell numbers were unaffected by neutrophil depletion. Despite this impaired immune response, neutrophil depleted mice were still able to expel the parasites, comparable to the isotype-treated controls. IL-17−/− exhibited a similar phenotype to neutrophil-depleted WT mice, failing to recruit neutrophils and subsequently exhibiting an impaired type 2 immune response against N. brasiliensis.

Conclusion

Our data demonstrate that neutrophils and IL-17 play are necessary for the development of a type 2 immune response during a lung-migrating nematode infection.

P44 Effect Of Structural Stability Of Allergenic Proteins On Antigen Processing And T-Cell Reactivity

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Keywords: Tropomyosin, Food Allergy, Shrimp, DustMite, Antigen Presentation

Introduction

Allergens have been shown to be stable proteins, with most of them capable of withstanding heat and enzymatic degradation. Altered structural stability of these allergens can influence their processing and presentation in antigen presenting cells, thereby influencing their T-cell reactivity and immunological properties. Tropomyosin (TM) is the major invertebrate pan-allergen present in shellfish, dust mites and insects, demonstrating a high degree of structural conservation. Yet, the frequency of IgE reactivity and allergenicity to members of the tropomyosin family is highly variable. This phenomenon presents an excellent opportunity to investigate the relation between structural stability and generation of stable allergen peptides for antigen presentation.

Method

Three allergenic TMs; Pen m 1 (shrimp), Der p 10 (dust mite) and Bla g 7 (cockroach) were analysed for structural properties using CD spectroscopy, SEC-Small angle x-ray scattering analysis, DLS measurements and FTIR spectroscopy. pH dependent endolysosomal allergen degradation and peptide generation was analysed using the degradome assay with JAWS II dendritic cell line. T-cell immuno-reactive regions of Pen m1 were elucidated using a overlapping peptide library, and T-cell cross-reactivity was analysed using a T-cell clone to Pen m 1(244-259).

Results

Thermal stability was highly variable among the TMs. TM showed high resistance but differential patterns of endolysosomal degradation over 48 hours; however complete degradation was observed at pH 4.5 and 5.2. Differential T-cell reactivity was observed to a conserved region of TM (aa 244-259) indicating presentation of peptides dependent on pH-based denaturation of the different TMs.

Conclusion

This research aims to delineate the factors that control the generation of allergen-derived T-cell epitopes that possess a high affinity to MHC class II molecules, with the potential to induce strong T-cell responses. Furthermore, it provides a platform for the development and validation of novel hypoallergenic constructs, assisting in modulating their stability and structure to control their fate in antigen presentation and consequently the immune response.

P45 Granulomatous And Lymphocytic Interstitial Lung Disease In Common Variable Immunodeficiency (CVID): A Single Center Retrospective Study

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Keywords: Granulomatous And Lymphocytic Interstitial Lung Disease, Common Variable Immunodeficiency, Lymphocytes, Immunoglobulin

Introduction

Granulomatous-lymphocytic interstitial lung disease (GLILD) is a recently described specific complication of CVID associated with reduced survival. Investigators have used different diagnostic criteria, including diffuse radiological abnormalities and/or histologic evidences. Management is primarily based on what reported in small case series.

Method

In this retrospective single-centre study, chest HRCT scans of a cohort of patients with diagnosis of CVID were double-blind reviewed by two radiologists. In patients with HRCT patterns consistent with GLILD, clinical and laboratory parameters were evaluated. The results obtained were compared with a cohort of CVID patient without radiological GLILD patterns.

Results

34 patients with diagnosis of CVID where enrolled (mean age 54±12.42 years). 15/34 patients presented radiographic findings raising the suspicion of GLILD. Compared to controls, 14/15 GLILD-patients had normal lung volume despite marked radiographic abnormalities on HRCT, but average DLCO was reduced. Splenomegaly was found in 53.3% of GLILD patients and in 15% of the controls. 3/15 GLILD-patients had autoimmune cytopenia, which was not found in control group. IgG medium levels at diagnosis was lower in GLILD-patients than in controls. Compared to controls, in GLILD-patients we found a reduced percentage of switched memory B lymphocytes and an higher percentage of activated B lymphocytes in peripheral blood. 33/34 patients were in Ig replacement therapy, the medium dose of Ig to reach similar trough levels of IgG was higher in GLILD-patients than controls. 8/15 GLILD-patients underwent bronchoscopy. BALF analysis revealed an increase of lymphocyte fraction in 6/8 patients, with an increased CD19+ fraction in 3 of them (mainly ly B activated) and a CD4/CD8 ratio increased in 2 patients. These last parameters have not yet been evaluated in the control group.

Conclusion

The results observed in our cohort of CVID patients support the view that GLILD represents a pulmonary manifestation of a generalized, multisystemic lymphoproliferative disorder with evidence of an immune dysregulation. GLILD radiological patterns should always be sought in routinely performed HRCT scans of CVID patients, even in presence of normal pulmonary function. Early diagnosis could indeed help addressing patients' personalized follow-up protocols and discovering new prognostic patterns, thus indentifying high risk patients that might deserve a more aggressive therapeutic approach.

P46 Characterization Of The Immune Response Associated With Oral Tolerance Upon Low Dose Oral Allergen Exposure In An Experimental Model

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Keywords: Food Allergy, Oral Tolerance, Oral Allergen Exposure

Introduction

It is widely accepted that oral exposure to food antigens is primarily resulting in prevention of food allergy by tolerance development. However, even to date mechanistic knowledge remains limited and has been mainly evaluated after single or repeated high dose antigen exposure in experimental models. Thus, we aimed at evaluating the immunological changes during oral tolerance induction in mice after oral exposure to low amounts of food proteins, which were previously described to be associated with food allergy development.

Method

For oral tolerance induction 24 mice were fed daily with 200µg of the model allergen ovalbumin (OVA) for 2 weeks. Immediately afterwards, one group (n=8) was sacrificed for evaluating the OVA-specific local and systemic immune response. Two groups were subsequently either subjected to food allergy induction by OVA feeding under gastric acid suppression or received repeated cycles of oral OVA gavages. All measured values were compared to the background levels determined in naïve controls

Results

After 14 days of oral OVA feeding we observed a significantly elevated local OVA-specific IgA production

measured in intestinal lavages and higher percentage of Foxp3 positive CD4 T cells. Even though the mice were protected from development of OVA-specific IgE and IgG levels after OVA feeding under gastric acid suppression, we measured significantly elevated OVA-specific IgG1 and IgG2a levels after exclusive oral OVA gavages.

Conclusion

Oral low dose exposure to food allergens is associated with profound induction of oral tolerance and changes in local and systemic immune response in an experimental model.

The work was supported by the BMWFW-AWS PRIZE Project P1621673.

P48 Neutrophil Hypersensitivity To Toxicants In COPD And Asthma

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Keywords: COPD, Asthma, Toxicant, Hypersensivity

Introduction

Neutrophil accumulation in the lung is a prominent feature of chronic obstructive pulmonary disease (COPD) and the activation of these cells, producing proteases and oxygen-derived free radicals, is thought to be important in the pathogenesis of the disease. The myeloperoxidase (MPO) activity has been found to correlate with neutrophil activation.

This study aimed to investigate the effects of toxicants, particularly of cigarette smoke extract, tobacco extract and exhaust gas solution on MPO release of neutrophils in vitro.

Method

We studied the effects of toxicants on MPO release of neutrophils from COPD patients, asthma patients, healthy smokers.

Cigarette smoke extract (CSE) was prepared using a modification of the method described by Su and coworkers (1998). CSE was prepared 30 min prior to use. Extract of tobacco free snuff (TE) was prepared using a protocol previously described Petro (2002), with a few modifications. Exhaust gas solution (EGS) was prepared using a protocol previously described Golohvast (2012), with a few modifications. Peripheral blood was obtained from 22 of the current-smokers subjects with COPD and 12 patients with allergic asthma. Healthy current smokers (n=8) with no evidence of COPD, asthma or other lung disease were also recruited.

Isolation of granulocytes was performed on heparinized blood. The mononuclear leukocytes were separated by percoll gradient centrifugation. The erythrocytes were lysed by icecold, sterile water and then washed.

The cells were exposed to 1% CSE or 1% TE, or 0.1% EGS at 37°C for 1 hour. MPO release were measured in cell-free supernatants using ELISA.

Results

The release of MPO by CSE increased significantly in both patients with COPD and asthma, but not in healthy donors (Table). The release of MPO by TE increased only in patients with COPD. The release of MPO by EGS increased in patients with asthma. The number of patients with a positive MPO release was higher in both COPD patients and asthma patients compared to healthy smokers (Figure).

Conclusion

Taken together, our data indicate that MPO release from neutrophils by toxicants determines the hypersensitivity of neutrophils in patients with COPD and asthma. In addition, the difference in the release of MPO between healthy smokers and patients-smokers allows to be used in predicting the development of the disease and the diagnosis of COPD and asthma.

Toxicants	COPD (n=22)	Asthma (n=12)	Healthy (n=8)
1% Cigarette smoke extract (CSE)	64(34;106)%**	139(53;172)%**	47(25;80)%
1% Tobacco extract (TE)	30(12;55)%**	7(0;22)%	6(0;20)%
0.1% Exhaust gas solution (EGS)	32(12;47)%	41(29;50)%**	30(24;47)%

*MPO release in % of control
**p-value<0.05

P49 Cytokines In Pulmonary Remodelling Following An Influenza Virus Infection And Potential Implication In Asthma Development

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Keywords: IL-10, Influenza Virus, Repair And Remodelling, Asthma

Introduction

Pulmonary immune homeostasis is maintained by a network of tissue-resident cells that monitor the external environment and instruct tolerance to innocuous inhaled particles. This network must also ensure an appropriate response against invading pathogens, including respiratory viruses. The inflammatory response to lung infection is followed by a phase of repair and regulation that is essential to maintain lung morphology and function. The long-term consequences of post-infection repair programmes are poorly understood. Exaggerated repair processes can culminate in pathogenic remodelling. Our hypothesis is that the molecular mechanisms controlling post-infection repair are contributory factors in asthma development.

Method

Our aim is to establish how the immune system interacts with stromal cells in the lung to promote effective remodelling and repair. We have used in vitro and in vivo, murine infections with Influenza A virus to assess transcriptional and functional changes in lung epithelium following respiratory viral challenge. In vivo cytokine blockade was used to assess the impact of IL-10 signalling on epithelial proliferation, barrier integrity, and tissue remodelling.

Results

Our data suggest that IL-10 signals promote epithelial proliferation and barrier repair during influenza infection. Our next experiments will assess the impact of these changes on subsequent responses to other respiratory pathogens and allergens.

Conclusion

Our data provide new insight into the mechanisms that drive recovery from Influenza and predispose to asthma following respiratory viral infection. Our approach has the potential to identify new regulatory pathways for therapeutic manipulation of infection and remodelling in individuals suffering from chronic lung disorders.

P50 Electrical Impedance Measurements For The Assessment Of Skin Epithelial Barrier Defects

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Introduction
Allergic disorders, such as atopic dermatitis, asthma and rhinitis, have been associated with an impaired epithelial barrier function. Defects in epithelial barriers, mainly due to disruptions of tight junctions in skin and mucosas or to a reduced expression of filaggrin in the skin, allow allergens, pollutants or microbes, to enter the tissue, and thus activate a chronic immune response. The aim of this study is to develop a method to assess the epithelial barrier function in vivo by electrical impedance (EI) spectroscopy, a relatively new technique for the characterization of epithelial tissue. After transmitting a harmless electrical signal at several depths and frequencies through the skin, electrical impedance is measured, which is influenced by several cellular properties.
Method
Epithelial barrier of C57BL/6 was damaged by epicutaneous application of several doses of different proteases, such as trypsin and papain, and electrical impedance and transepidermal water loss (TEWL) were measured after 1, 3, 5 and 24 hours. In addition, histological analysis, immunofluorescence staining and quantitative RT-PCR were performed in skin biopsies of the mice to evaluate the epithelial barrier.
Results
Protease application on the back skin of mice caused a decrease of electrical impedance consistent with the type and degree of the damage. Only 1 hour after the treatment with papain, a reduction of EI was detected, reflecting the decreased epithelial barrier function, which showed changes dependent on papain doses. The strongest decrease was observed after 5 h from the treatment. At the same time, 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. After 24 hours from the treatment, EI showed a tendency to increase, suggesting tissue healing and 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 qPCR and immunofluorescence staining, that showed downregulation of Occludin and Claudin-1, as well as of Filaggrin and other molecules involved in the barrier function. Same results were seen after treatment with other proteases.
Conclusion
EI spectroscopy can be used to detect epithelial barrier defects in the skin.

P51 An Adult Patient With Sideroblastic Anemia And Antibody Deficiency
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Keywords: Antibody Deficiency
Introduction
The congenital sideroblastic anemias (CSAs) are a heterogeneous group of syndromic and nonsyndromic inherited diseases characterized by pathologic iron deposition in the mitochondria of red blood cell precursors in the bone marrow. All identified causative CSA genes encode structural RNAs or proteins that are involved in 1 of 3 mitochondrial pathways.
Case description
A 42 years old male patient was admitted at the Referral Care Centre for Primary Immune Deficiencies because of a severe hypogammaglobulinemia (IgG 2.69 g/L, IgA 0.7 g/L, IgM 0.4 g/L). He was presenting with repeated sinopulmonary bacterial infections responding to antibiotic treatments. At 25 years he was diagnosed with a congenital sideroblastic anemia. In the preceding year he has repeatedly suffered from several episodes of a fever lasting for several weeks, dyspnea with cough and hemoptysis, elevated inflammatory markers, hypoxemia, weight loss. These manifestations were not responsive to intravenous antibiotics and required steroid treatment. A CT scan and pulmonary biopsy allowed the diagnosis of

bronchiolitis obliterans organizing pneumonia. He was investigated for a suspected primary antibody defect. Despite the hypogammaglobulinemia and the low B cell count the patient was able to mount a serological response to pneumococcal polysaccharides unconjugated vaccination. PBC immunophenotype constantly showed significant B-cell lymphopenia (CD19+ <0.1%) in the context of a normal total lymphocyte count and other lymphocyte subsets within the normal range. No BTK gene mutations were found. Biochemical investigation showed a marked hyperferritinemia; Magnetic Resonance Imaging revealed cardiomegaly and hepatomegaly with iron overload and abdominal ultrasound showed splenomegaly.

In addition, a novel heterozygous mutation (c.1234 C>T p.R412* Germinal) and a deletion was found in TRNT1 gene by Sanger sequencing. TRNT1 is the gene encoding the CCA-adding enzyme that is essential for maturation of both nuclear and mitochondrial transfer RNAs. Mutations in TRNT1 have been associated to retinitis pigmentosa and to a syndromic form of congenital sideroblastic anemias associated with B-cell immunodeficiency, periodic fevers, developmental delay, and high mortality during infancy, called SIFD.

How this report contributes to current knowledge

We describe a novel mutation in gene encoding TRNT1 in an adult, with an atypical form of SIDF, without development delay and fevers, characterized by severe respiratory systemic manifestation and T-lymphoprolipheration.

Sunday 28 January 2018

Oral abstract presentations

09:20 - 11:00: Asthma

O17 Plasmacytoid Dendritic Cells Drive Acute Exacerbations Of Asthma

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Keywords: Plasmacytoid Dendritic Cells, Asthma, Rhinovirus, Animal Models Of Allergic Airway Disease, Asthma Exacerbations

Introduction

Although acute exacerbations, mostly triggered by viruses, account for the majority of hospitalizations in asthma, there is still very little known about the pathophysiological mechanisms involved. Plasmacytoid DCs (pDCs), prominent cells of antiviral immunity, exhibit pro-inflammatory or tolerogenic functions depending on the context, yet their involvement in asthma exacerbations remains unexplored.

Method

Animal models of allergic airway disease (AAD) and virus-induced AAD exacerbations were employed to dissect pDC function in vivo and unwind potential mechanisms involved. Sputum from asthma patients

with stable disease or acute exacerbations was further studied to determine pDC presence and correlation with inflammation.

Results

pDCs were key mediators of the immuno-inflammatory cascade that drives asthma exacerbations. In animal models of AAD and RV-induced AAD exacerbations, pDCs were recruited to the lung during inflammation and migrated to the draining lymph nodes to boost Th2-mediated effector responses. Accordingly, pDC depletion post-allergen challenge or during RV infection abrogated exacerbation of inflammation and disease. Central to this process was IL-25, induced by allergen challenge or RV infection that conditioned pDCs for pro-inflammatory function. Consistently, in asthma patients pDCs were markedly increased during exacerbations, and correlated with the severity of inflammation and the risk for asthmatic attacks.

Conclusion

Our studies uncover a previously unsuspected role of pDCs in asthma exacerbations with potential diagnostic and prognostic implications. They also propose the therapeutic targeting of pDCs and IL-25 for the treatment of acute asthma.

O18 Airway Epithelial Barrier, Mucins And Inflammasome In Distinct Eosinophilic, Neutrophilic And Mixed Inflammatory Phenotypes Of Asthma

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Keywords: Airway Epithelial Barrier, Asthma Phenotypes, Mucin, Inflammasomes

Introduction

Asthma is a complex, chronic respiratory disease with marked clinical and pathophysiological heterogeneity. Specific pathways are thought to be involved in the pathomechanisms of different inflammatory phenotypes of asthma, yet direct in vivo comparison has not been performed. In the present study, we aimed to investigate the expression of tight junction molecules, mucins and inflammasome activation in different phenotypes of airway inflammation.

Method

We developed models of three different phenotypes of airway inflammation in mice, namely eosinophilic, mixed, and neutrophilic asthma via different methods of house dust mite sensitisation and challenge. Transcriptome analysis was performed using whole lung tissues, followed by quantitative RT-PCR, western blot analysis and confocal microscopy.

Results

By unbiased whole genome transcriptomic approach, we found that airway tight junction (TJ) molecules, mucins and inflammasome-related genes are differentially expressed in distinct phenotypes of allergic airway inflammation. Detailed analysis of several molecules from these families revealed that (i) Zo-1 and Cldn18 were downregulated in all phenotypes, while Cldn4 upregulation was characteristic for neutrophilic airway inflammation; (ii) mucins Clca1 (Gob5) and Muc5ac were upregulated in eosinophilic and even more in neutrophilic asthma, and (iii) upregulation of inflammasome-related molecules such as

Nlrp3, Nlrc4, casp-1 and IL-1β was characteristic for neutrophilic asthma. Finally, we showed that inflammasome/Th-17/neutrophilic axis cytokines, namely IL-1β and IL-17, might impair epithelial barrier function and increase mucins expressions in primary hBECs from normal and asthmatic donors.

Conclusion

Our findings suggest that differential expression of TJs, mucins and inflammasome-related molecules in distinct asthma phenotypes might be mechanistically linked and could further reflect the differences observed in the clinic.

O19 T2 And T17 Induced Bronchial Epithelial Gene Signatures Are Reflected In Released Exosomes

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Keywords: Asthma, Epithelial, Exosomes, Signatures, T2/T17

Introduction

Asthma is a heterogenous disease with different immune response driven phenotypes, including T2 and T17 driven asthma, respectively. Previous studies have shown that bronchial epithelial cells can express surrogate markers for the ongoing inflammation that can be used for asthma patient segmentation. Exosomes are cell-derived vesicles that can mediate cell-cell communication through the transfer of molecules between cells, and they also have potential as biomarkers of disease. The aim of this study was to generate T2 and T17 gene signatures in human bronchial epithelial cells (HBEC) and to further investigate if exosomes released from such in vitro-stimulated cells contain stimulus-specific cargos that act as disease drivers for the two asthma phenotypes or can be used for biomarker discovery.

Method

Primary HBECs cultured at air-liquid interface (ALI) were stimulated with T2 (IL-4+IL-13) or T17 (IL-17A+TNFa) cytokines. RNA was isolated from cell lysates and gene expression analysed by RNAseq and quantitative PCR. Exosomes were isolated from apical cell washes by density cushion followed by size-exclusion chromatography and protein profiling was performed using mass spectrometry. Proteomic data was interpreted by pathway analysis tools.

Results

Several of the top upregulated genes in HBECs stimulated with T2 (SERPINB2, NOS2, ALOX15 and DPP4) and T17 (CSF3, Pendrin, CEACAM7 and DUOX2) cytokines were also increased on protein level in the released exosomes. However, some proteins encoded by genes only modestly induced by the T2 and T17 cytokines were strongly increased on protein level in the exosomes. Pathway analysis revealed that exosomes from stimulated cells were enriched in proteins related to viral infection and cell homeostasis and viability, exosomes from T17-stimulated cells specifically showed increased levels of proteins related to cellular movement and synthesis of reactive oxygen species. Furthermore, the T2 and T17 cytokine induced gene signatures show similarities with previous publications.

Conclusion

The gene signatures and exosome protein profiles induced in HBEC/ALI cultures in vitro by T2 and T17 cytokines may be used for respiratory patient segmentation. Lung epithelium-derived exosomes might have a disease driving role by spreading pathogenic signals through the cargo they carry.

O20 Exhaled Breath Condensate MicroRNAs As Potential Biomarkers To Identify And Endotype Asthma In School-Aged Children

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Keywords: MicroRNA, Exhaled Breath Condensate, Asthma, Children

Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that act as regulators of gene expression, modulate almost all biological processes and are essential for maintaining cellular homeostasis. As such, miRNA profiling in disease states constitutes a powerful tool of diagnostic and prognostic value. Detection and quantification of miRNAs in exhaled breath condensate (EBC) has been poorly explored. We aimed to assess the profile of specific miRNA in EBC as a potential biomarker to diagnose and endotype asthma in school-aged children.

Method

Asthmatic children and controls (n=191, 50.8% female, 34% asthmatics, mean age=8.7) were selected from a study that included 1602 participants attending 71 classrooms from 20 local schools. All participants underwent spirometry with bronchodilation, determination of exhaled level of nitric oxide (NObreath; Bedfont Scientific Ltd., Rochester, Kent, UK), skin-prick testing, respiratory symptoms assessment (wheezing, dyspnea or dry cough) and had exhaled breath condensate collected (EcoScreen, Viasys, Germany). Based on previous studies ten specific miRNAs were chosen and analyzed in EBC by reverse transcription-quantitative real-time PCR. Asthma was defined by i) at least a 12% increase in FEV1 after bronchodilation and over 200mL, or ii) asthma diagnosed by a physician and reported symptoms in the past 12 months occurred, or iii) exhaled NO above 35ppb. Comparisons between groups were performed with nonparametric tests.

Results

Levels of miRNA-328-3p, miRNA-145-5p and miRNA-155-5p in exhaled breath were significantly increased in asthmatic children ($p<0.026$, $p<0.036$ and $p<0.048$, respectively). In children with non-allergic asthma levels of miRNA-21-5p and miRNA-126-3p were increased when compared with subjects with an allergic endotype ($p<0.044$ and $p<0.040$, respectively). The presence of respiratory symptoms and increased

exhaled NO was associated with lower levels of miRNA-21-5p in asthmatic children ($p<0.049$).

Conclusion

Our findings provide support for the potential use of EBC miRNA-328-3p, miRNA-145-5p and miRNA-155-5p to screen asthma, miRNA-21-5p and miRNA-126-3p to identify asthma non-allergic endotypes and for miRNA-21-5p to assess asthma control.