Estrogen protects against airway inflammation via upregulation of SLPI and downregulation of IL-33

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Airway epithelium (AE) can modify airway responses through production of anti-inflammatory mediators like secretory leukoprotease inhibitor (SLPI) and pro-inflammatory mediators like IL-33. Estrogen can modulate AE responses and we therefore investigated how estrogen affects severity of airway inflammation and SLPI and IL-33 production in mice.

Female balb/c mice were ovariectomized (OVX) or sham-treated and received a 0.1 mg estrogen (E2) pellet at OVX or not (all groups n=8). Four weeks after OVX, mice were sensitized i.p. with OV A and alum on days 1 and 7 and challenged with 1% OV A on days 14-20. On day 21, allergic inflammation (OV A-specific IgE, eosinophils) and production of IL-33 and SLPI were assessed.

Ablating estrogen significantly increased airway inflammation as compared to sham-treated mice. Treating OVX mice with E2 significantly reduced the higher airway inflammation induced by OVX as judged from lower eosinophil numbers in lung and lower OV A-specific IgE levels in serum. In the parenchyma of E2-treated OVX mice we found more type II alveolar epithelial cells (AECII) expressing SLPI than in nontreated OVX mice, which correlated with higher SLPI levels in lung and lower OV A-specific IgE levels in serum. In the parenchyma of E2-treated OVX mice we found more type II alveolar epithelial cells (AECII) expressing SLPI than in nontreated OVX mice, which correlated with higher SLPI levels in lung. The number of AECII producing IL-33 on the other hand was lower in E2-treated OVX mice as compared to nontreated OVX mice. This study shows that estrogen protects female mice against the development of airway inflammation and this is associated with higher SLPI and lower IL-33 production by AECII. We therefore postulate that estrogen has a protective effect on asthma development through induction of anti-inflammatory SLPI production and inhibition of pro-inflammatory IL-33 production by AECII.
P2359

IL-4 induces Th2 cells to resist the IL-27 counterregulation by downregulating STAT1 and STAT2 phosphorylation

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Rationale: Asthma is a chronic airway inflammation caused by overproduction of Th2 cytokines. IL-27 has been shown to inhibit differentiation of naïve CD4+ cells into Th2 cells in mice. However, it is not clear whether IL-27 can inhibit Th2 cell development in allergic patients.

Methods: Purify CD4+ T cells from human PBMC and mouse spleen were cultured under Th2 or Th2+IL-27 conditions. IL-4 and IFN-γ were detected by ELISA. IL-27 and p-STATs were determined by qRT-PCR and Western blot respectively.

Results: Human IL-27 suppressed Th2 differentiation in healthy subjects (p=0.006), but failed to do so in asthmatics (p=0.064). The suppressive effect of IL-27 on Th2 development was independent of IFN-γ, IL-10 and T-bet. However, studies with STAT1-knockout mice showed that this inhibitory effect of IL-27 was STAT1-dependent. IL-27 resistance to Th2 differentiation in asthmatics was not dependent on impairment of IL-27R. Further, it was found that Th2-inducing conditions could induce resistance to IL-27 in a dose-dependent manner and IL-4 is the most critical factor. Although IL-2 is imperative in Th2 cell priming, it does not contribute to induction of IL-27 resistance. We demonstrated that high dose of IL-4 treatment resulted in up-regulation of STAT1 phosphorylation, but not STAT3 or STAT4 phosphorylation.

Conclusions: IL-27 suppresses the development of Th2 immune response in both mice and human, which is STAT1-dependent, but independent of IFN-γ, IL-10 and T-bet. CD4+ T cells from asthmatics developed resistance to IL-27-mediated inhibition. IL-4 induced resistance to IL-27-mediated inhibition by impairing STAT1 signaling.

P2360

Regulatory role of antigen-induced IL-10, produced by Tr1 cells, in airway neutrophilia in a murine model for asthma

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It has been suggested that IL-10 exerts immunosuppressive effects on allergic inflammation, including asthma. In a model of experimental asthma utilizing multiple antigenic sensitization in sensitized BALB/c mice, as well as eosinophilia and neutrophilia in the lung were induced by the multiple challenges. In this study, we set out to reveal the cellular source of endogenously produced IL-10, and the roles of IL-10 in airway leukocyte inflammation using an anti-IL-10 receptor monoclonal antibody. Balb/c mice were sensitized i.p. with ovalbumin+Al(OH)3, and then challenged by intratracheal administration of ovalbumin 4 times. Flow cytometric analyses revealed that the cellular source of IL-10 was CD4+ T cells lacking the transcription factor, forkhead box P3, which should be Tr1 cells. Treatment with anti-IL-10 receptor monoclonal antibody prior to the 4th challenge significantly augmented airway neutrophilia as well as the production of IL-10 and CXC-chemokines, keratinocyte-derived chemokine (KC) and macrophage inflammatory protein (MIP)-2, but neither airway eosinophilia nor Th2 cytokine (IL-4 and IL-5) production. Approximately 40% of IL-10 receptor cells expressed the macrophage marker F4/80, whereas only 3-4% of the IL-10 receptor+ cells were granulocyte differentiation antigen (Gr)-1high cells (neutrophils). In conclusion, multiple airway antigen challenges induced the proliferation of IL-10-expressing Tr1 cells. It was suggested that IL-10 produced from induced the Tr1 cells by specific antigen challenge suppressed macrophages to produce CXC chemokines through activation of the IL-10 receptors on the cells.

P2361

Probiotics and synthetic: Effects on chronic asthma in mice

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Background: Asthma is a chronic inflammatory disorder of the airways characterized by structural changes of the airways which may contribute to airway obstruction and airway hyperresponsiveness. Modulation of the intestinal microbiota by probiotics and related products as a potential therapy for allergic disorders has been subject to investigation. Several murine models of asthma and clinical studies demonstrated beneficial effects of probiotics and synbiotics in asthma management. However, the effects on chronic symptoms of asthma have never been investigated in murine models.

Methods: Mice were sensitized twice (day 1 and 12) with ovalbumin (OVA)-inject allergen and challenged from day 17 till 23 daily with OVA. From day 24 till day 56, animals were challenged with OVA 3 times a week and on the same day, the animals were treated with either control solution or glucocorticoids (GCS) budesonide by oropharyngeal aspiration or probiotics (Lactobacillus or Bifidobacterium) or synbiotics by oral gavage. Pulmonary function, total and differential leukocyte counts in bronchoalveolar lavage were determined and the lung tissues were isolated to study airway remodeling.

Results: Treatment with probiotics or GCS significantly inhibited the OVA-induced increase in basal airway resistance and hyperresponsiveness. Probiotics, synbiotics and GCS significantly reduced pulmonary eosinophilia, and IL-4 was significantly increased in NK cells co-cultured with O3-exposed NEC cells. In untreated controls, IL-4 and IL-5 were increased in peripheral blood NK cells. Exposure to ozone significantly increased the expression of the NK cell ligands MICA/B and ULBP3 on NECs in co-cultures. NK cell surface markers NKG2D, but also type II cytokines, like IL-4. Yet, whether and how exposure to air pollutants affects this interactions and changes NK cell phenotypes is not known.

Conclusions: Differentiated NECs were exposed to ozone (O3: 0.4ppm, 4hrs). 2hrs after exposure peripheral blood NK cells were added to the apical side to establish co-cultures. At 24hrs post-exposure, both cell types were examined for changes in surface marker and intracellular cytokine expression by flow cytometry. Exposure to O3 significantly increased the expression of the NK cell ligands MICA/B and ULBP3 on NECs in co-cultures. NK cell surface markers NKG2D, NKP46 and CXCXR3 were not affected by co-culture with O3-exposed NECs. Expression of CD16, a cytotoxicity marker, and Granzyme B, a marker of cytolytic NK cells, were enhanced in co-cultures with O3-exposed NECs. Intracellular IFN-γ was decreased and IL-4 was significantly increased in NK cells co-cultured with O3-exposed NECs. O3-induced changes in the microenvironment of NECs change NK cell cytokine towards a type I phenotype (high IL-4) and towards a type II (high IL-4) immunophenotype. These data indicate that exposure of NEC to O3 changes interactions with resident immune cells, such as NK cells, shifting the immune phenotype which likely affects the ability to fight invading pathogens.

P2364

Immune modulation by mesenchymal stem cells in a mouse model of house dust mite allergic asthma

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Background: Mesenchymal Stem Cells (MSCs) display immunomodulatory features in ovalbumin-induced allergic asthma models, by inducing Th1 and/or Treg 428s.
CCK is a chemokine receptor initially thought specific to eosinophils but subsequently identified on Th2 cell subsets and mast cells. The prominent allergic inflammatory cells, eosinophils, mast cells, and Th2 cells exhibit preferential expression of CCR1, C-C chemokine receptor type 7, and C-C chemokine receptor type 8, respectively. After epicutaneous sensitization to HDM on day 0, 7, 14, and 21, mice were challenged with intranasal HDM on day 27 and 34. Asthmatic and sham non-asthmatic mice received an I.V. injection of 5.10^6 MSCs immediately prior first challenge. Higher levels of IFN-γ in airway lavage fluid (BAL), cytokine secretion and serum immunoglobulin were assessed on day 35 and 37.

Results: Treated asthmatic mice displayed a 1.8 fold decrease of airway resistance estimated by PEFN1 (<0.05) compared to controls with a return to baseline. Moreover, asthmatic mice treated with MSC had significantly fewer infiltrated cells in BAL than control mice (308±43 vs 754±119, N=10/mL, p<0.001), especially neutrophils (4±0.1%), T cells (p<0.05) and B cells (<p<0.05). An increase of IL-10 in BAL was observed in treated mice compared with controls (26.80±11.19 vs 10.39±2.75, pg/mL). In parallel, non-asthmatic treated mice showed neither significant changes in BALF eosinophils and airway hyperresponsiveness, and with the smallest particle distribution of each different rhTM was measured by Spraytec.

Conclusions: Taken together, these results demonstrate that MSC reduced AHR in a relevant allergic model of HDM asthma.

P2367
Allergic inflammatory cells use different GATA factors to activate CCR3 transcription
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CCR3 is a chemokine receptor initially thought specific to eosinophils but subsequently identified on Th2 cell subsets and mast cells. The prominent allergic inflammatory cells, eosinophils, mast cells, and Th2 cells exhibit preferential expression of CCR1, C-C chemokine receptor type 7, and C-C chemokine receptor type 8, respectively. After epicutaneous sensitization to HDM on day 0, 7, 14, and 21, mice were challenged with intranasal HDM on day 27 and 34. Asthmatic and sham non-asthmatic mice received an I.V. injection of 5.10^6 MSCs immediately prior first challenge. Higher levels of IFN-γ in airway lavage fluid (BAL), cytokine secretion and serum immunoglobulin were assessed on day 35 and 37.

Results: Treated asthmatic mice displayed a 1.8 fold decrease of airway resistance estimated by PEFN1 (<0.05) compared to controls with a return to baseline. Moreover, asthmatic mice treated with MSC had significantly fewer infiltrated cells in BAL than control mice (308±43 vs 754±119, N=10/mL, p<0.001), especially neutrophils (4±0.1%), T cells (p<0.05) and B cells (<p<0.05). An increase of IL-10 in BAL was observed in treated mice compared with controls (26.80±11.19 vs 10.39±2.75, pg/mL). In parallel, non-asthmatic treated mice showed neither significant changes in BALF eosinophils and airway hyperresponsiveness, and with the smallest particle distribution of each different rhTM was measured by Spraytec.

Conclusions: Taken together, these results demonstrate that MSC reduced AHR in a relevant allergic model of HDM asthma.

P2366
VKL-40 induces IL-8 expression from bronchial epithelium via MAPK (JNK, ERK1/2) and NF-κB pathways, causing bronchial smooth muscle proliferation and migration
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Our previous study, consistent with others, has shown that the serum YKL-40 levels in asthmatics were significantly elevated and were associated with asthma severity. Although these studies raise the possibility that YKL-40 may influence asthma, the mechanisms remain unknown. In this study, we investigated the mechanisms involved in YKL-40-mediated IL-8 production from human bronchial epithelial cells (BEAS-2B) and analyzed the soluble factors (including IL-8) secreted by BEAS-2B exposed to YKL-40 that were responsible for increasing proliferation and migration of primary normal human bronchial smooth muscle cells (BSMCs). We found BEAS-2B treated with YKL-40 resulted in a significant increase of IL-8 expression and release. Moreover, YKL-40 markedly phosphorylation of JNK, ERK, but not p38 in BEAS-2B. Transfection using a NF-κB-deactivator gene also showed YKL-40 induced IL-8 at the transcriptional level. Furthermore, BEAS-2B pretreated with inhibitors of JNK, ERK or NF-κB decreased IL-8 release upon YKL-40 treatment. In addition, we treated BEAS-2B with YKL-40 and added the conditioned culture media (YKL-40–BEAS-2B-CM) to BSMCs, which led to increased proliferation and migration of BSMCs. By comparison, IL-8-depleted YKL-40–BEAS-2B-CM failed to induce the proliferation and migration of BSMCs.

In summary, our data provided the first evidence of YKL-40-induced IL-8 expression in BEAS-2B via MAPK (JNK, ERK) and NF-κB pathways, and the induced IL-8 was found to further stimulate the proliferation and migration of BSMCs. Our results raise the possibility that YKL-40 may play a role in asthma by inducing IL-8 production.

P2368
 Efficiency of a DNA vaccine on allergic asthma
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Specific immunotherapy is an attractive therapeutic option in allergic asthma. It reduces symptoms and the use of medication. Nevertheless the efficiency of immunotherapy is less satisfactory and it may cause anaphylactic adverse effects when administered subcutaneously. One of the new modalities of immunotherapy is based on DNA vaccination which consists in the administration of DNA sequences encoding an antigen. It enables a targeted immunogenicity a Th1 bias and a good tolerance profile. In this study we used a DNA plasmid encoding Der f 1, a major allergen from the house dust mite dermaphagoides farinae, formulated with a synthetic vector. This vaccine was tested on mice and it was used as control. The effect of induced low dose to high dose rhTM was assessed by administering it prior to OVA exposure. Airway inflammation was evaluated by measuring the number of inflammatory cells and the levels of cytokines in bronchoalveolar lavage fluid (BAL). Airway hyperresponsiveness was measured using a plethysmograph. Particle size distribution of each different rhTM was measured by Spraytec. Results: The number of eosinophils in BALF and airway hyperresponsiveness was decreased by rhTM in a dose-dependent manner compared to saline treated mice. rhTM concentration of 3.75μg/mL was associated with the lowest number of BALF eosinophils and airway hyperresponsiveness, and with the smallest particle size.

Conclusion: These results suggest that the effect of rhTM in murine asthma is dose- and particle size-dependent.

P2369
ROG negatively regulating the expression of T cell cytokines through modulation on ICOS
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Background: ROG, can simultaneously suppress expression of Th1 and Th2 cytokines. Since suppression of Th2 cytokines by ROG via suppression of GATA-3 expression is well understood, it is postulated that there are other molecular targets of ROG that can suppress expression of the Th1 cytokines. Based on the current theory, we hypothesized that ROG might suppressing CD3, CD28, or ICOS, which can up-regulate the expression of T lymphocyte cytokines, or indirectly stimulate the expression of these pro-inflammatory cytokines. In order to rule out the possibility of artifactual cytokine infiltration and airway hyperresponsiveness, DNA vaccination seems therefore promising to prevent allergy.

Methods: Real-time quantitative PCR and Western Blot were performed to evaluate the mRNA and protein levels of CD3, CD28, ICOS, CTLA-4, and CD45 in Th1 and Th2 cells under various levels of ROG expression. ELISA was performed to measure the levels of IFN-γ and IL-4 in culture media of Th1 and Th2 cells. Results: The mRNA and protein levels of ROG were relatively low in Th1 and Th2 cells (<P<0.01). After ROG-pcDNA3.1 transfection, the mRNA and protein level of ROG was significantly elevated, while the expression of ICOS, IFN-γ, and IL-4 was markedly down-regulated (<P<0.01). Transfection of ROG-siRNA led to inhibition of ROG expression and up-regulation of ICOS, IFN-γ, and IL-4 (<P<0.01). The expression levels of CD3, CD28, CTLA-4, and CD45, however,
Materials and methods: We studied 96 patients; from them 48 were patients of ENT department, who suffered from drug hypersensitivity reactions of various severity, from mild urticaria to anaphylaxis (study group). The control group consisted of 48 ENT patients with same diseases, but without drug hypersensitivity. Patients were determined into “study” and “control” group according to “case-control” principle. Seven day before histamine sensitivity tests cell antihistamine preparation were stopped. Histamine serial dilutions test was performed with standardized prick-needle. We used 0.1 % histamine preparation. The end point was histamine dilution that caused papule ≥ 2mm in diameter.

Conclusion: In the control group in 54.2 % the end point was 10-2 histamine dilution, in 29.2 % - 10-3, in 7.3 % - 10-4, in 5.1 % - 10-5. There were few cases, when our patients debit react by papule formation to 0.01% histamine dilution and reacted only to 0.1% of histamine. In patients with drug allergy we found an elevated skin sensitivity. Thus, the end-point 10-2 was found in 39.5 % of patients, 10-3 in 21.6 %, 10-4 in 9.9 %. The difference in 10-2 dilutions and 10-3 dilutions between the control and studied group was statistically significant.

We concluded that in patients with drug allergy the increased skin sensitivity to histamine was found, and the level of this hypersensitivity should be considered in individual antihistamine preparations prescription.

P2374 New enzyme linked immunosorbent assay using recombinant antigens from Saccharopolyspora rectivirgula for farmer’s lung disease serodiagnosis

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Saccharopolyspora rectivirgula (SR) is the main etiologic agent of the Farmer’s Lung Disease (FLD). Serodiagnosis using immunoprecipitation techniques with crude antigens has proved effective but lacks of standardization. We aim at producing specific recombinant antigens (rAg) to develop a new standardized enzyme-linked immunosorbent assay (ELISA).

A database of putative proteins was first specifically created after partially sequencing of SR. Subsequently, proteins were analyzed by two-dimensional electrophoresis and revealed by Western-blot analysis with serum from 9 patients with FLD in comparison with the sera of 4 exposed controls. Among the 77 visible spots, 42 were analyzed by mass spectrometry and 28 proteins were identified. Sixteen FLD-specific proteins were produced as rAg in Escherichia coli. ELISA with 16 rAg were performed using sera of 20 FLD patients from France and Switzerland, and 27 controls. Results were analyzed by receiver operating characteristics curve. Five showed an area under the curve (AUC) above 0.75 and a sensitivity equal or greater than 75% (named SR1A, SR9, SR13, SR17 and SR25). The protein performing the best (SR9) reached 80% specificity and 85% specificity with an AUC of 0.90.

ELISA using rAg specific from SR were effective for serodiagnosis of FLD. The use of antigen panels including rAg from other micro-organisms involved in FLD (Aspergillus, Lichtheimia, Wallemia) may increase the diagnostic performance of the ELISA. A prospective study including FLD patients from other countries (Finland, Canada), exposed to other strains of SR, should be done for a large scale validation.

P2372 Histamine skin sensitivity in patient with drug hypersensitivity

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One of the main tests is the histamine serial dilutions sensitivity and exactly this may be very important for antihistamine preparations effectiveness study.
antigens in pigeon serum regardless of possessing PFL, and there was no difference in magnitude of response between the groups.

No pigeon-specific IL-4, IL-5 or IL-17 ELISPOT responses could be detected in any individual.

Interestingly, the IFNγ ELISPOT response could not be inhibited by anti-MHC class II antibody.

**Conclusion:** These findings show that an unconventional T cell response is generated in conjunction with pulmonary exposure to pigeon antigens that may be necessary but not sufficient to cause PFL disease, and so further analysis of these cells is warranted.

**P2375**

**Roles of periostin in vascular remodeling of allergic granulomatous angiitis in murine model**

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**Background:** We reported an allergic granulomatous angiitis (AGA) model of C57BL/6 mice (Exp Lung Res 2010). We also reported the suppressive effects of imatinib mesylate (IM) on vascular remodeling (ERS 2010). Periostin is a matricellular protein involved in airway remodeling in asthma and pulmonary fibrosis.

**Objectives:** To elucidate the role of periostin (PO) in vascular remodeling in AGA, we measured the concentrations of PO in bronchoalveolar lavage fluids (BALF) and serum in an AGA model of C57BL/6, in BALB/c as control and in C57BL/6 treated with IM.

**Methods:** C57BL/6 and BALB/c were sensitized with ovalbumin (OVA). They were exposed to aerosolized OVA daily for 7 days. C57BL/6 mice (IM treated) were also administered with IM (4.5mg/kg, p.o.) in parallel with daily exposure to aerosolized OVA for 7 days (n=12). On the 7th day, BAL was performed and the lungs were excised for pathological analysis. The concentrations of PO in BALF and serum were measured and the values were expressed as mean ± SE.

**Results:** The PO concentration in BALF of sensitized C57BL/6 mice exposed to OVA was significantly higher than that of BALB/c (C57BL/6 vs BALB/c; 39.3±0.58 vs 13.56±2.42%, p=0.003). PO in BALF of the IM treated C57BL/6 was significantly reduced compared to positive control mice (control vs IM treated; 40.3±1.85 vs 33.9±1.95%, p=0.019). The pathological scores were reduced significantly in the IM treated group compared to the control group (control vs IM treated; 3.67±0.2 vs 2.20±0.2, p=0.004).

**Conclusion:** The PO concentration in BALF was associated with vascular remodeling in a murine AGA model, suggesting its pivotal role in myofibroblast proliferation in pulmonary arteries.

**P2376**

**Challenges in the management of patients with ANCA-associated vasculitides**

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**Background:** Granulomatosis with polyangiitis (Wegener’s) and microscopic polyangiitis are antineutrophil cytoplasm antibodies (ANCA) – associated vasculitides with significant morbidity and mortality.

**Objective:** We evaluated the evolution of 12 patients diagnosed in our clinic between 2000 and 2011, treated with conventional treatment (prednisolone and pulse cyclophosphamide initially, and in remission with prednisolone and azathioprine).

**Results:** We evaluated 12 patients (10 females), median age of 46 years (range 20-74), with a median duration of follow up of 5.2 years (range 1-12 years).

Six patients had relapses (50%), 1 developed subglottic stenosis, 1 retro-orbital pseudo tumor and 2 patients developed lung abscesses inside a cavity which imposed lobectomy after unsuccessful antibiotic treatment. Two patients developed lung tuberculosis (one multi-drug-resistant) and 1 pulmonary nocardiosis linked to the immunosuppressive therapy. Two patients needed peritoneal dialysis for renal failure. Two patients died of stroke (1) and severe active vasculitis (1). Older age and renal failure were predictors of death.

**Conclusions:** The management of patients with ANCA-associated vasculitides is difficult, and marked by significant adverse effects of the therapy.