101. Animal models of airway inflammation

P954
T cell clone transfer model for steroid resistant asthma
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Background: Glucocorticoid (GC) action on asthma has been partly explained by the inhibition of T cell activation. We analyzed the steroid sensitivity of ovalbumin (OVA) reactive helper T (Th) cell clones both *in vitro* and *in vivo*.
Method: For *in vitro* experiments, Th clones were cultured with antigen presenting cells, OVA, and various concentrations of dexamethasone (DEX). The proliferative response of each Th clone was measured by ^{3}H-thymidine uptake. For *in vivo* experiments, unprimed BALB/c mice were transferred with Th clones, challenged with OVA, and administered with DEX subcutaneously. The number of infiltrating cells in bronchoalveolar lavage fluid (BALF) was measured.
Results: Six Th clones were classified into steroid sensitive and steroid resistant clones in terms of the effects of GC on the proliferative responses analyzed *in vitro*. Airway infiltration of eosinophils and lymphocytes of mice transferred with steroid sensitive clones were effectively inhibited by the administration of DEX. In contrast, those of mice transferred with steroid resistant clones were not significantly inhibited by DEX, except that the number of eosinophils in the BALF of mice transferred with one steroid resistant clone, T5-1, was only partially reduced.
Conclusion: Steroid sensitivity of Th clones measured *in vitro* were consistent with that of adoptively transferred asthma model measured *in vivo*. Steroid sensitive and resistant asthma models seem valuable for understanding the mechanisms of steroid resistance in severe asthma.

P955
PARP-1 deficiency blocks IL-5 expression through calpain-dependent degradation of STAT-6 in a murine asthma model
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Background: We recently showed that poly(ADP-ribose)polymerase-1 (PARP-1) may play a role in allergen (ovalbumin)-induced airway eosinophilia, potentially through a specific effect on IL-5 production.
Objective: To explore the mechanism by which PARP-1 regulates IL-5 production and to determine how PARP-1 inhibition blocks allergen-induced eosinophilia.
Methods: This study was conducted using a murine model of allergic airway inflammation and primary splenocytes.
Results: PARP-1 knockout-associated reduction in IL-5 upon allergen exposure occurs at the mRNA level. Such an effect appears to take place after IL-4 receptor activation as PARP-1 inhibition exerted no effect on JAK1/JAK3 activation. Signal transducer and activator of transcription-6 (STAT-6) protein was severely downregulated in spleens of PARP-1(-/-) mice without any effect on mRNA levels, suggesting an effect on protein integrity rather than gene transcription. Interestingly, the degradation of STAT-6 in PARP-1(-/-) mice required allergen stimulation. Additionally, PARP-1 enzymatic activity appears to be required for STAT-6 integrity. The downregulation of STAT-6 coincided with mRNA and protein reduction of GATA-binding protein-3 and occupancy of its binding site on the IL-5 gene promoter. IL-4 was sufficient to induce STAT-6 downregulation in both PARP-1(-/-) mice and isolated splenocytes. Such degradation may be mediated by calpain, but not by proteasomes.
Conclusion: These results demonstrate a novel function of PARP-1 in regulating IL-5 expression during allergen-induced inflammation and explain the underlying mechanism by which PARP-1 inhibition results in IL-5 reduction.

P956
Function of cAMP response element modulator in a murine asthma model
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Introduction: Several isoforms of the cAMP response element modulator (CREM) act as transcriptional repressors or activators binding to the cAMP response element of different promoters. In contact dermatitis we previously demonstrated the importance of CREM for antigen presenting cell-dependent and independent T cell function and termination of T cellular immune response. In this study we investigated the role of CREM in murine ovalbumin (OVA)-induced airway inflammation.
Method: Male wild type (WT) and CREM-knockout animals (CREM-KO) were sensitized i.p. with 10μg OVA in aluminum hydroxide solution (day 0, 14 and 21) and with aerosol (1% OVA on day 28 and 29). On day 35 bronchial responses to nebulized acetylcholine (0.001-1mg) were examined using the flexiVent system (SCIREQ, Montreal, Canada). Inflammatory responses were evaluated by cell counts, cytokine- and IgE measurements in bronchoalveloar lavage (BAL) and serum. Changes in lung tissue were investigated by histology and calculation of the wet/dry-ratio.
Results: CREM-KO mice showed an increase in airway responsiveness by elevation in central airway (177%) and tissue resistance (214%) compared to WT (100%). In addition, higher numbers of eosinophils and lymphocytes as well as upregulated Th2 cytokines were found in the BAL of CREM-KO mice. Lung histology indicated increased pulmonary cell infiltration, stronger mucus production and goblet cell hyperplasia.
Conclusion: CREM deficiency drives Th2 immune response and influences airway tone as well as mucus production. Our findings suggest that the presence of CREM at least partially protects from the development of asthmatic disease by immunological and non-immunological mechanisms.

P957

Control of allergen-induced inflammation and hyperresponsiveness by the metalloproteinase ADAMTS-12

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Background: ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) constitute a family of endopeptidases related to matrix metalloproteinases (MMPs). These proteinases have been largely implicated in tissue remodelling associated to pathological processes. *ADAMTS-12* has been identified as an asthma-associated gene in a human genome screening program.
Objective: To investigate potential roles of ADAMTS-12 in experimental models of asthma.
Methods: In our study, two different *in vivo* protocols of allergen-induced asthma were applied to the recently generated *Adamts-12*-deficient mice, and corresponding wild-type mice.
Results: The results obtained provide evidence for a protective effect of this enzyme against bronchial inflammation and hyperresponsiveness. In the absence of *Adamts-12*, challenge with allergens (ovalbumin and house dust mite) led to exacerbated eosinophilic inflammation in the bronchoalveolar lavage fluid (BALF) and in lung tissue, along with airway dysfunction assessed by increased airway responsiveness following methacholine exposure. Furthermore, mast cells counts, ST2 receptor, and IL-33 levels were higher in the lungs of allergen-challenged *Adamts-12*-deficient mice.
Conclusion: The present study provides the first experimental evidence for a contribution of ADAMTS-12 as a key mediator in asthma, interfering with immunological processes leading to inflammation and airway hyperresponsiveness.

P958

Local inhibition of IL-4 and IL-13 protects lung function in OVA mouse model

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DARPinsTM (Designed Ankyrin Repeat Proteins) are a novel class of proteins that combine the affinity and specificity properties of antibodies with the solubility and tissue penetration properties of small molecules. The excellent biophysical properties of DARPin domains allows for simple engineering of multispecificity by linking domains that target different ligands. DARPin134 is a bispecific DARPin that inhibits binding of murine IL-13 and IL-4 to their receptors.
The bispecific molecule (DARPin134) or either of the mono-specific molecules was delivered to the lungs of mice via intra-tracheal instillation during an acute ovalbumin sensitization and challenge model. Free IL-4 and IL-13 levels were reduced in the BAL following treatment with DARPin134. While the anti-IL-4 and anti-IL-13 DARPins each showed a trend towards reducing eosinophils in the lungs of mice, the bi-specific DARPin significantly reduced eosinophil infiltration. In addition, an improvement airway hyper-responsiveness in mice treated with DARPin134 was observed.
A variety of studies have confirmed the unique and overlapping roles for IL-13 and IL-4 in the pathology of asthma. Molecules inhibiting IL-4 alone have failed in clinical trials of asthma and a variety of clinical studies with molecules targeting IL-13 are ongoing. In contrast, DARPin134 targets both IL-4 and IL-13 and in vitro data demonstrate the bispecific DARPin has high potency, affinity and excellent biophysical properties. Blockade of both IL-4 and IL-13 in the pulmonary compartment could provide a therapeutic option in pulmonary diseases without disruption of either cytokine systemically.

P959

Effect of dietary nitrite and nitrated fatty acids on airway hyperresponsiveness and inflammation in a mouse model of asthma

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Dietary nitrite (DN) generates NO that lowers blood pressure *in vivo*. Nitrated fatty acids (NO_2-FAs) are formed endogenously from NO and nitrite and display broad anti-inflammatory effects including protection in an *in vivo* model of inflammatory bowel disease.
Our aim was to investigate if DN or NO_2-FAs affect airway hyperresponsiveness and inflammation in a mouse model of asthma.
Female BALB/c mice were sensitized with OVA+$Al(OH)_3$ on day 0 and 7 and challenged with OVA or PBS i.n. on day 14-16. DN was administered day 10-17 by nitrite-enriched drinking water. NO_2-FAs (5 mg/kg/day) were administrated day 10-17 by a s.c. placed osmotic pump. Airway resistance (R_N) to metacholine and inflammatory cells in bronchoalveolar lavage (BAL) were determined on day 17.
OVA induced an increase in R_N compare to ctrl in all groups (OVA vs. PBS; 5.3±0.2 vs. 2.3±0.1, OVA-DN vs. PBS; 5.6±0.2 vs. 2.5±0.1, OVA-NO_2-FAs vs. PBS; 5.4±0.2 vs. 2.2±0.1 (cmH_20/mL), all $p<0.001$). No differences in R_N were observed between the OVA groups with or without administration of DN or NO_2-FAs ($p>0.05$). OVA induced an increase in BAL eosinophils (EOS) compared to ctrl in all groups (OVA vs. PBS; 25±5.5 vs. 0±0, OVA-DN vs. PBS; 21±4 vs. 0±0, OVA-NO_2-FAs vs. PBS; 45±7 vs. 0.1±0 (10,000 cells/mL), all $p<0.001$). No difference in EOS were observed between OVA with or without administration of DN or NO_2-FAs ($p>0.05$). The number of EOS were higher in OVA- NO_2-FAs treated animals versus OVA alone ($p<0.001$).
To conclude, neither DN nor NO_2-FAs displayed anti-inflammatory effects in this asthma model, indicating that there is tissue specificity in the actions of these reactive nitrogen oxide species.

P960

Influence of acid sphingomyelinase deficiency in a murine model of allergen-induced asthma

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Introduction: Acid sphingomyelinase (ASM) deficency causes lipid storage diseases, called Niemann-Pick-disease (NPD) type A (neuronopathic) and B (non-neuronopathic). Previous studies demonstrated an association of NPD type B with respiratory failure and lung infections. We investigated the role of ASM deficiency in a murine model of allergic asthma.
Method: Male C57BL/6 (WT) and ASM knockout mice (ASM-KO) were intraperitoneally sensitized with ovalbumin (OVA)/Alum (10µg/1.5mg) on days 0, 14 and 21. Repeated aerosol challenges (1% OVA) followed for five weeks on two consecutive days every week. Bronchoalveolar lavage (BAL), serum and lung tissue were taken for cell counts, cytokine measurements, histology slides and calculation of wet/dry-ratio. Precision-cut lung slices (PCLS) were prepared to investigate early asthmatic response (EAR).
Results: Edema formation was less in ASM-KO (73.6±7.6%) compared to WT. In addition, sensitized ASM-KO showed significantly higher cell numbers in BAL and lung tissue primarily consisting of neutrophils and enlarged macrophages, whereas numbers of eosinophils were similar. The ratio of eosinophils and neutrophils was characteristic of a typical Th1-pattern. Differences in cytokine and IgE-levels in BAL and serum were not found. OVA stimulation of PCLS from both mouse strains resulted in a weak bronchoconstriction.
Conclusion: ASM-KO showed the typical characteristics of NPD, but their allergic inflammation was similar to that of WT mice, except for an increased neutrophil/eosinophil ratio. The major observation was reduced edema formation in ASM-KO mice. We conclude that ASM contributes to allergic edema formation, a key feature of asthma.

P961

Modulation of oral tolerance on the oxidative stress responses in distal lung parenchyma of guinea pigs with chronic allergic inflammation

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Rationale: We previously had shown that oral induced tolerance contributes to reduce distal lung responsiveness, inflammation and remodelling (Nakashima et al.,2008) in a model of chronic inflammation in guinea pigs (GP). In the present study, we evaluated if these responses were associated to alterations on the oxidative stress responses in distal lung.
Methods: GP were submitted to multiple inhalations of ovalbumin (OVA) or normal saline (NS) (2x/wk/4wks). At the same period oral tolerance was induced by offering GP *ad libitum* 2% ovalbumin in sterile drinking water during 4 weeks (OVA-T1) or starting oral ovalbumin after the 4th inhalation of ovalbumin (OVA-T2). Afterwards, lungs were removed, strips of distal lung were stained for iNOS and PGF2alfa (isoprostane) and analysed by morphometry.
Results: In OVA group there was an increase in the iNOS positive cells ($20.7±1.0/10^4 µm^2$) and PGF2alfa content (17.51±2.5%) compared to NS group ($p<0.05$). There was a decrease in iNOS positive cells in T1 ($12.8±1.9/10^4 µm^2$) and T2 ($14.3±2.2/10^4 µm^2$) compared to OVA ($p<0.05$). Considering PGF2alfa content, there was a decrease in T1 (6.17±0.4%) and T2 (5.81±0.7%) compared to OVA ($p<0.05$).
Conclusion: Oral tolerance attenuates the oxidative stress responses in distal lung in this animal model of chronic pulmonary inflammation. These results may clarify the mechanisms involved in the attenuation of mechanical responsiveness, inflammation and remodeling of distal lung by oral tolerance, as previously shown in this animal model.
Supported by: FAPESP, CNPq, LIM-20-HC-FMUSP.

P962

AQP5 role in ovalbumin-induced airway inflammation and secretion of MUC5AC in mice

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Background: Airway inflammation and mucus hypersecretion are two important characteristic features of the pathogenesis of asthma. Aquaporin5 (AQP5) is known to be a water channel protein expressed widely in lung epithelium and submucosal glands.

Objective: The present study aimed at investigating the involvement of AQP5 in asthma.

Methods: The ovalbumin (OVA)-induced allergic pulmonary inflammation and MUC5AC production were examined in AQP5$^{+/+}$ or AQP5$^{-/-}$ mice. The expression of AQPs in lung tissue and their regulation were detected. In addition, epidermal growth factor receptor (EGFR) expression and osmotic water permeability in lung were evaluated.

Results: Lower expression of AQP1, 4, 5 while higher AQP3 was significant in lung tissue from AQP5$^{+/+}$ model mice. Only AQP1 and AQP5 were up-regulated by anti-asthmatic agents (dexamethasone, ambroxol and terbutaline) significantly. However, AQP5 knockout had significantly low airway inflammation and less lung edema induced by OVA, as compared with those in AQP5$^{+/+}$ mice. In addition, lower expression of MUC5AC in airway epithelium, less secretion of MUC5AC were found in AQP5$^{-/-}$ model mice. Moreover, the expression of EGFR on airway epithelium was prevented by AQP5 knockout in asthmatic model.

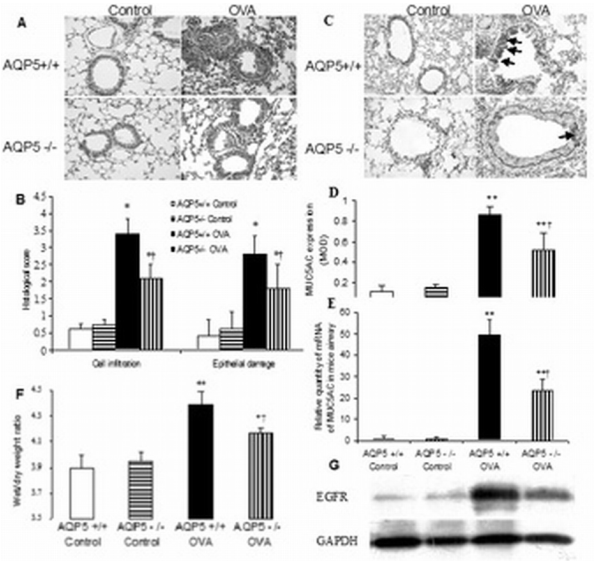

Figure legends: (A) Lung histology pictures. (B) Histological scores. (C) MUC5AC protein was measured by immunohistochemistry staining. (D) Score of staining for MUC5AC expression. (E) MUC5AC mRNA expression. (F) Wet-to-dry weight ratios of lung. Significant lower airway inflammation, MUC5AC production and lung edema were found in AQP5 -/- model mice compared with AQP5+/+ model mice. (G) EGFR protein expression by Western blot. Values are expressed as mean ± SEM, n = 5-8, per group in experiments. * and ** stand for $P < 0.05$ and 0.01, respectively, for OVA challenged model mice compared with control mice. † stands for $P < 0.05$ for AQP5 -/- model mice compared with AQP5+/+ model mice.

Conclusion: Our data indicate that AQP5 is involved in the development of allergic airway inflammation and mucus hypersecretion by regulating osmotic water permeability and expression of EGFR.

P963

Effects of increase gradual OVA doses in the inhalations in a model of asthma to long term

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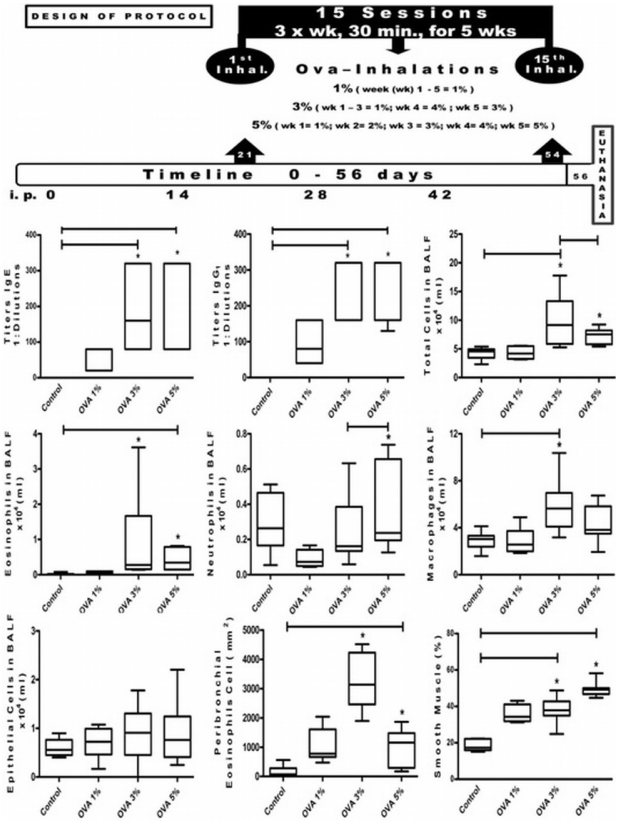
Mice are used to develop models of pulmonary allergic disease.

Objective: To evaluate the increase gradual OVA doses in a protocol of experimental asthma.

Methods: Balb/c mice male were divided in Groups: Control, OVA1%, OVA3%, OVA5%. Sensitized animals received i.p.(OVA+Sal+AlumHidrox; Days: 0,14,28,42). Inhalations sessions (OVA+Saline; 3x wk/30min) were performed between 21st and 54th day. The OVA1% group received inhalation with concentration of OVA1%. The OVA3% began with 1% for 3 weeks (wk), in the 4th wk was increased for 3%. The OVA5% began with 1% in the 1st wk, 2sd wk was increased for 2%, 3rd wk with 3%, 4th wk for 4%, 5th wk for 5% of OVA. The Control group received i.p. and Saline inhalations. In the study were evaluated IgE and IgG1 titers by PCA technique, cells in BALF and peribronchial eosinophils, and smooth muscle of the airways for image analyses.

Results: IgE and IgG1 were increased in sensitized groups, but in the groups 3% and 5% the levels were highest and shown increased of cell migrations presented increase of eosinophils, neutrophils and trickiness of the smooth muscle when compared with others two groups ($p<0.01$), just Group 3% shown high index of peribronchial eosinophils ($p<0.01$).



Conclusions: Groups OVA3% and OVA5% are more efficient for develop features of experimental asthma, and seems that Group OVA3% develop important increase of eosinophil migration in the airways.

P964

Chronic airway inflammation alters the peripheral distribution of transferred mast cells in deficient C57BL/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice

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Mast cells (MC) play important roles in chronic inflammatory diseases such as asthma. This study tested if transfer of bone-marrow derived MC (BMMC) to the lung could provide a new method to explore MC involvement in chronic allergic airway inflammation (CAAI).

We addressed this issue by comparing MC-distribution in wild-type C57BL/6 (WT), Wsh (MC-deficient) and intravenously BMMC-transferred Wsh (Wsh+MC) mice. CAAI was induced by ovalbumin/alum injections and challenges during 90 days. Lung sections were histochemically stained to detect MC.

In control mice, number of MC in the lungs was significantly higher in Wsh+MC [MC/section; Mean±SEM, 36±4] compared to WT [2±1]. No MCs were detected in Wsh mice. MC in WT mice were located around the central airway (CA) [1±0] and in the perivascular space (PVS) [1±0] whereas they were negligible in parenchyma (PA) and smaller airways (SA). In contrast, MCs in Wsh+MC mice were predominantly found in PA [16±2] and PVS [15±2] but also around CA [3±1] and SA [3±1]. Induction of CAAI in Wsh+MC mice caused increased MC number in the PA [28±6 vs. 16±2] and decreased MC number in the CA [0±0 vs. 3±1] and PVS [5±1 vs. 15±2] compared to controls. Importantly, these findings suggest that MC transfer causes a completely different condition, which might not be comparable to the WT and thus not serve as an adequate control. Instead,

Wsh+MC mice may be more suitable in experiments for studying CAAI, since mast cell number, distribution and relocation in response to inflammation more closely resemble findings in the human lung.

P965

Effects of chronic allergen exposure on the airway hyperreactivity in sensitized rats

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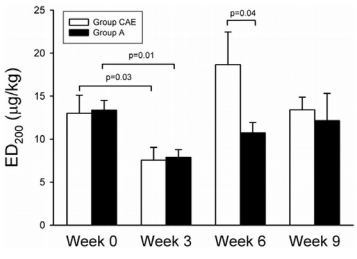
We aimed at investigating whether chronic allergen exposure (CAE) leads to the development of tolerance or enhances the airway hyperreactivity (AH) in sensitized rats. The airway resistance (Raw) was determined from the total respiratory system impedance under the control condition and following iv methacholine provocations (MCh 2-16 μg/kg) to establish the basal lung responsiveness. The rats were then sensitized to ovalbumin (OVA) and they were assigned into two groups: the rats in Group CAE (n=6) were exposed to aerosolized OVA three times a week throughout the study period, while the OVA was withdrawn during the entire protocol in the rats enrolled in Group A (n=8). Assessment of lung responsiveness was repeated in both groups in an identical manner on weeks 3, 6 and 9. The equivalent dose of MCh causing a 200% increase in Raw (ED_{200}) was calculated from each challenge. OVA sensitization was proved to induce AH on week 3 in Groups CAE and A (decrease in ED_{200} of 33±14% and 40±7%, respectively). The subsequent OVA exposure affected significantly the MCh responsiveness with sustained decreases in ED_{200} on week 6 in Group A and gradual return to normal on week 9, whereas the ED_{200} in Group CAE was similar to the initial on weeks 6 and 9.



Our findings demonstrate a diminishment of AH following CAE suggesting its favourable influence on the reduction of an existing AH after allergic sensitization.
Supported by grant OTKA K81179.

P966

Poly I:C treatment causes fatal AHR in sensitized mice

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Respiratory infections are known to promote airway hyperresponsiveness (AHR) in asthmatic patients. Toll-like receptors (TLRs) are parts of the innate immune system that recognize viral and bacterial components. This *in vivo* study explores the relation between TLR activation and AHR in a model of allergic airway inflammation.
Female BALB/c mice where sensitized on day 1 and 8, then challenged with either ovalbumin (OVA) or PBS on day 15-17. PolyI:C activating TLR3, LPS triggering TLR4 or PBS were then given i.n. during 4 consecutive days. On day 22 the outcome of metacholine (Mch) induced airway resistance was investigated with *flexi*Vent® technique. Cells and inflammatory mediators were analysed in bronchoalveolar fluid (BALF).
PolyI:C and LPS treated mice developed a marked AHR not seen among control mice. The airway resistance reached critically high levels in mice concomitantly challenged with OVA (Rmax at 1mgMch/kg: PBS: 2.1±0.1, PBS-LPS: 2.9±0.3, PBS-polyI:C: 3.6±0.2, OVA:5.6±0.2, OVA-LPS: 9.3±0.6 and OVA-polyI:C: 7.3±0.5 cmH_2O s ml^{-1}). The OVA-polyI:C mice also displayed circulatory collapse ($p<0.05$). PolyI:C increased the amount of lymphocytes and LPS neutrophils in BALF. Multivariate analysis of a panel of inflammatory mediators could not reveal a clear separation between PBS and OVA groups. However, polyI:C treatment induced a specific increase of IL-12 and KC in both treatment groups.
Costimulation with PolyI:C and LPS, representing viral and bacterial activation, respectively, caused AHR above the effect of OVA which in by polyI:C caused fatal circulatory responses. It is therefore tempting to suggest that TLRs might play a vital role in virus-induced exacerbation of allergic asthma.

P967

The influence of female sex hormones on the number of alternatively activated lung macrophages and airway inflammation in a mouse model of asthma

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The chance of developing asthma increases in girls during puberty. A role for sex hormones has been suggested. Recently we have shown that alternatively activated macrophages (aaMP) contribute to the development of asthma in female mice. Here we have investigated how sex hormone depletion before puberty affects asthma in mice and whether this correlates with aaMP numbers in lung.
Female Balb/c mice were ovariectomized (OVX) or sham treated before puberty. OVX animals were left untreated, received a 0.1 mg estrogen (E2) pellet, or a 15 mg progesterone (PG) pellet at the day of OVX (all groups n=8). Four weeks later, mice were sensitized i.p. with ovalbumin (OVA) and alum on days 1 and 7 and challenged with 1% OVA on days 14-20. On day 21, allergic inflammation (OVA-specific IgE, eosinophils) and aaMP numbers were assessed.
Ablating sex hormones before puberty significantly increased airway inflammation as judged from higher eosinophil numbers in bronchoalveolar lavage fluid and higher OVA-specific IgE levels in serum. aaMP numbers were unaffected by OVX. Treating OVX mice with E2 significantly reduced eosinophilic airway inflammation with a concomitant reduction in aaMP numbers, whereas PG did not change airway inflammation or aaMP numbers.
This study surprisingly shows that OVX in mice before puberty amplifies OVA-induced airway inflammation. This is a consequence of E2 depletion since PG substitution does not reduce the increased allergic inflammation while E2 substitution does. Our data also shows that the effect of OVX does not involve aaMP, whereas reduction of eosinophilic inflammation after E2 substitution appears to involve aaMP.

P968

Transcription factor FOXp3 is over expressed in BALT of systemic sclerosis model induced by collagen V nasal tolerance

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Background: To evaluate the transcription factor FOXp3 expression in lymphocytes of bronchus-associated lymphoid tissue (BALT) and correlate with the inflammatory process and the amonts of collagen in pulmonary interstitium of systemic sclerosis (SSc) model after type V collagen (COL V)-induced nasal tolerance.
Methods: Female New Zealand rabbits (N=12) were immunized with 1mg/ml of COL V in Freund's adjuvant (IM). After 150 days, six animals were tolerated by nasal administration of COL V (25 mg/day) (IM-TOL), daily during 60 days. Animals (N=6) not immunized and COL V tolerated served as control (CT). Immunohistochimistry and morphometric analysis was used to evaluate the T lymphocytes FOXp3 expression in BALT structures and inflammatory cells in pulmonary interstitium. Types I, III and V collagen expression were evaluated by Real-time PCR.
Results: We observed the BALT lymphocytes FOXp3 expressed in all of IM-TOL animals when comapred with 33,3% of CT (p=0,03) group. In the pulmonary interstitium, IM-TOL presented a significant decreased of lymphocytes (4.33±1.71 *vs.* 11.45±2.52), macrophages (5.7433±2.27 *vs.* 7.66±1.568) and monocytes (1.9158±0.7332 *vs.* 27.67±3.72) when compared with IM. COLI (0.10±0.07 *vs.* 1.0±0.528, p=0,002) and COLV (1.12±0.42 *vs.* 4.74±2.25, p=0,009) mRNA expression were reduced in IM-TOL when compared with IM.
Conclusions: COL V-induced nasal tolerance in the experimental SSc induced FOXp3 regulatory T cells in BALT which can trigger an immune regulatory mechanism resulting in decreased inflammation and collagen expression. It suggests that tolerance with COL V could be a promising therapeutic option for human scleroderma treatment.

P969

The respiratory allergen glutaraldehyde in the local lymph node assay: Sensitization by skin exposure, but not by inhalation

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Previously, a selection of low molecular weight contact and respiratory allergens had tested positive in both a skin and a respiratory local lymph node assay (LLNA), but formaldehyde was negative for sensitization by inhalation. To investigate whether this was due to intrinsic properties of aldehyde sensitizers, the structurally-related allergen glutaraldehyde (GA) was tested. BALB/c mice were

exposed by inhalation to 6 or 18 ppm GA (respiratory LLNA), both generated as a vapor and as an aerosol. Other groups received 0.25% or 2.5% GA on the skin of the ears (skin LLNA). Lymphocyte proliferation and cytokine production were measured in the draining lymph nodes. GA was positive in the skin LLNA and its cytokine profile (IL-4: IFN-g) skewed towards a Th2-type immune response with increasing dose. Inhalation exposure did not result in increased lymphocyte proliferation or increased cytokine levels, despite comparable tissue damage (irritation) in the skin and respiratory tract. We hypothesize that the highly reactive and hydrophilic GA oligomerizes in the protein-rich mucous layer of the respiratory tract, which impedes sensitization but still facilitates local irritation. Within the context of risk assessment in respiratory allergy, our results stress the importance of prevention of skin – besides inhalation – exposure to aldehydes like GA.

P970

Overexpression of peroxiredoxin 6 protect mice from ovalbumin-induced airway inflammation and hypersecretion of MUC5AC by reducing ROS levels

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Background: Oxidative stress plays an important role in the pathogenesis of asthma. Peroxiredoxin 6 (Prdx 6), as a newly identified peroxidase, protect cell or organ from reactive oxygen species (ROS)-induced oxidative stress.
Objective: The present study aimed at investigating the involvement of prdx 6 in asthma.
Methods: The ovalbumin (OVA) - induced allergic airway inflammation and MUC5AC production were examined in wild-type (WT), overexpressing (Prdx 6+/+) or Prdx6 null (Prdx 6–/–) mice. Prdx 6's expression in lung and intracellular ROS levels in bronchoalveolar lavage fluid (BALF) were evaluated.
Results: The expression of Prdx 6 was reduced significantly and up-regulated by dexamethasone and ambroxol in lung from WT mice. Prdx 6+/+ mice had significantly low airway inflammation, low levels of IL-13 in BALF as compared with those in WT mice. In addition, lower expression of MUC5AC in airway epithelial cells, less secretion of MUC5AC in BALF were found in Prdx6+/+ model mice, especially with fewer intracellular ROS levels in BALF. However, Prdx6–/– mice showed no significant difference compared to WT mice.

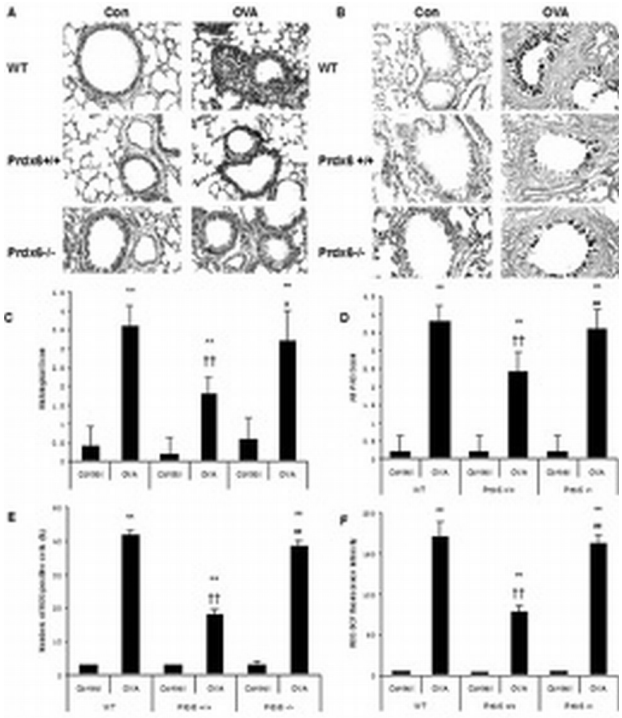

Figure legends: (A) Lung histology pictures. (B) AB-PAS staining of bronchus. (C) Histological scores. (D) AB-PAS staining scores. Overexpression of Prdx 6 inhibits significantly murine ovalbumin-induced airway inflammation and hypersecretion. (E) Ratio of ROS-positive cells to total cells in BALF. (F) Dichlorofluorescein (DCF) fluorescence intensity. There were fewer ROS-positive cells and fluorescence intensity in BALF from Prdx 6 +/+ model mice compared with WT and Prdx 6 -/- model mice. ** $P < 0.01$ as compared with Control group, †† $P < 0.01$ as compared with WT OVA group, and * and ** stands for $P < 0.05$ and 0.01 as compared with Prdx6 +/+ OVA group. Original magnifications were ×400.

Conclusions: Our data indicate that overexpression of Prdx 6 prevent allergic airway inflammation and hypersecretion by reducing ROS levels. While due to several compensatory mechanisms, targeted disruption of Prdx 6 fails to increase OVA-induced asthma.

P971

Crucial role of phospholipase Cε in the development of asthma in mice

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Phospholipase Cε (PLCε) is an effector of Ras and Rap small GTPases. Studies using genetically-modified mice of PLCε have shown its crucial role in skin inflammation. The purpose of this study is to examine whether PLCε is involved in the pathogenesis of inflammation in the respiratory system. First, we analyzed the location of PLCε in the respiratory system. By immunohistochemical analyses, we found that PLCε is expressed highly by alveolar epithelial cells and moderately by bronchial epithelial cells and smooth muscle cells in the bronchioles and the pulmonary arteries. Next, we experimentally induced asthma in PLCε$^{+/+}$ and PLCε$^{-/-}$ mice in the C57Bl/6J background by sensitization with ovalbumin (OVA) followed by boost with OVA by inhalation, and performed pathological analyses at 24 h after the last inhalation. Pathohistological studies of the sections of the lung and bronchi showed that infiltration of leukocytes and mucus production by the goblet cells were greatly suppressed in PLCε$^{-/-}$ mice. Also, characterization of inflammatory cells in bronchoalveolar lavage fluid demonstrated that the infiltration of leukocytes, particularly that of eosinophils, was suppressed in PLCε$^{-/-}$ mice. On the other hand, the serum levels of IgG and IgE specific for OVA were not affected by PLCε-deficiency. These results suggest that PLCε has a crucial role in the pathogenesis of asthma and that PLCε would become a molecular target for the treatment of patients with allergic asthma.

P972

Grape seed proanthocyanidin extract attenuates airway inflammation and hyperresponsiveness in a murine model of asthma: Downregulating inducible nitric oxide synthase

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Background: Allergic asthma is characterized by hyperresponsiveness and inflammation of the airway with increased expression of inducible nitric oxide synthase (iNOS) and overproduction of nitric oxide (NO). Grape seed proanthocyanidin extract (GSPE) has been proved to have antioxidant, anti-inflammatory and other pharmacological effects.
Aims and objectives: The purpose of this study was to examine the role of GSPE on airway inflammation and hyperresponsiveness in a mouse model of allergic asthma.
Methods: BALB/c mice, sensitized and challenged with OVA, were intraperitoneally injected with GSPE. HE staining and PAS staining were used to observe airway inflammation in lung tissue and airway mucus secretion, respectively. Quantification of cytokines in bronchoalveolar lavage fluid (BALF) and total serum immunoglobulin E (IgE) were detected by ELISA. The protein expression of iNOS was evaluated by immunohistochemistry and Western Blot analysis.
Results: GSPE remarkably suppressed airway resistance, and reduced the total inflammatory cell and eosinophil counts in BALF. Treatment with GSPE significantly enhanced interferon (IFN)-γ level and decreased interleukin (IL)-4 and IL-13 levels in BALF and total IgE levels in serum. GSPE attenuated allergen-induced lung eosinophilic inflammation and mucus-producing goblet cells in the airway. The elevated iNOS expression observed in the OVA mice was significantly inhibited by GSPE.
Conclusions: GSPE decreases the progression of airway inflammation and hyperresponsiveness by downregulating the iNOS expression, promising to be a potential in the treatment of allergic asthma.

P973

The effects of bacilli Calmette Guerin-polysaccharide nucleic acid on nasal airway inflammation and resistance in allergic rhinitis mice

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Background: Lots of data can be found to approve that exposure to mycobacteria has the potential to suppress the development of allergic rhinitis or atopy.But the influence of bacilli Calmette Guerin-polysaccharide nucleic acid (BCG-PSN) especially on nasal airway inflammation and resistance (R_{NA}) on mice,are poorly understood.

Objective: Investigate the effects of BCG-PSN on nasal airway inflammation and resistance in allergic rhinitis mice.

Method: Balb/c mice were sensitized by intraperitoneal injection of ovalbumin (OVA)/saline,then challenged by intranasal administration under conscious to establish an allergic rhinitis model.The mice were intervened with BCG-PSN by intranasal administration (1, 5, 10μg) before sensitization. A novel method of R_{NA} was applied to measure nasal function. Then the inflammation of nose was accessed by nasal tissues histology.

Result: In 3 interventions groups the R_{NA} had no statistical difference compared to rhinitis group.But compared to the normal group,the R_{NA} of expiration in 10μg,5μg group and the R_{NA} of inspiration in 10μg group had no statistical difference.The number of eosinophils (Eos) in 10μg group was lower than rhinitis group as well as mucosal thickness.

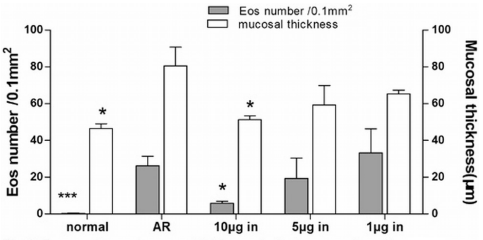

Fig.1. Eos number and mucosal thickness in the lower portion of nasal septum in all groups.AR=allergic rhinitis,in=intranasal adminstration. Data are shown as means ± SD.Statistical significance was measured by Student's t test. * p<0.05 vs. AR group. *** p<0.001 vs. AR group. n=3-4 mice per group.

Conclusion: BCG-PSN can inhibit nasal airway inflammation and decrease nasal airway resistance in allergic rhinitis mice.