258. Asthma: mechanisms of airway inflammation

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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 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 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 OVA-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.

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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 differentiation in asthmatic patients.
Methods: Purify CD4+ T cells from human PBMC and mice spleen were cultured under Th2 or Th2 + IL-27 conditions. IL-4 and IFN-gamma were detected by ELISA. IL-27R 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-r, 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.We further 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 the IL-27 resistance. We demonstrated that high dose of IL-4 treatment resulted in impairment 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-dependant, but independent of IFN-r, 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 intratracheal antigen challenges in sensitized mice, IL-10 production 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-1β, 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)-1^{high} 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 the induced Tr1 cells by the specific antigen challenge suppressed macrophages to produce CXC chemokines through activation of the IL-10 receptors on the cells.

P2361

Probiotics and synbiotics: 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 diseases 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)-imject alum and challenged from day 17 till 23 daily with OVA. From day 24 till day 56, the animals were challenged with OVA 3 times a week and on the same days 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 leukocytes infiltration by 50% especially eosinophilia. Neutrophilia in the airways was reduced by GCS and probiotics. Effects on airway remodelling are in process.
Conclusion: The probiotics and synbiotic used in this study seem to be as potent as GCS in reducing cell infiltration in mice with chronic asthma.

P2362

Short-chain fatty acids are potent modulators of allergic airway inflammation

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Over the past few decades, there has been a clear increase in the prevalence of asthma in westernized countries. Both experimental and epidemiological data indicate that environmental factors, such as an individuals diet and commensal flora can have profound effects upon the susceptibility and progression of inflammatory diseases. Short-chain fatty acids (SCFAs), end-products of the colonic fermentation of dietary fibers by bacteria, have been shown to exert protective effects upon cardiovascular and intestinal inflammation; however, their impact on lung inflammation remains to be determined. We have found that mice treated with SCFAs exhibit an accelerated resolution of house-dust mite (HDM) induced allergic airway inflammation. Although the antigen presentation capacity of dendritic cells (DCs) isolated from SCFA-treated mice was normal, such DCs failed to fully polarize cells towards Th2 or Th17 subtypes. Comparatively, mice that were fed a low-fiber diet (LFD)- reducing the availability of endogenous SCFAs - exhibited an exacerbated airway inflammation when exposed to HDM. Indeed, LFD-fed mice had an increased bronchoalveolar lavage (BAL) cellular infiltrate and eosinophilia, along with significantly more systemic HDM-specific IgE and IgG1 antibodies. Our results show that fiber and SCFAs have intrinsic immunomodulatory functions with the ability to accelerate the resolution of allergic airway inflammation.

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Exposure to ozone shifts natural killer cells co-cultured with epithelial cells towards a type II phenotype

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Nasal epithelial cells (NECs) are one of the first targets for air pollutants, such as ozone. Previous studies in our lab have demonstrated that natural killer (NK) cells are an important immune cell population in the nose. NK cells and NECs interact with each other via receptor-ligand interactions as well as soluble mediators. NK cells are an important source for type I cytokines, like IFN-γ, 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.
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 CXCR3 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 phenotypes from a type I (high IFN-γ) to 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

polarization. However, little is known about their role in pneumallergen adjuvant free animal models.
Objectives: Our aim was to evaluate whether MSC could suppress inflammation in an adjuvant-free mouse model of house dust mite (HDM) allergic asthma. We hypothesized that alleviated inflammatory responses could trigger a decrease in airway hyper responsiveness (AHR) in treated mice.
Methods: 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 5.10^5 MSCs immediately prior first challenge. Effects on AHR, pulmonary inflammation, bronchoalveolar lavage (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 PENH ($p<0,05$) 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 ± 34 vs 734 ± 119, $N.10^3$/mL, $p<0.001$), especially neutrophils ($p<0.01$), 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 ($26,80\pm11,19$ vs $10,39\pm2,75$, pg/mL). In parallel, non-asthmatic treated mice showed neither significant pulmonary cell infiltration (227 ± 38 vs 152 ± 18, $N.10^3$/mL) nor development of AHR compared to controls.
Conclusions: Taken together, these results demonstrate that MSC reduced AHR in a relevant allergic model of HDM asthma.

P2365
Dose dependent effect of thrombomodulin in a murine model of allergen-induced asthma
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Background: Thrombomodulin (TM), the thrombin receptor on the endothelial cell surface, plays an important role in coagulation and inflammation by inactivating thrombin and activating protein C. Previously we reported that recombinant human TM (rhTM) is protective against murine asthma. However, the effect of different concentrations of rhTM on asthmatic inflammation remains unclear.
Objective: To evaluate the dose-dependent effect of inhaled rhTM on airway inflammation and hyperresponsiveness in a murine asthma model.
Methods: Bronchial asthma was induced by sensitization and challenge with ovalbumin (OVA). Mice treated with saline were used as control. The effect of inhaled 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 (BALF). 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 $3.75\mu g$/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.

P2366
YKL-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 mediated phosphorylation of JNK, ERK, but not p38 in BEAS-2B. Transfection using a NF-κB-luciferase reporter 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.

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 GATA-1, GATA-2, and GATA-3, respectively. We have previously demonstrated GATA-1-mediated CCR3 transcription with functional mapping of a GATA element in the regulatory region of CCR3 gene. Here, we investigated whether GATA factors other than GATA-1 play a major role in CCR3 transcription in these cell types. Knockdown assay showed that GATA-2 siRNA reduced the CCR3 reporter activity in EoL-1 eosinophilic cells, and GATA-1 and GATA-2 siRNAs reduced in EoL-1 and HMC-1 mast cells, while GATA-3 siRNA suppressed it in Jurkat T cells. In parallel, EMSA and ChIP analyses revealed DNA binding to and occupancy on the functional GATA element of different GATA factors. These results highlight that different GATA factors participate in CCR3 transcription in a cell type-speciifc fashion among the major allergic inflammatory cells.

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 remains limited and it may cause anaphylactic adverse effects when administrated subcutaneously. One of the new modality 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 dermatophagoides farinae, formulated with a synthetic vector. This vaccine was tested on a murine model of allergic asthma with a prophylactic approach. We evaluated the response to treatement by airways hyperresponsiveness (AHR) measurements, and by systemic (Ig assays) and in situ (cytokine assay) immunological responses.
Vaccinated mice showed a decreased AHR. The cellularity of the bronchio-alveolar lavage (BAL) was also decreased in the vaccinated group with a decrease of neutrophils and eosinophils. IgG2a/IgG1 ratio was increased in the vaccinated group in favor of Th1 bias confirmed by increase of IFN-γ secretion by splenocytes restimulated in vitro with purified Der f 1. Analyses of cytokines in BAL showed a decrease of Th2 and Th17 cytokines and an increase of IL-10 and IFN-γ. Total IgE levels were not modified.
This formulation of DNA vaccination induces cellular and humoral specific response with a pro-Th1 bias accompanied by a decrease of the Th17 and Th2 responses. It enables an improvement of lung cellular infiltrate and airway hyperresponsiveness. DNA vaccination seems therefore promising to prevent allergy.

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 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 suppress CD3, CD28, or ICOS, which can up-regulate the expression of T lymphocyte cytokines, or indirectly stimulate the expression of CTLA-4 or CD45, which can down-regulate the expression of T lymphocyte cytokines.
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,

did not change in both ROG-pcDNA3.1- and ROG-siRNA-transfected Th1 and Th2 cells ($P>0.05$).
Conclusions: ROG can inhibit the expression of Th1 and Th2 cytokines by down-regulating the expression of ICOS, which could be a petential regulating target for asthma treatment.

P2370
Long-term bortezomib treatment decreases allergen-specific IgE but fails to amend chronic asthma in mice
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Since allergen-specific immuno-globulin E (IgE) enables mast cells and eosinophils to react on allergen-contact it plays a critical role in the formation of allergic inflammation and has been identified as a target for asthma therapy. By inhibiting the proteasome complex Bortezomib efficiently depletes Ig-secreting plasma cells and, thus, reduces Ig-serum titers. The present study evaluates the therapeutic potential of Bortezomib in a mouse model of chronic experimental asthma.
Therefore, BALB/c mice were sensitized to ovalbumin (OVA) and challenged with OVA-aerosol for twelve weeks. Bortezomib treatment was started after six weeks of challenge, and continued for one week (short-term) or six weeks (long-term), respectively, with a dosage of 0.75 mg/kg body weight with two intra-venous injections weekly. Airway responsiveness to metacholine, lung histology, Ig serum titers, and plasma cell numbers were assessed.
In mice with chronic experimental asthma short-term treatment resulted in decreased eosinophil numbers in BAL fluids, while long-term treatment significantly lowered serum titers of anti-OVA IgE. Nevertheless, neither short-term nor long-term treatment significantly diminish ed plasma cell numbers, anti-OVA IgG1 serum titers, allergic airway inflammation or improved lung function.
These results demonstrate that Bortezomib has no therapeutic effect on chronic experimental asthma in mice. Therefore, Bortezomib treatment could have only limited value as plasma cell depleting therapy against allergic bronchial asthma.

P2371
A low total sputum cell count is a marker for asthma remission during adolescence
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An improved understanding of the physiological and pathological characteristics underlying adolescent asthma phenotypes, especially those who grow out, is essential.
Methods: A Birth Cohort (n=1456) was established on the Isle of Wight, UK and seen until 18-years. Relevant tests at 18-yrs included ISAAC questions, spirometry, exhaled nitric oxide (FeNO), skin test, bronchial responsiveness (BHR) and (in a subset) sputum induction and processing. Asthma groups were "never asthma" (no asthma since birth), "persistent asthma" (asthma at age 10 and 18), "remission asthma" (asthma at age 10 but not at 18) and "new-onset asthma" (asthma at age 18 but not at age 10). This analysis is confined to subjects who underwent sputum induction.
Results: Asthma (new-onset or persistent) group (n=40) had a higher eosinophil count (1.73% (interquartile range [IQR] (25-75): 0.25-5.88) vs.0.25% (IQR: 0 – 1.25); p=0.01) and ECP (median 115.00 (62.08-374.95) vs. 62.34 (25.44 -229.30); p=0.04) compared to never asthma (n=46) at age 18. Importantly, those with asthma remission (n=18) had a lower total cell count compared to never asthma (31.50 (12.88-40.38) vs. 47.00 (19.50-181.25); p=0.03), even though both groups were asymptomatic at age 18 and their lung function, BHR and FeNO were not significantly different. Remission group also had a significantly reduced eosinophil 0.25% (IQR: 0 – 1.44) vs. 3.00% (0.66-6.56); p=0.05) compared to those with new-onset asthma (n=20) and a lower ECP when compared to those with asthma at age 18 (median 35.48 (18.74-229.63) vs. 115.00 (62.08-374.95); p=0.05.
Conclusion: A low sputum total cell count is indicative of asthma remission during adolescence.

P2372
Histamine skin sensitivity in patient with drug hypersensitivity
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One of less studied is the test of histamine serial dilutions sensitivity and exactly this test may be very important for antihistamine preparations effectiveness study.
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 ten 0,1% histamine preparation. The end point was histamine dilution that caused papule $\geq$ 2mm in diameter.
Results and discussion: 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.

P2373
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 serum 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 rAgs were performed using serums 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 SR1FA, SR9, SR13, SR17 and SR25). The protein performing the best (SR9) reached 80% sensitivity and 85% specificity with an AUC of 0.90.
ELISA using rAgs specific from SR were effective for serodiagnosis of FLD. The use of antigen panels including rAg specific from other micro-organisms involved in FLD (*Aspergillus, Lichtheimia, Wallemia*) may increase the diagnosis 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.

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Characterizing T cell responses associated with hypersensitivity pneumonitis in pigeon fanciers
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Background: Around 10% of pigeon fanciers are affected by a form of hypersensitivity pneumonitis known as pigeon fanciers' lung (PFL). PFL is an aberrant inflammatory immune response to dust particles that contain pigeon antigens, inhaled during pigeon husbandry, and manifests in fever and dyspnoea. A lymphocytic infiltrate has been observed in lung biopsies and bronchoalveolar lavage as well as Th1 cytokines. However, little is known about the implicated pathogenic T cells and whether a systemic response can be measured that indicates disease presence or severity.
Methods: Disease history and blood samples were taken from 72 pigeon fanciers at a local pigeon show, 36.1% of whom had PFL. Effector T cell responses were examined by ELIspot.
Results: All pigeon fanciers showed an Interferon (IFN)gamma response against

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.
CFSE dilution demonstrated proliferation of CD4 cells in response to pigeon antigen that also possessed CD49d, a potential lung-homing integrin.
Interestingly, the IFNg 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.

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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.

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Challenges in the management of patients with ANCA- associated vasculitides
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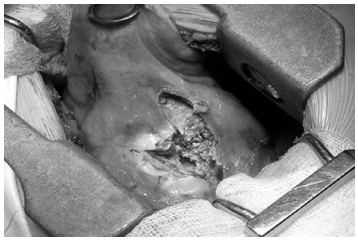
Background: Granulomatosis with polyangiitis (Wegener's) and microscopic polyangiitis are antineutrophil cytoplasm antibodies (ANCA) – associated vasculitides with significant morbidity and mortality.
Objective: We evaluated the evolution of 12 patients diagnosed in our clinic between 2000 and 2011, 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.