96. Cell biology and inflammatory gene expression in chronic lung disease

P800

Human alveolar macrophages express mucin5B

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Introduction: This study investigated whether alveolar macrophages (AM), in addition to epithelial cells, express mucin5B (MUC5B) in human lung environment influenced by long-term cigarette smoke.
Methods: We analyzed MUC5B expression at the level of apomucin and mRNA in human BALF cells from fifty subjects (20 non-smokers, 17 patients with chronic bronchitis [CB] and 13 patients with chronic obstructive pulmonary disease [COPD]).
Results: Apomucin5B was observed in BALF mononuclear cells in 60% of all subjects whereas significantly higher frequency of apomucin5B+ cells was found in CB (95% CI 4.5-24.9) and COPD (95% CI 6.2-39.6) subjects than in non-smokers (95% CI 0.5-2.5). Apomucin5B+ mononuclear cells showed strong expression of CD163, confirming their identity as AM. MUC5B mRNA expression was detected in AM of subjects investigated by in situ hybridization. qPCR showed MUC5B mRNA expression in purified AM of subjects investigated. An inverse correlation between apoMUC5B+ AM levels and FEV1 was found ($r = -0.46$, $p = 0.002$ in whole study group). The correlation between apoMUC5B+ AM levels and smoking pack-years was positive in whole study group ($r = 0.65$, $p < 0.001$).
Conclusion: Under injuring circumstances of cigarette smoking human alveolar macrophages can changes their expression profile in the lung.

P801

Molecular mechanisms of plasminogen activator inhibitor-1 elevation in COPD sputum

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Background: Plasminogen activator inhibitor-1 (PAI-1) is an important regulator of fibrinolysis at sites of vascular injury and thrombus formation. Oxidative stress is known to involve in PAI-1 expression. We previously reported that mean PAI-1 levels in sputum of COPD was significantly higher than that of age-matched controls and significantly correlated both with sputum malondialdehyde (a oxidative stress marker) and NF-kB DNA binding activity in sputum macrophages. However, the precise mechanisms of PAI-1 elevation in COPD were not clarified. We hypothesised that HDAC2 reduction in COPD involves in PAI-1 elevation. The aim of this study was to investigate the association between HDAC2 reduction and elevated PAI-1 expression.
Methods and results: A549 cells were transfected with SiRNA of HDAC2 to knockdown HDAC2 and followed by treatment with TGF-β. HDAC2 knockdown (KD) significantly upregulated PAI-1 release (Wild type (WT) *vs.* HDAC2 KD: 2.9±0.2 *vs.* 4.1±0.3 ng/ml with 0.01ng/ml TGF-β, 7.4±0.4 *vs.* 8.4±0.5 ng/ml with 0.1ng/ml TGF-β). To investigate the association between NF-kB DNA binding activity and HDAC2, HDAC2 KD cells were stimulated by 10 ng/ml TNF-α for 2hrs, and NF-kB DNA binding activity and p65 acetylation were evaluated with TransAM NF-kB p65 Activation Assay kit and Western blot, respectively. NF-kB DNA binding activity was significantly increased in HDAC2 KD cells (activity(OD)/protein: WT *vs.* HDAC2 KD; 76±4 *vs.* 98±4). Acetylation of p65 also significantly upregulated in HDAC2 KD cells (acetyl-p65/p65: WT *vs.* HDAC2 KD; 2.3±0.1 *vs.* 3.1±0.1).
Conclusion: HDAC2 reduction in COPD seems to cause PAI-1 elevation in COPD via activation of NF-kB DNA binding by p65 acetylation.

P802

The complex role of SOCS1 expression in peripheral blood mononuclears of patients with bronchial asthma

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Aim: The aim of the study is to analyze an expression of a negative regulator of cytokine signaling SOCS1 in peripheral blood of patients with allergic (ABA) and non-allergic bronchial asthma (NABA).
Materials and methods: 14 healthy controls, 40 patients with allergic (atopic) and 33 with non-allergic BA were examined. The expression of SOCS1 mRNA was analyzed by RT-PCR. Primers of SOCS1 have been developed on the basis of known sequences (GenBank). Expression level of mRNA SOCS1 was estimated relatively to level of β-actin mRNA. Preparation of cell lysates, and the Western blotting were performed through the standard procedure. Anti-SOCS1 antibodies (Santa Cruz Biotechnology, UK) were used. The level of protein was analyzed relatively to β-actin using anti-actin antibodies (Sigma Aldrich, USA).
Results: We determined the decrease (in 5,4 times) of SOCS1 expression in peripheral blood mononuclears in patients with ABA in comparison with healthy persons (p=0,036) and NABA patients (in 5,3 times) (p=0,024) (U-criterion). NABA patients had no significant difference in comparison with healthy persons. SOCS1 mRNA expression level in peripheral blood mononuclears in ABA patients was significantly lower than in the healthy persons (in 1,4 times) (p=0,048; crit. Tukey) and NABA patients (in 1,3 times) (p=0,041; crit. Tukey).
Conclusion: SOCS1 may play an important role in pathogenesis of bronchial asthma. Decrease of SOCS1, and SOCS1 mRNA expression in patients with ABA may be sign of defect in negative regulation system of cytokine signaling. It may be caused by genetic based impairment of SOCS1 expression regulation either on transcription or further levels (translation, protein levels).

P803

Reduced expression of interferon stimulated genes in refractory asthma

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Introduction: Patients with refractory asthma are more susceptible to allergen- and infection-induced exacerbations. This susceptibility is poorly understood, but it may be related to an inefficient activation of innate host defence pathways. Interferon-stimulated genes (ISGs), such as myxovirus resistance (MX1), 2'5'-oligoadenylate synthetase (OAS) and viperin are associated to biological activities, including antiviral, antiproliferative, and proapoptotic effects.
Objective: The aim of the study was investigate the expression of antiviral genes in patients with refractory compared with moderate and mild asthma.
Methods: The mRNA expression of the ISGs (MX1, OAS and viperin), interferon (IFN) type I (β) and type III (IL-28 and IL-29) were measured by RTq-PCR in cells of induced sputum from 19 healthy subjects, 19 mild, 22 moderate and 22 refractory asthmatics.
Results: The mRNA expression of Mx1 and viperin was significantly reduced in refractory asthmatics (p=0,04 and p= 0,03, respectively), while the mRNA expression of OAS, IFNβ, IL-28 and IL-29 was not different between groups.
Conclusion: The results point to a deficient innate immune response in refractory asthmatics demonstrated by a decreased expression of some interferon stimulated genes.

P804

Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi

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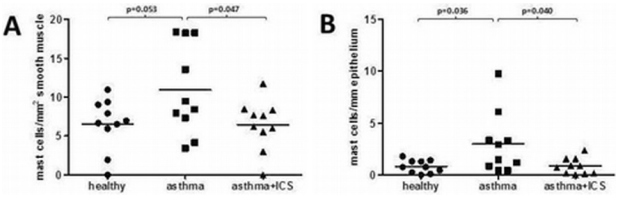
Background: Mast cells are important in the pathophysiology of airway inflammation and evidence suggests their sub-localisation within the airway is altered in asthma. However, little is known about the effect of corticosteroids on mast cell localisation within the bronchi.
Aims and methods: We aimed to examine mast cells numbers within the smooth muscle, epithelium and submucosa in bronchial biopsies of healthy subjects (n=10) and well-characterised asthmatic patients, using either b_2-agonists alone (n=10) or b_2-agonists and inhaled corticosteroids (n=10). Immunohistochemical analysis of tryptase positive mast cells was performed.
Results: Patients using inhaled corticosteroids (asthma+ICS) displayed significantly lower numbers of mast cells within their smooth muscle (fig 1A) and epithelium (fig 1B) compared to those not treated with inhaled corticosteroids (asthma). Submucosal mast cells were not affected by corticosteroid treatment. Numbers of smooth muscle mast cells correlated with bronchial responsiveness (rho -0.6, p=0.008) and epithelial mast cells with exhaled NO (rho 0.8, p<0.001).



Conclusions: We demonstrate that glucocorticosteroids differentially affect mast cell numbers within specific airway sub-locations highlighting the importance of mast cell and smooth muscle/epithelial interactions in asthma pathogenesis.

P805

Estimation of activity apoptosis genes based on expression of Bcl2, Bax, caspase-3 activity in bronchial epithelium in asthma

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Aim: The aim is to reveal the disorders of apoptosis in bronchial epithelium in bronchial asthma (BA) based on the estimation of expression of Bcl2, Bax, caspase-3.
Methods: In 21 patients a fibreoptic bronchoscopy was performed (patients have signed the ICF). Expression of Bcl2, Bax, CPP32 (caspase-3 activity) in bronchial epithelium was performed by immunohistochemical analysis of bronchus biopsies taken in fibrobronchoscopy using DAKO kits.
Results: In allergic BA elevation of Bcl2 expression and decrease of Bax expression compared to nonallergic BA and oral glucocorticoids taking patients were found. Activity of Bax expression was significant decreased in allergic BA compared to that in other groups. The same data were revealed on analysis of Bcl-2/Bax index. Expression level of caspase-3 was high in both groups.
Conclusion: Features of apoptosis in bronchial epithelium in different variants of BA could indicate to different pathogenetic mechanisms of apoptosis in allergic inflammation persistence.

P806

Role of altered level of oxidant-antioxidant in disease prognosis of asthma

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Background: Asthma is a chronic airway disorder which is associated to airway obstruction. Inflammatory and immune cells generate more reactive oxygen species in asthma and lead to contribute in tissue injury.
Aims and objectives: The aim of this study was to investigate the role of oxidant-antioxidant imbalance with disease severity in asthmatic patients.
Methods: In this study 140 asthmatic patients and 70 healthy controls were documented. Malondialdehyde level, total protein carbonyls and sulfhydryls were measured as the indicator of oxidative stress in plasma. Antioxidants were evaluated by the activity of catalase, glutathione peroxidase, total blood glutathione and total antioxidant capacity.
Results: The present work showed that the levels of malondialdehyde (4.51±0.12 nmol/mL) and protein carbonyls (1.30±0.02 nmol/mg) were found to be significantly higher in asthmatic patients while protein sulfhydryls (0.54±0.01 mmol/L) decreased as compared to controls (2.84±0.12 nmol/mL, p<0.001; 0.79±0.02 nmol/mg, p<0.001 and 0.60±0.02 mmol/L, p<0.01 respectively). We also observed increased total blood glutathione (0.98±0.02 mmol/L), decreased glutathione peroxidase (41.22±1.10 U/g Hb) and catalase activity (4650±81.36 U/g Hb) in erythrocytes compared with control (0.84±0.04 mmol/L, p<0.01; 48.37±2.47 U/g Hb, p<0.01 and 4946±89.94 U/g Hb, p<0.05 respectively). The level of total antioxidant status (714.70±23.75 μmol/L) in plasma were also decreased as compared to control (840.40±28.39, p<0.001).
Conclusions: These results support the hypothesis that an imbalance in oxidant-antioxidant is associated to the oxidative stress which plays a significant role in severity of the disease.

P807

Leptin, adiponectin and ghrelin levels in female patients with asthma during stable and exacerbation periods

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Introduction: Adipose tissue-derived hormones may be involved in the relationship of obesity with asthma. No definite conclusions regarding the role of leptin and adiponectin with asthma are available. No studies have examined the role of ghrelin in asthma.
Methods: We assessed the concentrations of leptin, adiponectin and ghrelin in 32 post-menopausal stable asthma patients (mean age±SD:57.5±8.9years), 37 female asthmatics during exacerbations (51.24±14.50years) and 8 weeks later, and 22 controls (57.6±10.8years). We examined the relationship between the three peptides and indexes of airway inflammation and atopy.
Results: Stable asthma patients exhibited higher leptin and lower ghrelin concentrations compared to controls (24.8±14.8vs11.2 (5.7-21.7),p=0.04 and 470.1 (353.0-578.6) vs 739.0 (614.6-955.5), p<0.001, respectively). Patients with severe asthma had higher leptin and lower adiponectin levels vs patients with mild to moderate asthma (31.1±15.5 vs 19.2±12.1, p=0.021 and 6.7 (4.6-12.9) vs 16.6±9.3, p=0.017, respectively). Increased BMI was associated with increased asthma severity, however when adjusted for leptin levels this association did not persist. During asthma exacerbations serum leptin levels and leptin/adiponectin ratio were elevated and adiponectin and ghrelin levels were decreased compared to stable state (19.8 (15.2-29.1) vs 9.8 (6.1-16.2), p<0.001 and 2.6 (1.9-4.6) vs 0.6 (0.4-1.1), p<0.001, and 7.9±3.2 vs 15.0±7.0, p<0.001, 662.6±227.5 vs 884.0 (660.4-1018), p<0.001, respectively).
Conclusion: Our data suggest that leptin, adiponectin and ghrelin may play a significant role to the pathogenesis of asthma during stable state and asthma exacerbatio independently of obesity.

P808

Tobacco smoking alters the relationship between airway inflammation and airway hyperresponsiveness in asthma

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Background: Smoking in asthma patients constitutes a major health problem, due to impaired steroid responsiveness, poor symptom control and an accelerated loss of lung function. This may relate to alterations in the airway inflammation. The aim of the present study was to evaluate the effect of smoking on the relationships between airway inflammation and airway hyperresponsiveness in asthma patients not on steroid treatment.
Material and methods: A group of smoking asthma patients (n=27) was compared to a group of non smoking asthma patients (n=34) with induced sputum, exhaled nitrogenous oxide (eNO) and airway hyperresponsiveness with a bronchial challenge with mannitol. Airway eosinophilia was defined as a sputum % eosinophils >2%.
Results: A significantly higher proportion of patients with non-eosinophilic asthma was observed in the smoking group than in the non-smoking group (44% and 18%, p=0.023). The mean eNO was lower in the smoker group compared to the non-smoker group (11.7 ppb vs 38.2 ppb (p=0.001)). The proportion of subjects with a positive mannitol test was comparable among smokers (68%), and non-smokers (50%) (p=0.19). Smokers with a positive mannitol test (n=15) had a lower mean eNO (15.4 ppb) than the non-smokers (mean eNO=46.3 ppb, n=15), p=0.006.
Conclusion: Our data showed that despite of a lower proportion of eosinophilic phenotype amongst the smoking asthma patients than amongst the non-smokers, no reduced degree of airway hyperresponsiveness to mannitol in the smoker group was observed, indicating that pathogenetic mechanisms other than eosinophilic airway inflammation are responsible for the tendency to airway narrowing in smoking asthma patients.

P809

Lipid laden macrophages and lipase as markers of aspiration in patients with chronic cough

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Introduction: Gastroesophageal reflux disease (GERD) causes chronic cough by several mechanisms such as reapeated microaspiration.Some authors question that IL exclusively reflects this phenomenon.Our aim was to evaluate the lipase as a marker of microaspiration.
Material and methods: We included 79 patients with chronic cough who underwent of clinical history, spirometry with bronchodilator test, chest radiography, determination LI in sputum and measurement of lipase in blood and sputum.
Results: We obtained 77 samples of sputum with cellularity enough and 42 (54%) had lipid laden. The average LI was 110. In 55 patients (71%) blood lipase (36 (16)U/L) and sputum (14 (35) U/L) could be determined. But there was no significant correlation between them. Lipase in sputum was not associated with LI, although patients with LI> 30 had higher levels of lipase. Patients with symptoms of reflux had LI and lipase higher in sputum than patients without clinical reflux (mean difference of 26 units (p = 0.03) and 9 U/L (0.001), respectively). Also, patients taking proton pump inhibitors (PPIs) had not lower LI and lipase levels in sputum.Patients with a LI> 30 had lower FVC (mean difference 533ml, p = 0.033) and FEV1 (mean difference 507 ml, p = 0.019) and an inverse relationship between LI and lung function when adjusted for the presence of asthma that is not could be confirmed for the lipase.
Conclusions: Lipase in sputum is detectable in most patients with chronic cough.Patients with clinical reflux have higher levels of both markers, but therapy with PPIs modifies the results.Elevated IL affects lung function.

P810

Regulation of immune response plasticity – A path to success in pulmonary diseases management

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Disorder of the immune response plasticity is the basis of pathogenesis of pulmonary diseases with inflammatory component.
Objective: To assess the possibility of immune response plasticity management in pulmonary diseases with inflammatory component by means of microenvironment components of alveolar macrophages (AM).
Methods: AM of patients with chronic obstructive pulmonary disease (n=18), bronchial asthma (n=17), sarcoidosis (n=23) were cultured with various concentrations of standard serum FBS containing TGF-β and surfactant protein D (SP-D)–serum model of reprogramming. Quality of macrophages bioprogramming was measured by morphological characteristic (light microscopy), cytokine production and surface macrophages markers expression (flow cytometry, Beckman Coulter FC500).
Results: Changing of macrophages microenvironment with FBS concentration and the level of TGF-β and SP-D, respectively, purposefully programmed macrophages phenotype on M1 proinflammatory, or M2 antiinflammatory, shifting the immune response to Th1 or Th2, respectively. Decreasing FBS concentration to 0% programmed macrophages to M1 phenotype which was confirmed by morphologic characteristic, cytokine production and macrophages markers expression. The consecutive increasing of FBS concentration from 20% to 40% led to bioprogramming of macrophages on M2 phenotype and Th2 response development with close relationship to concentration.
Conclusions: Serum model of reprogramming was effective in regulation of immune response plasticity in pulmonary diseases and can be used as the new approach in pathogenetic therapy of pulmonary diseases with inflammatory component.

P811

Change of phenotype and phenotypic plasticity of alveolar macrophages in COPD

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Development of chronic obstructive pulmonary disease (COPD) is due to imbalance of Th1/Th2 immune responses which suppose disorder in M1 and M2 macrophages phenotypes.
Objective: Assessment of macrophages phenotype and phenotypic plasticity of alveolar macrophages (AM) in COPD.
Methods: In vitro experiments were carried out on AM cultures of COPD patients (n=18, 59.7±3.56 y.o.). AM phenotype was assessed by flow cytometry (Beckman Coulter, FC500) by surface macrophages markers CD80, CD25 and CD163, CD206, typical for M1 and M2 phenotype, respectively. Phenotypic plasticity of AM was measured as percentage change of markers during 36 hours of AM reprogramming in the presence of standard serum (FBS) in concentrations 0%, 10%, 40%.
Results: In COPD patients AM of M1 phenotype prevailed, thus AM population was not monophenotypical: CD80 44.76%±1.80%, CD25 70.26%±2.20%, CD163 31.71%±1.74% and CD206 37.5%±1.23%. During AM reprogramming decreasing of FBS concentrations from 40% to 0% significantly changed expression of M1 phenotype markers: CD80 from 38.09%+0.93% to 65.42%+1.07% and CD25 from 41.51%+1.24% to 72.36%+1.43%. Increase of FBS concentrations from 0% to 40% significantly increased M2 markers expression CD163 and CD206. After 36 hours of culturing with 10%FBS only CD80 pointed to M1 phenotype prevalence in AM of COPD patients.
Conclusions: In changing environment the previous condition of macrophages phenotype is longer fixed by M1 marker CD80, and CD80 can be used for phenotypic plasticity definition. Phenotypic plasticity should be assessed specific to certain factors so that different markers show different picture of phenotypic plasticity in COPD.

P812

Phenotypic plasticity of fibrocytes upon culture with airway smooth muscle

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Asthma is a major cause of morbidity and mortality worldwide and its prevalence is increasing. Increased airway smooth muscle (ASM) mass is a hallmark of asthma, which increases with disease severity and is associated with decline in lung function. Fibrocytes (FCs) are elevated in the peripheral blood and ASM in asthma and ASM has the potential to mediate FC recruitment (Saunders *et al*, J Allergy Clin Immunol, 2009;123:376-84). We hypothesised that once recruited to the ASM FCs differentiate into a more ASM like phenotype under the influence of local factors.
FCs were isolated from peripheral blood, ASM from bronchial biopsies and lung resection material. FCs were labelled with CFSE prior to culture with ASM cells for 7d, allowing identification by gating following flow cytometry.
24h after isolation from the peripheral blood cells are predominantly $CD14^{high}$/α-smooth muscle actin $(\alpha SMA)^{low}$. Subsequent monoculture yields $CD14^{low}/\alpha SMA^{high}$ cells, consistent with differentiation to fibrocytes. Coculture with ASM from both non-asthmatic (NA) and asthmatic (A) donors yields $CD14^{high}$ FCs, whereas ASM from NA donors yields αSMA^{low} FCs and ASM from A donors yields αSMA^{high} FCs (Table 1).

Table 1

	Cells (% positive)				
	24h after isolation	ASM - NA		ASM - A	
		FC	FC+ASM	FC	FC+ASM
CD14	78±5	12±8*	70±6§	22±3*	71±6§
αSMA	15±10	52±10*	14±3§	80±8*	74±5*#

$p<0.05$ compared to *24h after isolation, §paired FC data, #FC+ASM, t-tests, n=4-7.

Our results show that FCs have the capability to undergo phenotypic plasticity, depending on culture conditions. Further work is required to understand the factors affecting FC differentiation upon localisation to the ASM in asthma and the resultant contribution of FCs to ASM dysfunction.

P813

Expression of interferon regulatory factor 8 in human lung dendritic cell subsets

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Disease-causing mutations in the transcription factor *interferon regulatory factor 8 (IRF8)* gene impair differentiation of mononuclear phagocytes into dendritic cells (DC) and confer susceptibility to mycobacterial disease. In autosomal recessive IRF8 deficiency, density of $CD1a^+$ DC in the skin is very low, whereas numbers of Langerhans cells are normal, implicating important heterogeneity in dermal DC (Hambleton *et al*., N Engl J Med 2011). Since infection with *Mycobacterium tuberculosis* occurs via inhalation, we investigated the expression of IRF2, IRF4 and IRF8 in the 3 major resident pulmonary DC populations: Langerhans-type myeloid DC (LDC), interstitial-type myeloid DC (intDC) and plasmacytoid DC (pDC).
Lung tissue was obtained from 4 subjects who underwent pneumectomy. DC were isolated by FACS from mononuclear cell suspensions of lung digests to obtain highly purified (>95%) DC subsets. LDC, intDC and pDC were identified as respectively $langerin^+$, DC-$SIGN^+$ and $BDCA2^+$ cells in the low autofluorescent, $CD3^-$ and $CD19^-$ fraction. RNA was extracted and expression of IRF2, IRF4, IRF8 and housekeeping genes GADPH, HPRT1 and PPIA was analyzed by RT-PCR.
Expression of IRF8 was significantly higher in pDC compared with both LDC and intDC. LDC had the lowest IRF8 expression (Figure 1). Expression of IRF2 and IRF4 did not differ between the DC subsets.

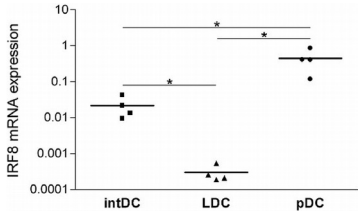

Figure 1

These results suggest distinct roles of IRF8 in the development of human lung DC subsets.

P814

Expression of factor XIIIa+ cells, CD207+ Langerhans cells and CD83+ mature dendritic cells in fatal asthma

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Rationale: Dendritic cells (DCs) are a heterogeneous class of antigen presenting cells that initiate immune responses in asthma. Little information is known about the expression of different DCs in the airways and lymph nodes (LNs) of asthmatics. Our aim was to study the expression of CD83+ mature DCs, CD207+ Langerhans cells, and factor XIIIa+ reticular DCs in mediastinal LNs and airways of patients that died of asthma.
Methods: We studied 10 non-smoker fatal asthma patients (FA) and 8 non-smoker individuals that died of non-pulmonary causes (controls, CTRLs). Immunohistochemistry was performed with antibodies against CD83, CD207 and factor XIIIa. The total area stained with anti-CD83/CD207 antibodies was measured on the cortical area of the LNs and on the internal, airway smooth muscle and external layers of the cartilaginous airways. Factor XIIIa was analyzed only on LNs.
Results: CD83 and CD207 stained cells in the airways and all antibodies stained cells in the LNs of FA and CTRLs. No differences were found in the areas stained with CD83, CD207 and factor XIIIa between FA and CTRLs. In fatal asthma CD83+ stained area in LNs correlated with CD207+ stained area in LNs ($r = 0.65$; $p = 0.04$) and CD207+ stained area in LNs correlated with CD207+ stained area in the internal ($r = 0.79$, $p = 0.006$), muscle ($r = 0.78$, $p = 0.008$) and external ($r = 0.73$, $p = 0.02$) layers of the airways.
Conclusion: A fatal asthma episode is not associated with an increased expression of Langerhans cells, factor XIIIa+ reticular cells and mature CD83+ DCs in cartilaginous airways or thoracic LNs. In asthma however, some DC cell trafficking between airway mucosa and LNs seems to occur.

P815

TCR V-beta usage in patients with sarcoidosis

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Sarcoidosis is a granulomatous disease of unknown aetiology, mainly affecting the lungs. Elevated numbers of activated T cells are found in bronchoalveolar lavage (BAL) fluid. HLA-DRB1*0301+ (DR3+) patients, who typically have Löfgren's syndrome, are characterized by good prognosis and an accumulation of lung CD4+ T cells using the T cell receptor (TCR) gene segment Valpha2.3 (AV2S3). However, the corresponding β-chains that are part of the TCR have been poorly characterized, and there is only limited knowledge about the TCR usage in non-acute patients.
We used antibody staining and flow cytometry to characterize TCR Vbeta usage in CD4+ and CD8+ T cell subsets in blood and BAL fluid samples from different sarcoidosis patient groups as well as from healthy controls.
Overall, the TCR Vbeta usage of Valpha2.3 + CD4+ T cells was quite diverse, indicating a predominant role in antigen recognition only for the alpha chain in these T-cell expansions. However, a preference for selective use of Vbeta 22 was noted in one patient.
A higher degree of selective Vbeta expression in both BAL and blood of non-acute patients may be due to epitope spreading over time, with more antigenic epitopes available to trigger distinct T cells. The higher non-random TCR Vbeta usage in the lung compared to blood could be related to antigenic triggering at the site of active disease, although more BAL samples of healthy individuals should be analyzed to estimate the normal differences between lung and blood in this respect.

P816

CD4+/CD8+ T-cells ratio in patients with pulmonary tuberculosis

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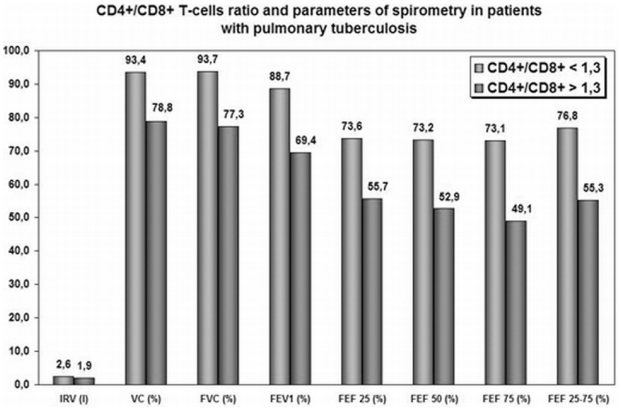
Introduction: A wide range of immune components are involved in an effective immune response against M. tuberculosis. CD4+ and CD8+ T-cells are central for protection against active tuberculosis.
The purpose of this research was studying CD4+/CD8+ T-cells ratio and parameters of spirometry in 34 patients with pulmonary tuberculosis.
Material and methods: Parameters of spirometry, CD4+/CD8+ T-cells ratio were analyzed.
Depending on CD4+/CD8+ T-cells ratio patients with pulmonary tuberculosis have been shared into 2 groups:
1 group - 18 patients CD4+/CD8+ ratio < 1,3;
2 group - 16 patients CD4+/CD8+ ratio > 1,3.
There was no difference in age between groups.
Student's t-test were used in the statistical analysis. Significance level was set at 0,05.
Results: Mean values of parameters spirometry in each group are submitted on the diagram.



It is revealed, that groups authentically ($p < 0,05$) differed on parameters of spirometry IRV, VC, FVC, FEV1, FEF25, FEF50, FEF75, FEF 25-75. CD4+/CD8+ T-cells ratio correlated with parameters of spirometry (IRV, VC, FVC, FEV1, FEF25, FEF50, FEF75, FEF 25-75) in patients with pulmonary tuberculosis. At parameter CD4+/CD8+ T-cells ratio > 1,3 the lowest parameters of spirometry were marked, and at parameter CD4+/CD8+ T-cells ratio < 1,3 parameters of spirometry were the highest.
Conclusion: Connection between CD4+/CD8+ T-cells ratio and parameters of spirometry is revealed in patients with pulmonary tuberculosis.

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Airway smooth muscle behaviour is altered following coculture with fibrocytes

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Asthma is a major cause of morbidity and mortality worldwide and its prevalence is increasing. The airway smooth muscle (ASM) in asthma is dysfunctional with increased mass and contractility. Fibrocytes (FC) are elevated in the peripheral blood and ASM in asthma and ASM has the potential to mediate FC recruitment (Saunders *et al*, J Allergy Clin Immunol, 2009;123:376-84). We hypothesised that FCs modify ASM behaviour to contribute to ASM dysfunction in asthma.
FCs were isolated from peripheral blood, ASM from bronchial biopsies and lung resection material. ASM contraction was assessed by collagen gel contraction, and

potential mechanisms for increased mass by migration, proliferation and survival assays.
Contraction of collagen gels impregnated with ASM cultured with FCs for 3-4d vs ASM alone increased significantly (i.e. reduction in area under curve of gel size as a percentage of the original size plotted over 30 min (AUC_{30}), Table 1). ASM wound healing (WH) increased significantly in the presence of supernatants (SNs) from FC±ASM 7d cultures vs control media, but no additional WH was seen in the presence of FC or FC+ASM SNs vs ASM SNs alone (Table 1). ASM proliferation and survival was unaffected by culture with FCs/FC SNs for 7d.

Table 1

	Control	FC	FC+ASM	ASM
Contraction (AUC30)	2931±32 (no cells)	2909±20	2295±116*¶#	2522±146
WH (cells moved into wound at 6h)	25±4	41±7*¶	44±8*	44±8*

$p<0.05$ vs *control, #FC, ¶ASM, t-tests, n=3-5.

Localisation of FCs to the ASM-bundle could contribute to the hypercontractility of ASM in asthma, which is integral to the pathophysiology of asthma. The mechanism by which this occurs requires further investigation, but has the potential to provide future therapeutic targets.

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Airway wall responses to tidal breathing and deep inspiration

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Introduction: Mechanical stretch attenuates airway smooth muscle (ASM) force production, which may explain the reversal of bronchoconstriction (i.e. bronchodilation) following a deep inspiration (DI) in vivo. We measured the effect of simulated DI on both narrowing and ASM force in isolated bronchi.
Methods: Bronchial segments were dissected from pig lungs and maintained in organ bath chambers. Airway narrowing (% volume) to acetylcholine (ACh, 10^{-7}M - $3x10^{-3}$M) was measured under static transmural pressure (P_{tm}) conditions and during fixed P_{tm} oscillations simulating tidal breathing ($\Delta 5cmH_2O$) with intermittent DI ($\Delta 25cmH_2O$). In a separate group of experiments, the above protocols were repeated using fixed volume oscillations to simulate tidal and DI breathing whilst measuring the increase in P_{tm} produced by ASM contraction. Under each condition, airway wall stiffness was measured from the change in P_{tm} and volume during tidal oscillations.
Results: Under static conditions, maximal response to ACh was 92.3*m*4.3% narrowing (n=6) and 73.5±9.2cmH_2O P_{tm} (n=4). DI to $\Delta 25cmH_2O$ reversed ~60% narrowing at low doses of ACh but had no affect at moderate or high doses, whereas fixed volume DI attenuated ASM force to 12.7±5.6cmH_2O (t-test, $p<0.01$) even at high doses. At maximal contraction to ACh, stiffness increased ~10 fold in both groups of which DI had no affect under fixed P_{tm} conditions but reversed 60.9±4.4% ($p<0.001$) under fixed volume oscillation conditions.
Conclusions: The mechanical loading conditions present during tidal breathing and DI influence the airway response to mechanical stretch. Inhibition of bronchoconstriction to mechanical stretch is minimal during fixed Ptm oscillations particularly at high levels of ASM activation.