89. COPD: human studies

P722

Various phenotypes of COPD are closely related to regulatory T cells

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Background: Regulatory T-cells (T-reg) play an important role in physiology and pathology of immune system and take essential place in pathogenesis of various autoimmune, tumoral and allergies diseases. However, T-reg subpopulations in separate phenotypes of COPD remain insufficiently studied.
Aim and objectives: To investigate different subpopulations of T-reg in patients with various phenotypes of COPD.
Methods: Levels of natural CD4+CD25high and inducible CD4+FoxP3+ T-reg were analyzed in 60 subjects with COPD (including 13 subjects diagnosed with emphysematous phenotype, 28 patients with bronhitich phenotype,19 subjects with mixed phenotype of COPD) and 17 healthy subjects (HS) using the flow cytometry analysis.

Results: CD4+CD25high in COPD were significantly increased compared to HS (2,82±0,24%, p = 0,005; 1,56±0,24%, p = 0,005, respectively). Increased level of CD4+CD25high was shown for persons with emphysematous phenotype (3,45±0,40%, p = 0,021) compared to persons diagnosed with bronhitich (2,83±0,22%, p = 0,021) and mixed (2,38±0,22%, p = 0,014) phenotypes of COPD. As for CD4+FoxP3+ T-reg no difference between different phenotypes of COPD and HS was observed.
Conclusions: Different subpopulations of T-reg may determine various phenotypes of COPD and this study indicates that increased level of CD4+CD25high is associated with emphysematous phenotype of COPD to support this hypothesis.

P723
IL-33 has a nuclear distribution and is not increased in peripheral lung of COPD patients
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Interleukin-33 (IL-33), a member of the IL-1 cytokine family, is crucial for induction of Th2-type immune responses but is also involved in the induction of non-Th2-type inflammation as a pro-inflammatory cytokine, similar to IL-1 and IL-18. The aim of our study was to investigate by immunohistochemistry (IHC), RT-qPCR, ELISA and Western blotting (WB) the expression and the amount of IL-33 in peripheral lung, and bronchoalveolar lavage (BAL) from 15 age- and smoking history-matched smokers with normal lung function and 15 smokers with mild to moderate stable COPD not treated with ICS and/or theophylline. The IHC staining was mainly confined to the nuclei of endothelial cells and small airway epithelial cells. The number of IL-33 positive cells and the level of mRNA expression was not significantly different in patients with COPD compared to controls. IL-33 was under detection limits using ELISA (BAL) and WB (peripheral lung). These data suggest that an IL-33-mediated immune response, may not be critical in COPD and its nuclear role is uncertain.

P724
Decrease of soluble adhesion molecules levels and bacterial mixt-infection in patients with COPD
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The soluble forms of intracellular adhesion molecules (sICAM) such as membrane forms are involved in modulating the cell interaction. In order to investigate adhesion disturbances the serum levels of sICAM-1 (sCD54) and sICAM-3 (sCD50) were analyzed in 48 patients with exacerbation of COPD and 86 healthy donors using ELISA- method. Quantitative culture of sputum or bronchoalveoalar lavage fluid were performed.
The median levels of sCD50 and sCD54 were significantly lower in COPD patients than in donors. The sCD50 and sCD54 values below the normal range were registrated in 60% of the patients. The decrease of sICAM levels was associated with longer smoking history compared with patients having normal/elevated values, purulent sputum and leucocytosis. The patients with low sICAM values had significantly more exacerbations pro year than other patients (2,3±0,7 vs 1,3±0,5). Respiratory mixt-infection was observed more often in patients with decreased sICAM levels. The associations of 5-6 kinds of pathogenes were isolated in 20% of patients with reduced sCD50 and sCD54 levels and in none of patients having normal/elevated sICAM values. It led to longer course of antibacterial therapy and increase of hospitalization days in patients with low sICAM concentration compared with other patients.
Thus, the reduced serum levels of sCD50 and sCD54 were found in 2/3 of patients with exacerbation of COPD, correlating with longer smocking history, purulent bronchitis, bacterial mixt-infections, and prolonged therapy duration. The low serum levels of the soluble adhesion molecules reflecting the impaired expression of the membrane forms can be considered as unfavorable factor.

P725
Value of serum and induced sputum surfactant protein-D in chronic obstructive pulmonary disease
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Aim: In the present study, we aimed to investigate the correlation between SP-D in serum and induced sputum and severity of COPD.
Methods: 20 healthy subjects older than 40 years of age with at least 10 pack- year smoking history (group 1), 20 stage I-II COPD patients (group 2) and 20 stage III-IV COPD patients (group 3) were enrolled to the study. All subjects performed pulmonary function tests and C-reactive protein (CRP) and blood SP-D levels measured. Induced sputum samples were obtained to determine induced sputum SP-D level. COPD patients were followed up for exacerbation for 6 months.
Results: Serum SP-D levels of group 3 were highest and and induced sputum SP-D levels of group 2 were lowest among three groups. However, there were no statistically significant differences among three groups *($p>0.05$).* SP-D levels of induced sputum decreased in patients with high number of cigarette pack-year, whereas serum SP-D levels increased in these patients ($p<0.05$). Induced sputum SP-D levels of COPD patients receiving inhaler corticosteroid treatment were higher than patients who were not receiving inhaler corticosteroid treatment ($p<0.05$). An inverse correlation between serum SP-D levels and FEV_1 (L,%) was found and there was a positive significant correlation between the serum SP-D levels and exacerbation frequency in 6-month follow up period in our study *($p<0.05$).*
Conclusion: Our study demonstrates adverse effects of smoking on local SP-D levels. Low levels of local SP-D in the group of current smokers, who were not receiving inhaler corticosteroid treatment indicate the importance of airway inflammation in COPD pathogenesis.

P726
Proinflammatory cytokines in induced sputum and COPD phenotype
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Chronic obstructive lung disease (COPD) does not have a uniform clinical and morphological nature. Airflow limitation is caused by inflammation and parenchymal destruction, there are patients with predominance of emphysema and those with predominance of chronic bronchitis. The aim of the study was to characterize the inflammatory process in two distinct forms of COPD.
35 COPD patients were investigated. They were divided into two groups: emphysema (E, n=25) and chronic bronchitis pts (CB, n=10); the presence of emphysema in HRCT and hyperinflation in pletysmography were the distinguishing criteria. The concentration of cytokines (IL-8, IL-1β, IL-6, IL-10, TNFα and IL-12) in induced sputum (IS) was measured using flow cytometry with Cytometric Bead Array method.
The median concentration of proinflammatory cytokines (IL-8, IL-6, IL1β and TNFα) did not differ between two groups. The concentration of IL-10 and IL-12 was slightly, but not significantly, higher in the E when compared with CB group (median: 57 vs 41 pg/mL, 1553 vs 1122 pg/mL, respectively). There was a significant correlation between the concentrations of IL-8, IL-6, IL1β and TNFα in the CB group.
When we analysed the relation of cytokine concentration with the clinical parameters, we observed a difference between the E and CB group. There was a significant correlation of IL-10, TNFα and IL-12 concentration with the degree of emphysema (RV, TLC, Raw) and FEV1 only in the E group. A significant correlation of IL-6 concentration with pack/years, TLC and with the inflammatory cell total count was observed only in the CB group.
The differences in cytokine profile and correlations indicate a possible different character of inflammation in two COPD phenotypes.

P727
Relationship between % neutrophils in induced sputum and IL-6 and TNF-α in COPD
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Introduction: The percentage of sputum neutrophils are increased in patients by Chronic Obstructive Pulmonary Disease (COPD), and is a biomarker of airway inflammation. The aim of this study is the relationship between sputum% neutrophils and levels of IL-6 and TNFα in induced sputum in COPD with moderate-severe airflow obstruction.
Method: Sputum samples were obtained using 3% saline, according to international guidelines., from a total of 60 subjects 40 COPD patients (criteria for diagnostic and classification of disease severity based on the Global Initiative for Chronic Obstructive lung disease (GOLD) and 20 healthy men (control group) who had no story of lung disease and normal spirometry. All the patients were smokers of >20 pack/year, and were free from acute exacerbations in three months prior to the study. In each subject BMI (kg/m^2), dyspnoea according to the modified Medical Research Council scale (MRC), oxygen saturation (SatO2), spirometry before and after 400μg inhaled salbutamol, lung volumes and carbon monoxide transfer factor coefficient (KCO) measurements were measured. TNF-α and IL6 were meassured by RIA.
Results: The sputum neutrophil% was statistic significative higher in COPD patients than group control p<0,05. We find a negative correlation between the percentage of the non-scamous cell count and% neutrophil whit the FEV1% and the% FEV1/FVC p< 0,05, but not with the grade of dyspnea (MRC), KCO, and SatO2. There was a weak association between sputum neutrophils and Il-6 r= 0,385, p<0,003, and TNF r= 0,468 p= 0,001
Conclusion: Neutrophils in sputum in COPD patients are associated with the airflow limitation and whit the levels of IL-6 and TNF-α.
Proyect FFIS/EMER09/18

P728

Expression of the phosphorylated myristoylated alanine-rich C kinase substrate (MARCKS) in COPD bronchial glands

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Myristoylated alanine-rich C kinase substrate (MARCKS) is a central regulatory molecule involved in mucin granule release by normal human bronchial epithelial cells in vitro. Upon stimulation, activated PKC phosphorylates MARCKS (p-MARCKs), causing translocation of p-MARCKS from the plasma membrane to the cytoplasm, where it is then dephosphorylated, thus interacting with mucin granule membranes and mediating their subsequent exocytosis. The aim of our study was to investigate by immunohistochemistry (IHC) the expression of protein p-MARCKS in the bronchial rings, obtained during lung resection surgery, of smokers (current and ex) with or without COPD compared with non-smoker subjects. We examined formalin-fixed paraffin-embedded bronchial rings by IHC for identification of total p-MARCKS+ cells. The number of p-MARCKS+ve cells was determined among the mucous cells in the bronchial submucosal glands. Samples from 10 age-matched non-smokers subjects, 29 smokers with normal lung function and 29 smokers with COPD were obtained. We were unable to detect any significant differences in p-MARCKS in the bronchial mucous glands between the 3 groups of subjects. This data suggests that the p-MARCKS pathway is not critical for mucin secretion in the bronchial glands of COPD patients.

P729

The effects of morphological remodeling of the bronchi on the neocollagenesis processes in patients with COPD stage 2 during exacerbation

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Background: Chronic obstructive pulmonary disease (COPD) is the third most common cause of death (8%) in the 25 member states of the European Union.
Purpose of the study: To study the effects of morphological remodeling of the bronchi on the neocollagenesis processes in patients with COPD stage 2 during exacerbation.
Materials and methods: To study the morphological processes of bronchial remodelling there was conducted an electron microscopy of bronchial mucosa bioptic materials that had been conducted at the bifurcation of the proximal bronchi during fiber-optic bronchoscopy in 9 patients with COPD stage 2 at the moment of hospitalization. The content of type IV collagen was investigated in bronchoalveolar fluid of 43 patients with stage 2 COPD before and after 1, 3 and 6 months of treatment with tiotropium bromide using ELISA.
Results of the study: The results of our research showed that in patients with COPD on early stages (2nd stage) there were damage of bronchial mucosa with a significant growth of connective tissue in lamina propria and was accompanied by increased content of collagen type IV in bronchoalveolar fluid in 6.19 times compared with healthy. This is an evidence of increased activity of fibroblasts as a result of microcirculation disturbances, activation of lipid peroxidation and effects of hypoxia.
Conclusions: Due to the morphological remodeling there is great activity of neocollagenesis during exacerbation of COPD, evidenced by significantly increased production type IV of collagen in bronchoalveolar fluid.

P730

Differences in biomarkers of systemic inflammation in two phenotypes of COPD patients

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Introduction: Classification of chronic obstructive pulmonary diseasse (COPD) is usually based on the severity of airflow limitation, whit no reflect the heterogeneity of this disease. The aim of this study was to establish differences in biomarkers of systemic inflammation in two phenotypes of COPD patients, through determination protein-C reactive (PCR), fibrinogen, IL-6 and TNF-α in plasma, in stable COPD.
Method: A total of 60 subjects, 40 stable COPD (diagnostic and severity based on the Global Initiative for Chronic Obstructive Lung Disease) and 20 controls.We established two groups of patients based in the carbon monoxide gas transfer/alveolar volume (KCO) and visual detection of emphysema in computed tomography (CT). A: Phenotype emphysema (n=19), KC0<70% an CT compatible; B: phenotype chronic bronchitis (n=21), KC0<70% and CT without emphysema. Plasmatic determination of fibrinogen, PCR,% neutrophils,IL-6, and TNF (RIA).
Results: The values of fibrinógeno/PCR did not show significant differences between both phenotypes of COPD (B 432,200±0,470 mg/dl / 0,542±0,14 mg/l), (A 452,368±70,503 mg/dl / 0,842±0,935 mg/l), but compared with controls (C 346,000±48,098 mg/dl / 0,206±0,175 mg/l) we have found significant differences (p<0,05). IL-6/TNF-α in pg/ml (B 29,985±4,123 / 39,814±17,354) (A 30,694±3,612 / 33,784±13,871) were significant higher in the COPD than controls (21,277±6,004 / 18,900±6,617) p<0,05, but non differences were show between the two phenotypes. Leukocytes, %neutrophils, PCR and fibrinogen in blood showed a negative correlation with the %FEV1 (p<0,05).
Conclusion: No differences in the two phenotypes of COPD stable was show in the biomarkers of inflammation studied.

P731

LSC 2011 Abstract: The relationship of CD4+/CD8+ ratios and toll like receptor-2 expression with COPD and smoking

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Aim: Native immune system activates acquired immunity by Toll Like Receptors (TLR). There may be some alterations in T cell profile and TLR expression in peripheral blood monocytes of COPD patients. We aimed to evaluate the utilization of the ratio of CD4+, CD8+ T cells and TLR-2 expression as a marker of lung pathology as well as their relationship between pulmonary function tests in COPD patients and smokers.
Method: Forty stable COPD patients admitted to our university's outpatient clinic and 40 volunteers were included in the study. The study population was evaluated in 4 groups according to their smoking status. CD4+, CD8+ T cells and monocyte TLR-2 expression was measured by flow cytometry in patients and control groups. Spirometry was performed in the whole study population except for nonsmoker control group.
Results: TLR-2 expressions investigated in CD14+ cells were found%56±25 in Group 1 (nonsmoker COPD),%65±23 in Group 2 (smoker COPD),%64±24 in Group 3 (healthy smoker) and%52±25 in Group 4 (healthy nonsmoker), any difference was not observed between the groups. CD4+, CD8+ T cells and CD4+/CD8+ ratios were not found to be different between the groups. CD4+ T cells and FEV_1, FEV_1/FVC had a positive correlation (r=0,311, p=0,01; r=0,293, p=0,023, respectively). CD4+/CD8+ ratios and FEV_1/FVC also showed positive correlation (r=0,295, p=0,022). Smoking amount and CD4+/CD8+ ratios had a negative correlation (r= -0,274, p=0,034).
Conclusion: The results of our study demonstrated that CD4+/CD8+ ratio may be used as a biomarker to evaluate the pathogenesis of COPD, however TLR-2 is not convenient for this purpose.

P732

Peripheral neutrophil stiffness in severe COPD exacerbations

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Rationale: Exacerbations of COPD (EXCOPD) are characterized by activated pulmonary neutrophils (NEUT) with potentially increased cellular stiffness.
Objective: The aim of this study was to assess NEUT stiffness in patients with COPD during and after exacerbations.
Methods: 12 COPD patients (age, 70±9 yrs; 83% male; all smokers, 71±36 pack-yrs) hospitalized for EXCOPD were included. Stiffness of peripheral NEUT was assessed using the atomic force microscopy technique within the first 72 h of admission and 12 weeks after discharge (Study Group [SG]). If patients suffered a new episode within this period of follow-up, measurements were repeated at this time in point (mean, 7±4 weeks) (Re-exacerbation Group [RG]). The COPD Assessment Test (CAT), the ADO Index and dyspnoea (MMRC) were also assessed.
Results: There were no differences on admission between the two subsets of patients. Increased NEUT stiffness in SG patients (n, 6) decreased from admission (890±293 Pa) to discharge (616±226 Pa) (p<0.05) compared to that measured in the RG subset (n, 6) (from 999±424 to 842±343 Pa). The ADO Index was associated with NEUT stiffness on admission in the two subsets of patients (r=0.60, p<0.05).
Conclusions: Changes in peripheral NEUT stiffness during COPD exacerbations and convalescence indicate that the activation of NEUT underlies enhanced pulmonary inflammation.
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P733

Increase of extracellular IL-17A and anti-microbial peptides in the peripheral airways of smokers

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Among several known cytokine-signalling pathways influencing innate effector

cells, T helper (Th) 17 cytokines have emerged as particularly interesting for the understanding of chronic inflammatory conditions. Cigarette smokers suffer from frequent infections, are often colonised with bacteria in their lower airways and display signs of chronic inflammation including increased tissue expression of the Th17 cytokine IL-17A. We evaluated whether long-term current cigarette smokers display increased extracellular concentrations of Th17 cytokines and how this relates to anti-microbial peptides in the peripheral airways.
Concentrations of IL-17A, -17F and -22 plus the downstream effector molecules human beta-defensin 2 (HBD2) and LL-37 (cathelicidin) were measured in bronchoalveolar lavage (BAL) fluid from non-smokers and from smokers with and without COPD (n=17-20/group). All study subjects were free from airway infections during the last 4 weeks prior to the study.
The extracellular IL-17A concentrations (median [range]) were higher in smokers without COPD (1.2 [0.3-3.5], pg/ml) than in non-smokers (0.4 [0.1-4.4, pg/ml]; $p<0.05$). Smokers with COPD displayed intermediate concentrations of IL-17A (0.7 [0.1-7.1], pg/ml) Compared to non-smokers (22 [4-384], pg/ml), HBD2 was higher in smokers without COPD (70 [6-500], pg/ml; $p<0.05$), but not in smokers with COPD (24 [4-364], pg/ml). Interleukin-17F, IL-22 and LL37 were undetectable in most of the samples.
In conclusion, long-term cigarette smokers without COPD display increased extracellular concentrations of IL-17A, and this may be linked to increased concentrations of the anti-microbial peptide HBD2.

P734

Comparison of airway cellular and mediator profiles between tobacco smoke-induced COPD and biomass fuel exposure-induced COPD in an Indian population

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Exposure to biomass fuel smoke is as an important risk factor for COPD (BS-COPD), but it is not known whether this phenotype is different from tobacco-smoke induced TS-COPD with reference to airway cellular and mediator profiles.
Aim: To compare cellular and mediator inflammatory profiles in induced sputum between BS-COPD, TS-COPD and healthy controls.
Methods: 15 subjects with BS-COPD (mean exposure 43 yrs), 30 subjects with TS-COPD (mean 43 pack yrs) and 13 healthy subjects underwent sputum induction with 3% saline. Sputum was processed with 0.1% DTT. Cytospin slides were prepared and stained with diff-quick satins, and inflammatory cells were identified and counted under light-microscope. IL8, IL6, MMP-9, 8-isoprostane and LTB4 in the induced sputum were measured using ELISA. Mann-Whitney and Kruskal-Wallis tests were used for inter-group comparisons of medians (inter-quartile range).
Results: Compared with healthy subjects, both BS-COPD and TS-COPD subjects showed increased sputum neutrophil counts ($p=0.05$ & $p=0.01$ respectively) and eosinophil counts ($p<0.0001$ and $p<0.0001$ respectively). Both BS-COPD and TS-COPD showed increased levels of IL-8 ($p<0.001$ and $p<0.0001$ respectively), MMP-9 ($p=0.004$ and $p=0.001$) and 8-isoprostane ($p=0.002$ and $p=0.007$) levels compared to healthy subjects. There were no differences in the cellular and mediator inflammatory profiles between BS-COPD and TS-COPD.
Conclusion: BS-COPD showed increased numbers of neutrophils and eosinophils in the induced sputum, which was accompanied by increased levels of IL8, 8-isoprostane and MMP-9 levels, and these levels did not differ with TS-COPD.

P735

Vascular dysfunction and systemic inflammation in patients with COPD

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Introduction: We studied the differences between COPD patients and healthy controls in order to establish an etiological association between systemic inflammation and vascular endothelial dysfunction.
Materials and methods: Observational case-control study involving COPD patients and controls without COPD or cardiovascular history. All of them were tested for serum and urinary markers. Peripheral arterial tonometry by oscillometric sphygmomanometer (ENDO-PAT 2000) was used to measure endothelial dysfunction and arterial stiffness values (Reactive Hyperemia Index-RHI and Augmentation Index-AI). We also performed anthropometric and pulmonary function measurements.
Results: This study involved 52 persons (33 COPD patients and 17 healthy controls). Mean age: 59 in the COPD group and 52,5 in the controls (p =0, 07). Mean BMI: 25, 4 and 26, 2 respectively (p= 0,55). Mean FEV1 value: 74, 3% and mean CO diffusion test value: 74, 6%. The results for the serum and urinary markers (Mann-Whitney test) comparison with median and quartiles were the following: CRP (COPD 0,69, controls 0,60, p=0,06), leucocytes (COPD 8500, controls 6800, p= 0,001), D-dimer (COPD 289, controls 242, p= 0,16), BNP (COPD 36,3, controls 119,6, p= 0,41), fibrinogen (COPD 416, controls 327, p= 0,01) and microalbuminuria (COPD 11,0, controls 3,0, p= 0,03). No statistically significant differences in vascular endothelial dysfunction (RHI, COPD 2,2, control 1,8, p= 0,1) neither in arterial stiffness (AI, COPD 22, controls 34, p= 0,4) were found.
Conclusions: Cardiovascular risk increase in COPD patients can be predicted by measuring serum and urinary markers. Tonometrical measurements of endothelial dysfunction and arterial stiffness were unpredictive.

P736

Influence of AECOPD to ROS production in neutrophils

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Introduction and background: Chronic obstructive pulmonary disease (COPD) is characterized by specific pattern of inflammation involving polymorphonuclear neutrophils (PMN), macrophages and other cells. PMN are the major reactive oxygen species (ROS) producing cells. Our previous data showed that ROS production is decreased in stable COPD (SCOPD). It is still not known about ROS production in acute exacerbation of COPD (AECOPD).
Aim: To investigate ROS production in PMN during AECOPD.
Methods: 10 patients with SCOPD, 5 with AECOPD and 10 non-smoking healthy individuals (HI) were studied. PMN were isolated from peripheral blood using a high-density gradient. ROS production was induced using phorbol-myriste-acetate (PMA, 0.1-30 nM) and *S. aureus* bacteria (1-167 bacteria/neutrophil). ROS production was measured flow cytometrycally by mean of fluorescence intensity in PMN population.
Results: The production of spontaneous ROS in PMN was 29.7±4.1% higher in AECOPD group than in SCOPD ($p<0.05$). PMA stimulated ROS production in all groups. The most significant increase of ROS production was observed between 0.3 and 1 nM PMA (AECOPD 172.5-fold, SCOPD 90.1-fold, HI 46.2-fold ($p<0.05$)). The higher ROS production in PMN after stimulation with different *S. aureus* concentration was found in AECOPD group compared with SCOPD and HI ($p<0.05$).
Conclusion: Our data shows that ROS production is increased in AECOPD. Chemical (PMA) and biological (*S. aureus* bacteria) factors activates more intensive ROS generation.

P737

Acute smoke exposure decreases bronchial extracellular proteasome concentration

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Existence of repair mechanisms resulting in bronchial and alveolar protein degradation induced by smoke exposure are largely unknown.
In the alveolar space the presence of an extracellular, biologically active 20S proteasome is recently reported [1,2]. The proteasome is involved in protein degradation and is able to degrade proteins at a high rate, possibly to minimize oncotic pressure [1].
We tested whether the 20S proteasome in the bronchoalveolar space of healthy subjects is influenced by inhalation of tobacco smoke.
Twelve healthy adult subjects underwent bronchoalveolar lavage (BAL) before and after cigarette smoking. To separate bronchiolar and alveolar proteasome concentration, first and second portion of lavage fluid were evaluated separately. Extracellular proteasome concentration was measured by an established in-house ELISA [2] targeting the 20S proteasome subunit a6 (HC2) in cell-free BAL fluid. For statistical evaluation the Wilcoxon signed rank test was applied.
Acute smoke induces a significant decrease in extracellular 20S proteasome concentration in the bronchiolar space (94±55 to 65±50 ng/mL; $p<0.05$), but no change in the alveolar space (87±48 to 93±55 ng/mL).
Unchanged proteasome concentration in the alveolar fluid showed no artificial elution of the proteasomes by the pre-smoking lavage.
Tobacco smoke inhalation reduces the concentration of extracellular proteasome in the bronchial space and suggests less bronchiolar protein degradation in the acute phase after smoke exposure.
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Neutrophil phagocytic activity in AECOPD

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Background: Neutrophils are the major inflammatory cells in pathogenesis of chronic obstructive pulmonary disease (COPD). It is known that phagocytic activity of these cells is decreased during stable COPD (SCOPD). Otherwise, it is unknown how neutrophils act in acute exacerbation of COPD (AECOPD).
Aim: To investigate neutrophils phagocytic activity in AECOPD.
Methods: 10 patients with SCOPD, 7 with AECOPD and 10 healthy individuals (HI) were studied. Neutrophils were isolated from peripheral blood by high-density gradient Ficoll. Phagocytosis was investigated using different concentrations of FITC labeled *S. aureus* bacteria (1-167 bacteria/neutrophil (b/n)). Phagocytic activity was analyzed using flow cytometer assessing the cellular immunofluorescence intensity in neutrophil population.
Results: Neutrophil phagocytic activity was decreased in patients with AECOPD in comparison with SCOPD and HI. The highest phagocytosis was obtained using 167 b/n concentration in AECOPD and SCOPD groups (6.52±2.5-fold and 8.84±1.8-fold), but phagocytic activity was lower compared with HI (13.5±2.2-fold) ($p<0.05$).
Conclusion: Our results shows that neutrophils phagocytosis is decreased during AECOPD.

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LSC 2011 Abstract: Suppression of antitumor immunity as a potential link between inflammation and cancer in COPD

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Background: COPD is an independent risk factor for lung cancer. Chronic inflammation facilitates tumor development through non immune and immune mechanisms including the perturbation of myelopoiesis resulting in deficiency in Ag-presenting cells and dysfunctional cell-mediated antitumor immunity. We hypothesize that chronic systemic inflammation characteristic of COPD is associated with an expansion of cell populations that suppress antitumor immunity and that these changes are even more evident in COPD with lung cancer (LC).
Methods: The percentage of myeloid derived suppressor cells (MDSC) and dendritic cells (DCs) from blood was quantified by flow cytometry in 18 patients with COPD (mean±SD,FEV1=62±23%); 21 smokers with normal lung function (SM) (FEV1= 106±15%pred);26 non-smokers (NSC) (FEV1= 111±14%pred); 19 COPD with LC (COPD LC) (FEV1=71±20%pred) and 8 smokers with LC (SM LC) (FEV1=103±21%pred).
Results: COPD patients had an increased number of MDSC (median, range: 79.2, 817 cells x mm^3) as compared to NSC (21.1, 338; p=0,02). In addition they showed a reduced percentage of DCs with respect to NSC (0.4, 1%; 0.6; 8%; p=0,02, respectively). To test whether the presence of LC influences these cell populations we compared COPD patients with patients with LC but we did not find any statistically significant result. However, the ratio between MDSC/DC progressively increased in patients with COPD and LC (NSC:0,6; SM:1,2; COPD:2; SM LC: 2,3;COPD LC:3,3).
Conclusions: In COPD patients there is an altered pattern of blood MDSC and DCs suggesting a blunted antitumor immunity in this disease as a potential link between inflammation and cancer. These findings are similar to those evident in patients with LC.

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LSC 2011 Abstract: BAFF in the interaction between T regulatory and B cells in COPD

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Background: COPD shares some features with autoimmune diseases, such as a blunted regulatory T-cell (Tregs) activity allowing autoimmune reactions. B cell Activating Factor (BAFF) is a key factor modulating B cell homeostasis, and is overexpressed in B-cells lymphoid follicles of lungs from COPD patients. We hypothesize that BAFF upregulation in B cells is due to a reduced activity of TRegs translated as a lower production of BAFF in these cells.
Methods: BAFF expression in B-lymphocytes and Tregs from bronchoalveolar lavage (BAL) and blood was quantified by Flow Cytometry in 38 patients with COPD (mean±SD,FEV1=68±14%pred); 17 smokers with normal lung function (SC) (FEV1=104±15%pred) and 20 non-smokers (NSC) (FEV1=101±20%pred).
Results: In BAL, Tregs from COPD patients showed lower BAFF expression (Median, Range: 7, 0-32 Mean Fluorescence, MF) as compared to SC (17, 10-27; $p=2\times10^{-4}$) and NSC (14, 10-22; p=0,005).
Conversely, BAFF was overexpressed in B lymphocytes from COPD patients (50, 23-121) when compared to SC (12, 0-28; $p=6\times10^{-5}$) and NSC (20, 6-95; p=0,001). In blood, COPD had less Baff+ TRegs (10, 0-22,5) and more Baff+ B lymphocytes (23, 5,6-102) compared to NSC(12, 10-5; 13, 10-53; p=0,01; p=0,006, respectively). To test whether cigarette smoke (CS) influences BAFF expression, we cultivated blood mononuclear cells from 4 NSC with 3% and 7%CS. At 24 hours, BAFF was increased in B lymphocytes stimulated with CS 3% and even more with CS 7% with respect to the control (p=0,03; p=0,02, respectively).
Conclusion: In COPD there is a blunted expression of BAFF in TRegs that leads, partly due to cigarette smoke, to its excessive production by B lymphocytes, showing an imbalance of its functioning that is a common feature of several autoimmune diseases.

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Is centrilobular emphysema the link to the Dutch hypothesis?

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Asthma and COPD are chronic obstructive diseases with a certain degree of physiologic and pathologic similarities and the Dutch Hypothesis proposes that these diseases could be phenotypes of the same process. This hypothesis has never been universally accepted due to the evident differences between the two diseases. However COPD has two distinct pathological phenotypes, Panlobular Emphysema (PLE) where airflow obstruction is secondary to loss of elasticity and Centrilobular Emphysema (CLE) where airflow obstruction is secondary to bronchiolar remodeling with increased muscle and smaller diameter. We hypothesize that CLE could have features of Asthma different from PLE, especially as far as mast cells (MC) inflammation and their relation to airway reactivity. Therefore, we quantified tryptase stained MC in all layers (submucosa, smooth muscle and adventitia) of small airways in 27 lungs with CLE, 24 with PLE, 8 smokers without emphysema (SNE) and 8 non smokers (NS). MC in smooth muscle of small airways were significantly higher in CLE (206±41 cells/mm^2) than in PLE (104±21 cells/mm^2; p=0.01), SNE (107±54 cells/mm^2; p=0.05) and NS (105±27 cells/mm^2; p=0.05). Moreover, the degree of MC infiltration in the smooth muscle was related with the degree of hyperresponsiveness, as it has been shown in Asthma (PC20<3mg/ml: 396±116 cells/mm^2, PC20 3-8mg/ml: 89±69 cells/mm^2 and PC20>8mg/ml: 107±21 cells/mm^2; p=0.02 for all). In conclusion, MC infiltration of the muscle layer and its relation to airway reactivity, an important mechanism in the pathogenesis of Asthma, is a prominent feature of COPD patients with CLE, but not with PLE. These findings suggest that CLE could be the COPD link to the Dutch Hypothesis.