88. New mechanisms in airway disease

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microRNA regulation of the Alpha-1 antitrypsin gene

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Introduction: Alpha-1 antitrypsin (AAT) deficiency is a conformational disorder characterised by chronic inflammatory lung disease and liver disease. microRNAs (miRNA) represent drug targets that function as post-transcriptional negative regulatory molecules of target gene expression.

Aims and Methods: To identify miRNAs that target AAT gene expression using in silico analysis, to quantify AAT gene and protein expression in monocytic THP-1, 16HBE14o- bronchial epithelial and HepG2 liver cells using qRT-PCR and ELISA, to quantify specific miRNAs expression in these cells, to modulate AAT expression using premiRs and to validate miRNAs targeting AAT using a AAT-3'UTR luciferase reporter plasmid in HEK293 cells.

Results: In silico analysis predicts multiple miRNAs that target the AAT gene such as miR- 940 (with multiple binding sites in the 3'UTR), miR-132 and -212. Different cells expressed different levels of AAT and miRNAs. AAT and miR-940 are reciprocally expressed in the three cell lines, with miR-940:AAT ratio decreasing with increased AAT expression. Over-expression with premiR-940, but not premiR 132 and 212, led to a decrease in AAT mRNA and protein levels. All three miRNAs could inhibit expression of an AAT 3'UTR luciferase reported plasmid in HEK293 cells. A combination of pre-miRs over-expressed in HepG2 only resulted in a decrease of AAT mRNA and protein expression involving premiR-940, however, not significantly more than cells transfected with premiR 940 alone.

Conclusion: AAT mRNA is a true target for miR 940, 132 and 212. miR 940 is a more effective inhibitor than miR-132 or miR-212 due to attractive inherent properties compared to the other miRNAs tested.

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Inflammatory mediators in COPD patients with and without alpha-1-antitrypsin deficiency

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Introduction: Systemic inflammation is present in a large proportion of COPD patients and is discussed to be the missing link between COPD and its extrapulmonary manifestations. Little is known about differences in systemic inflammation in COPD patients with and without alpha-1 antitrypsin deficiency (AATD).
Aims: We tested the hypothesis that systemic inflammation in COPD would differ in patients with and without AATD and that markers of inflammation would be associated with quality of life (QoL).
Methods: In this cross-sectional study we included 102 participants. We measured lung function, quality of life (QoL) using the St. Georges Respiratory Questionnaire (SGRQ), and inflammatory mediators in peripheral blood. We compared concentrations of inflammatory mediators in four groups: AATD patients without augmentation therapy ($AATD_{unsub}$; n=20), AATD patients with augmentation therapy ($AATD_{sub}$; n=20), patients with COPD (n=46) and healthy controls (HC; n=16).
Results: Geometric mean high sensitivity C-reactive protein (hsCRP) was significantly elevated in COPD (3.72 mg/l) and $AATD_{unsub}$ (4.19 mg/l) compared to $AATD_{sub}$ (0.96 mg/l) and HC (0.82 mg/l). Significant differences could also be detected for alpha-1 antitrypsin ($p<0.001$). Interleukin-6 (IL-6), IL-8, and tumor necrosis factor-alpha did not differ significantly.
HsCRP levels were positively correlated with SGRQ in COPD patients without AATD (Spearman's $r=0.46$; $p<0.005$).
Conclusion: Low grade systemic inflammation is present in COPD with and without AATD. Despite different causes of these diseases, a common inflammatory pathway may exist. In patients with COPD, but not in AATD patients, systemic inflammation is associated with QoL.

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Polymorphism of collagen I gene and glutation-S-transferases T1 and M1 in chronic obstructive pulmonary disease associated with cardiovascular disease

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The aim of the research was to establish association between presence of mutant genotype COL1A1 (locus G1546T), null-genotypes of glutation-S-transferases (GSTs) GSTM1 and GSTT1 and stage of chronic obstructive pulmonary disease (COPD). The group of patients with COPD (II and III stage of COPD, n=30) associated with I-II functional groups of stable stenocardia (I and II subgroup, n=30) was examined.
In the research of gene COL1A polymorphism pathological allele T was found in 15% of healthy persons. Genotype GT was revealed at 1/3 of control group while pathological homozygous genotype TT in the same group hadn't been registered. In both groups frequency of genotype GT was higher than in the control group ($\chi^2=6.5$, $\rho=0.01$). Genotype TT was found in II group only ($\chi^2=12$, $\rho=0.01$). The OR of severe COPD in the presence of pathological allele T COL1A1 was authentically higher in II group, than in I (OR=1.74, OR=1.65, accordingly; $p<0.05$). Patients with pathological genotype of COL1A1 had been screened for the presence of combined null-genotype of GSTs. The homozygous deletion of both genes was found at four patients (all patients from II group). Respectively these patients had pathological genotype GT of COL1A1 demonstrated by functional inadequacy of collagen 1A1. Presence of combined null-genotype GSTM1 and GSTT1 at these patients, in turn, can lead to the total absence of enzymes GSTs, contributing accumulation of toxic intermediate metabolites of xenobiotics in the cell without their further neutralization and development of oxidative stress and destruction of connective tissue.

P705

Sputum biomarkers in stable COPD

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Characterization of the COPD proteome could help early diagnosis of the disease and aid the development of specific treatment options. The aim of this study was to detect sputum biomarkers characteristic for COPD.
Induced sputum was collected from 19 stable COPD patients (GOLD I-III, ex-smokers, mean age 62 years, pack year 52, FEV_1 64% predicted, FEV_1/FVC 50%) and 19 healthy controls (mean age 62 years, pack year 29, FEV_1 110% predicted, FEV_1/FVC 77%). CRP level (9.8mg/L vs. 5.1mg/L) and total sputum cell number (3.5×10^6 vs. 1.3×10^6) were significantly different between the two groups.
Sputum supernatants were subjected to cytokine antibody microarray analysis. Of the 120 cytokines investigated 96 was detectable above background level in the majority of samples. A greater subset of factors was down regulated while a smaller subset was up regulated in COPD. 14 cytokines exhibited a difference of at least 50% in relative expression level. IL-6, IL-1α, GRO and GROα expression was significantly different ($p<0.05$) in COPD patients vs. healthy controls. These results suggest that a CODP specific cytokine pattern might exist.

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Polymorphism of glutation-S-transferases T1 and M1 in chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is the result of genetic factors and environmental conditions influence on a human organism. Glutation-S-transferases (GST) play significant role in cell resistance to oxidative stress, and in prevention of DNA damage from products of tobacco smoke.
80 patients have been examined. They were divided into two groups: 1 group - smoking persons without COPD; 2 group – smoking patients with I or II stages of COPD (2 subgroups). The control group consisted of healthy non-smoking persons.
In control group homozygous deletion of GSTT1 was found in 5% of patients. GSTT1 null-genotype has been revealed in 14.3% in first group and in 17.6% of cases in second group. Reliability of distinctions between groups was not received. However in comparison of the control group with subgroups of COPD patients statistically significant difference between frequency of GSTT1 null-genotype in control group and in II stage COPD patients had been received ($\chi^2=10$, $p=0.02$). For smoking persons without COPD and patients with I stage of COPD OR equaled 1, and for smoking persons and II stage COPD patients OR was 1.5 that indicated on increased risk of illness with severe stage of COPD at presence of GSTT1 null-genotype. Also OR, identical to smokers without COPD and patients with COPD stage I, means equal risk of disease that was especially significant for smokers without COPD.
Frequency of GSTM1 null-genotype in all groups was equal (46.6, 46.4, 47% accordingly).
The results of our research demonstrate that null-genotype of GSTT1 could be recommended as a marker of chronic obstructive pulmonary disease fast progressing in smokers.

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Gene regulation of apoptosis and telomere length in COPD and lung cancer

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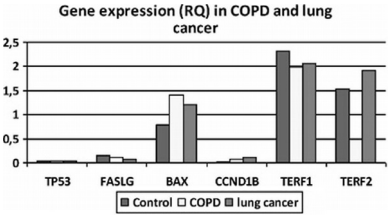
The aim of this study was to investigate the role of gene regulaton of apoptosis (TP53, TP53 I3, FASLG, BAX, CCND1) and telomere function (TRF1, TRF2) in peripheral blood lymphocytes in COPD and lung cancer.
Methods: Groups included 16 COPD patients (FEV1 41-64), 5 lung cancer and 12 controls. Relative telomere length and apoptosis in peripheral blood cells were analyzed by flow cytometry. Relative quantification (RQ) of gene expression was investigated by the RT-PCR using 7900 HT System and TaqMan technology.
Results: In COPD a decreased RQ was shown for FASLG, TP53 and TP53 I3 genes with overexpression of BAX ($p<0,001$) and CCND1 genes and increased presentation of BAX-protein and MAPK14 (p38) on CD16+56+ cells by flow cytometry.
A significant decrease was demonstrated TERF1 and especially TERF2 expression (Fig. 1). In lung cancer high Ki-67+ and HER2+ cell counts were combined with a decrease of BIRC5 and FASLG expression and MADD, MAPK14 and CDKN1B over-expression. The RTL was higher in lung cancer as comparing with COPD and control but no correlation was seen with TERT or TERF2 gene expression.



Conclusion: In COPD the association is demonstrated between the cytotoxic cells activation, MAPK and CCND1 genes and decreased apoptosis. Some independence is shown between TERT and T-loop genes expression and the telomere length in lung cancer and COPD, supposing the influence of different regulation pathways.

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Peripheral insulin resistance accounts for impaired glucose tolerance and is associated with systemic inflammation in COPD

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Introduction: Impaired glucose tolerance is common in COPD [Archer & Baker, Resp Med: COPD Update 2009;5:67-74]. We assessed pancreatic function, hepatic and peripheral insulin resistance and determined the relationship of these with systemic inflammation.
Methods: Participants were 8 stable COPD patients (C) (4 male, 66±8yrs, FEV_1 44±16%predicted, body mass index (BMI) 23±6kg/m^2) and 8 healthy volunteers (V) (8m, 24±5yrs, FEV_1 89±12%, BMI 22±2kg/m^2) without diabetes mellitus and with fasting glucose <7mM. Participants underwent 120min oral glucose tolerance testing (OGTT). Hepatic insulin resistance (HOMA2-IR) and pancreatic beta-cell function (HOMA2-%B) were derived from fasting values and the Matsuda Index (composite hepatic and muscle insulin sensitivity) from OGTT values.
Results: Fasting glucose (p=0.038), HbA_{1C} (p=0.013) and C peptide (p=0.028) were higher in COPD than in volunteers, but insulin was not different (p=0.234). HOMA2-%B was: C 126 [81-182], V 91 [86-113], p=0.279, HOMA2-IR was: C 1.3 [0.8-1.8], V 1.0 [0.7-1.2], p=0.161, Matsuda Index was: C 3 [3-5], V (8 [6-10], p=0.002. In COPD, but not volunteers, ln(CRP) was inversely correlated with ln(Matsuda) (R=-0.760, p=0.047) but not with other homeostatic measures after controlling for BMI.
Conclusion: Non-obese COPD patients without diabetes had increased composite, but not hepatic, insulin resistance compared to volunteers, indicating skeletal muscle insulin resistance. Possible mechanisms underlying the correlation between peripheral insulin resistance and inflammation include inhibition of insulin receptor signalling by inflammatory mediators or pro-inflammatory effects of elevated glucose.

P709

MKK3 is expressed in cells from patients with allergic asthma

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MKK3 is a member of the p38 MAPK signalling pathway. Studies have shown that MKK3 is an important factor in non allergic inflammatory and Th1 responses. Less is known about the role of MKK3 in allergic inflammation and allergic diseases. Proteomic analysis was performed on peripheral blood cells obtained from 22 healthy and 18 allergic asthmatic patients. Lysates from purified lymphocytes were separated by SDS-PAGE and the protein inventories of each sample were identified using mass spectrometry. A 3.65 fold increase in the expression of MKK3 was observed in $CD8^+$ T lymphocytes from asthmatic in relation to healthy volunteers, (% vol protein abundance). Western blot (WB) analysis showed MKK3 expression in human $CD4^+$ lymphocytes, human endothelial cells (HUVECs) and human epithelial cells (HBECs) but not in neutrophils or eosinophils. However, using densitometric analysis we found no differences between healthy and asthmatic subjects (n=10) in any of the cell types analysed. Real time PCR studies showed no expression of MKK3 isoform C in any tissue or cell type. However, large quantities of isoform B gene expression were found in neutrophils and lung tissue from patients with COPD (isoform A: 450 vs Isoform B: 1500 relative expression (ACT)). MKK3 expression was also measured in lung samples from mice treated with lipopolysacharide (LPS) or saline. Densitometric analysis of WB data showed that LPS treated mice developed an increased total MKK3 protein expression compared to saline-treated mice (sham: 49 *vs* LPS: 60 A.U. p≥0.05). No significant increase in expression in these tissues was observed in mice allergic to ovalbumin. In conclusion, MKK3 is differentially expressed under non-allergic and allergic conditions.

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Airway inflammation and airway pathophysiology in older asthmatics

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Background: Asthma morbidity increases with ageing, which may partially result from alterations in airway pathophysiology. We aimed to describe determinants of ventilatory function and airway responses in older asthmatics to further our understanding of pathophysiological mechanisms.
Methods: Bronchoconstriction was induced with hypertonic saline (HTS) in 30 asthmatics aged over 54 years (75% female). The response dose ratio (RDR,%change/mg saline) was used to quantify challenge response: RDRFEV1/FVC reflecting airway narrowing, and RDRFVC reflecting airway closure. These measures were regressed against markers of airway inflammation (Induced sputum (s-) neutrophils and eosinophils and exhaled NO).
Results: Average (SD) FEV1 was 78%pred (16), FVC 91% (12) with 13% (9) fall in FEV1 during HTS challenge. Baseline ventilatory function was inversely correlated with s-neutrophils (r=-0.61, -0.53 and -0.56 for FEV1%, FVC% and FEV1/FVC respectively, all p<0.05), as well as sputum markers of neutrophils (neutrophil elastase, IL-8 and MMP), but not s-eosinophils (p>0.05 for all).
In contrast, the challenge response was predicted by eNO (RDRFEV1/FVC: r= 0,42, p=0,05), indicating airway narrowing associated with airway inflammation. Age predicted an increased RDRFVC (r=0,47 p=0.02), suggesting an increasing tendency to airway closure with ageing.
Conclusions: In older asthmatics, the pattern of inflammation bears important relationships to airway physiology. Neutrophilic inflammation is an important determinant of baseline ventilatory function, whereas airway reactivity relates to eosinophilic inflammation. Age is also determinant of airway closure, consistent with an age-related reduction in parenchymal airway support.

P711

Investigation for candidate genes of glucocorticoid resistance by genome-wide gene expression profiling in animal model of asthma

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The prevalence of impaired glucocorticoid (GC) responsiveness or GC resistance (GR) is 5-10% among asthma patients, even so 50% of asthma-related health care costs are spent on these cases. We have developed a murine model of asthma with GR (Clin.Exp.Allergy 2006;36:951). Ovalbumin (Ova) challenge, preceded by intranasal lipopolysaccharide priming, resulted in more severe eosinophil inflammation in Ova sensitized BALB/c mice (LPS/OVA) than Ova provocation alone (OVA). Moreover, the airway inflammation was resistant to dexamethasone (Dex) treatment in LPS/OVA mice. To further investigate the mechanisms of GR in this model, we have performed 20 whole mouse gene expression microarrays (44K; Agilent, St.Clara, USA). Total lung tissue mRNA expression of GC sensitive (OVA) and resistant (LPS/OVA) mice, as well as their Dex-treated counterparts (OVA+Dex and LPS/OVA+Dex) were compared with the gene expression profile of control (C) animals using two sample t test (n=4/group). The obtained gene lists were further dissected by GeneSpring GX (Agilent), considering only the significantly altered genes (p<0.01 vs. C) that show at least 2-fold up or down regulation. From these genes 23 were identified to be GR-related, i.e. were found in the common cluster of uniquely changed genes of LPS/OVA (but not OVA) and LPS/OVA+Dex (but not OVA+Dex) mice. Members of known asthma-related (eg. Serpine1) and GR pathways (eg. transforming growth factor β-2) were found to be involved in our model supporting relevance of this model to human disease. We have also nominated new candidate genes as potential therapeutic targets for GR in asthma. Funded by Hungarian Respiratory Society.

P712

Protective effect of transcription factor PAX-5 (BSAP) on asthma severity

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The aim is to evaluate the association of B-cell specific transcription factor PAX-5 amount in patients with different bronchial asthma (BA) courses.
Materials and methods: Peripheral blood lymphocytes derived from 12 BA patients and healthy were examined before and after 30-minutes IL-4 action. To estimate the mRNA quantity of PAX-5, reverse transcription PCR followed by electrophoresis on agarose gel was performed. PhosphoSTAT6 analyzed by Western blot. Western blotting was performed through standard procedure using antyphosphoSTAT6-antibody (Cell Signaling, USA).
Results: PAX-5 mRNA levels have trend to increase in patients with mild BA, in comparison with those with moderate and severe BA (data not shown). We revealed important positive correlations of PAX-5 with some characteristics of PFT probably defining severity of BA (%FEV1/FVC after bronchodilator: R=0,755; p=0,031; n=8; PEF%pred. before bronchodilator: R=0,623; p=0,041; n=11; MMEF50% exp. after bronchodilator: R=0,69; p=0,02; n=11) and the level of phosphoSTAT6 that is crucial for Th2-activity (R=0,724; p=0,027; n=9; after IL-4 action: R=0,87; p=0,025; n=6). PAX-5 mRNA levels correlate negatively with serum IgE at the end of exacerbation (r=-1,0; p=0,042; n=4) and with IFN-γmRNA levels (r=-0,511; p=0,004; n=10).
Conclusion: We revealed the association of PAX-5 and asthma severity. It probably may be explained by the fact of PAX-5 inhibiting B-cell differentiation to IgE-secreting plasmacells that may lead to decreased serum IgE level. IFNγhaving Th2-supressing activity may cause decreasing of PAX-5 amount through additional inhibiting of B-cell activation.
The work was supported by Saint-Petersburg government grant 28-04/17 (Certif. PSP080591).

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GATA-3 or T-bet: Who is the crucial marker of allergic bronchial asthma

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The aim: to establish features of expression of GATA-3 and T-bet in bronchial asthma (BA).

Material and methods: 20 healthy, 44 patients with allergic and 42 with non-allergic BA were examined.

Transcription factors GATA-3 and T-bet expressed in peripheral lymphocytes were analyzed by Western blot after cells were lysed. Preparation of lysates, and the Western blotting were performed through standard procedure. Antibodies against GATA-3 (Abcam, UK), T-bet (Santa Cruz Biotechnology) were used. Level of protein analyzed according to β-actin using anti-actin antibody (Sigma Aldrich, USA).

Results: Expression of GATA-3 was significantly increased and expression of T-bet was significantly decreased in lymphocytes of patients with allergic BA compared to healthy ($p<0,04$) and non-allergic BA groups ($p<0,005$). The level of GATA-3 in allergic BA negatively correlated with the degree of airflow obstruction ($r =-0,4$; $p =0,011$; $n=41$) and positively correlated with intensity of steroid therapy ($r =0,33$; $p =0,033$; $n=42$). The level of T-bet in non-allergic BA positively correlated with the prevalence of asthmatic triad ($r =0,4$; $p =0,01$; $n=42$) and drug intolerance ($r =0,43$; $p =0,005$; $n=42$).

Conclusion: GATA-3 and T-bet may play a key role in the pathophysiology of BA. The expression of GATA-3 and T-bet may serve as markers of allergic BA to provide necessary dose of steroids in patients with BA. This study suggests that atopic BA underlie the high level of Th2-cytokines production in allergic disease.

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P714

Expression of CD38 and its participation in formation of endothelial dysfunction in patients with bronchial asthma

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In the article we studied the role of CD38/ADP-ribosilcyclase in formation of endothelial dysfunction in patients with bronchial asthma.

In the research we have been included patients with mild (1st group) and severe (2nd group) bronchial asthma. We found the increased expression of CD38 in peripheral blood lymphocytes in patients of both groups (in the 1st group 4 out of 300 cells, in the 2nd 6 out of 300 cells) in an exacerbation in comparison with the control group (2 out of 300 cells). Increase of level of expression CD38 promotes the realisation of the mechanism of interaction of the activated lymphocytes with the cells of endothelium expressing of non-substrated ligand CD38 – CD 31/sPECAM-1.

We note increase of sPECAM-1 in plasma of peripheral blood in the 2nd group to 7,19 ng/ml [6,8; 7,7] in comparison with results of the patients of the 1st group: 6,78 ng/ml [6,09; 8,35] ($p=0,022525$). We found the positive interrelation between the level of CD31 and the expression of CD38 ($r=0,3$; $p <0,05$), and also direct correlation relationship between the level of CD31 and the concentration of C-reactive protein in blood plasma ($r=0,39$, $p <0,01$). The result of such relationship is the damage of cells of endothelium and formation of endothelial dysfunction which, in its turn, determines the disturbance of mechanisms of endothelial regulation of vascular wall elasticity and increase of its rigidity.

P715

The role of genetic polymorphism of NO-synthase in the implementation of the "atopic march"

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NO-synthase genes are candidate for development bronchial asthma (BA) and atopic dermatitis (AD), because nitric oxide and its metabolites are involved in the formation of oxidative stress and nitrolizing. We planned and conducted study of the following polymorphisms: 276 C/T, -186 A/C gene nNOS; -954 G/C, (CCTTT) n, (TAAA) n gene iNOS; 894 C/G and VNTR gene eNOS.

The aim of the study. Set polymorphisms NO-synthase gene associated with clinical and functional manifestations of bronchial asthma and atopic dermatitis for the formation of molecular predictors of implementation of the "atopic march".

Materials and methods: Material study were DNA samples of patients with asthma (n = 929), BA (n = 847), AD + BA (n = 460). As a control, the DNA of healthy subjects (n = 720).

Results: Established genotypes increase the risk of formation of "atopic march": TT genotype polymorphism 276 C/T, AC polymorphism 186 A/C gene nNOS (RR = 4,56, p = 0,003); 220/220 polymorphism (TAAA) n, GC and CC polymorphism - 954G/C, x/x on the number of repeats (CCTTT) n gene iNOS (RR = 1,60, p = 0,002); aa polymorphism VNTR, CG polymorphism 894C/G gene of eNOS (RR = 3,75, p = 0,001).

Found haplotypes of genes involved in the implementation of the "atopic march"atopic march": (nNOS, iNOS, eNOS): T276 (276C/T, nNOS) + C186 (-186A/C, nNOS) + X (CCTTT) n, NOS + G954 (- 954G/C, iNOS) +220 (TAAA) n, iNOS, + G894 (894C/G, eNOS) + a (VNTR), eNOs.

P716

The influence of cigarette smoke on the polymorphism of matrix metalloprotease (MMP2) in asthmatic patients

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Introduction: Matrix metalloproteinases (MMPs) are a large family of proteolytic enzymes that degrade the components of extracellular matrix. The MMP2 is involved in the pathogenesis of both chronic lung disease: asthma and COPD. However, its role is not clear.

The aim of this study: Determine whether cigarette smoke plays a role in induction of polymorphism of MMP-2 in tunisian patients.

Patients and methods: A total of 150 asthmatic patients (36 smokers and 114 non-smokers) were included prospectively. MMP-2 (C-735T) genotypes of both groups were determined by the restriction fragment length polymorphism method.

Results: The mean age of asthmatic patients was 48 years. The sex ratioM/W was 0,44. The frequency of patients with severe asthma is 28,6%, moderate asthma is 42,6% and mild asthma is 20,6%. There was no difference in polymorphism of MMP-2 (C-735T) between smokers and non-smokers asthmatic patients.

Conclusion: The present study demonstrates that exposure to cigarette smoke has no influence on the polymorphism in the promoter region of MMP2. However, these results may be confirmed by further prospective studies.

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Genetic typing of bronchial asthma afflicted people according to polymorphous genes for the purpose of disease progress risk factors detection

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The article presents the data concerning frequency allocation.of gaplotypes and alleles of genes belonging to family glutathione-S-transferase (GSTM and GSTT), genes of cytokines (IL-6 and TNF-A), cytotoxic receptor of T-lymphocyte gene (CTLA-4) and gene of vitamin D receptor in case of afflicted people with uncontrolled bronchial asthma clinical course. The data are compared to the frequency allocation of gaplotypes and alleles of the foregoing genes in the citizens' population in different areas of Belarus. The received data concerning the frequency allocation of gaplotypes and alleles of genes CTLA-4, TNF-A, GSTM and GSTT didn't authentically differ from the frequency of Belarusian population. No authentic frequency allocation differences were detected. However there were authentically proved differences in frequency allocation of gaplotypes and alleles of genes between four polymorphous locuses of VDR-gene in case of bronchial asthma afflicted people from the one detected in the population. The VDR gene's frequency of homozygotes FF at FokI locus (36,0%) is higher than the population's one (29,1%). The VRD gaplotype TtAaBbFF appeared to be the bronchial asthma afflicted people's most spread one (17,4%) (in Belarusians' population selection the most spread gaplotype is TtAaBbFf). The received data indicate the connection of VDR- polymorphism with uncontrolled bronchial asthma and the utility of conducting the further research concerning the influence of vitamin D on treatment.

P718

The role of the epithelial barrier in allergic airway inflammation

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Aim: Identify the correlation of clinical and morphological data damage the mucous membranes of the nose and bronchi in patients with asthma.

Materials and methods: We studied scrapings of the mucous membrane of the nasal cavity, lavage and bronhobioptaty patients with allergic rhinitis (AR) and brochial asthma (BA). A comparative analysis between a group of teenagers and adults. Scanning (SEM) and transmission electron microscopy (TEM), semi-thin sections.

Results: We have shown that the surface of the epithelial cells of the upper and lower respiratory tract in AR and BA tend to have the same changes that, along with clinical data on hyperreactivity of these departments, as determined by provocative tests, and indicates a common mechanism of damage to the epithelium. By SEM we found the greatest damage to the exposed cilliated cells (CC) and by TEM detected and intracellular destructive processes. Significant changes in goblet cells (GC) is to enhance the secretory function. This reaction is more pronounced in the nasal mucosa. Clinical effect of allergen specific immunotherapy (ASIT) in adolescents was morphologically confirmed. After carrying out ASIT with middle-heavy and heavy forms BA at TEM and SEM are shown endocellular regeneration processes in CC and GC.

Conclusion: The intensity of changes is directly related to the severity of the disease, its early onset, dynamics and duration of disease. This whole complex of changes suggests major modifications in the morphology of the airway in allergic response to aggression. Timely basic therapy and ASIT contribute to a better clinical effect, helps to prevent potential complications, and also helps to restore a damaged nose and bronchial mucosa.

P719

Concomitant exposure to nicotine and endotoxin in vitro induces murine airway hyperreactivity possibly via nicotine-induced upregulation of toll-like receptors

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Introduction: Nicotine and endotoxin are important components of cigarette smoke, known to cause asthma and trigger exacerbations. The present study examined whether concomitant exposure to nicotine and endotoxin in vitro can cause airway hyperreactivity (AHR) in murine tracheal segments, and explored the involvement of Toll-like receptors (TLR) and MAPK signalling pathways in this process.
Methods: Isolated murine tracheal segments were exposed to nicotine (10 μM) and/or endotoxin (10 μg/ml) in organ culture for 4 days. Contractile responses to carbachol were recorded by myograph. In parallel, mRNA and protein expressions of the cell surface TLRs and several inflammatory mediators were semi-quantified by real-time PCR and confocal microscopy-based immunohistochemistry after exposure to only nicotine with/without specific inhibitors for MAPK pathways JNK, ERK1/2 or p38.
Results: Nicotine or endotoxin alone failed to affect contractile responses to carbachol, whereas AHR appeared after concomitant exposure. Incubation with nicotine increased expression of TLR2, 4 and 6, decreased TLR5, while TLR1 and 11 expressions remained unchanged. In parallel, MCP-1 and COX-2 expressions also increased. Inhibition of JNK, but not ERK1/2 or p38, attenuated nicotine's effects.
Conclusion: Long-term nicotine exposure induces local inflammation and derrangement of the airway cell surface TLR expression pattern via the MAPK JNK signaling dependent pathways. It is therefore tempting to suggest that the nicotine-induced up-regulation of TLR is responsible for the AHR observed after concomitant incubation with nicotine and endotoxin.

P720

Increased cough responsiveness in guinea pigs with respiratory syncytial virus infection and its neurogenic mechanism

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Objective: To investigate the role of neurogenic inflammation in increasing cough responsiveness in respiratory syncytial virus (RSV) -infected guinea pigs
Methods: Fifty guinea pigs were randomly divided into a control group and four RSV-inoculated groups: post-infection day (PID) 6, 12, 28 and 42. cough responsiveness, airway inflammation, RSV antigen and nucleic acid, substance P (SP) protein content and expression, and neurokinin receptor 1 (NK1) mRNA expression were evaluated and the correlation between cough responsiveness and SP and NK1 mRNA levels determined.
Results: Cough responsiveness of infected groups was significantly higher than control and reached a peak at PID 12. Inflammatory cells and lymphocytes increased in bronchoalveolar lavage fluid, and lung tissue pathology showed airway inflammation without pneumonia. RSV RNA content was highest at PID 6 and then gradually decreased. SP peaked at PID 28 and remained high at PID 42, SP protein staining could be seen in lung tissue. NK1 mRNA levels were also significantly increased at PID 12, 28 and 42. cough responsiveness was positively correlated with and SP and NK1 mRNA levels.
Conclusion: The increase in cough responsiveness was consistent with the clinical features of cough caused by RSV. The accompanying increase in SP and its receptor with the positive correlation between these parameters and cough responsiveness suggests that neurogenic inflammation may play a decisive role in the increase in cough responsiveness and cough induced by RSV.

P721

Immunomodulation capacity of alpha-1 antitrypsin in regulation of CD14 molecules expression and secretion in human monocytes in vitro

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Introduction: The recognition of bacterial lipopolysaccharide (LPS) is principally mediated by CD14 molecules. Recent findings suggest that AAT can also exhibit biological activity independent of inhibition of serine proteases.
Aims: To investigate influence of AAT on monocytes activity in vitro through regulation of CD14 secretion and expression.
Methods: Human monocytes CD14 expression was analysed by flow cytometry and RNR expression, soluble CD14 levels were analysed using ELISA kit.
Results: Short-term (up to 2h) monocyte exposure to AAT induces expression of CD14 and secretion of soluble CD14.

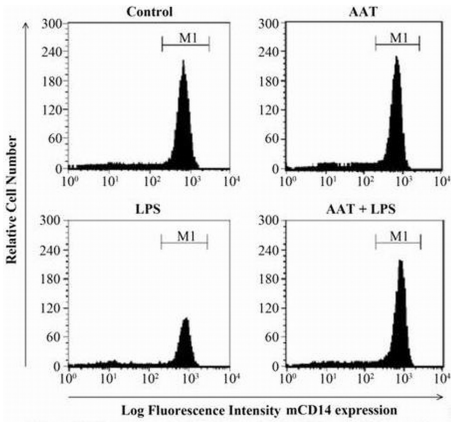

Figure 1. Short-term (2 h) effects of AAT, LPS and LPS + AAT combination on mCD14 expression (measured by flow cytometry)

It helps monocytes in neutralization of LPS. Longer term (18h) monocytes incubation with AAT decreases expression of CD14 and enhances soluble CD14 secretion, thus monocytes are protected from hyperstimulation of bacterial endotoxyn.

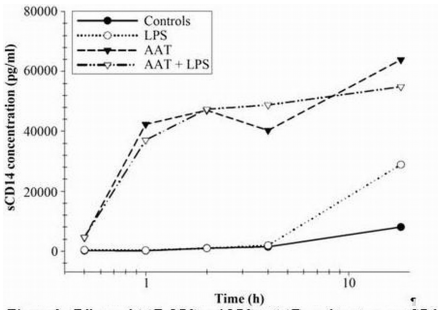

Figure 2. Effects of AAT, LPS and LPS + AAT combination on sCD14 secretion

Conclusions: These findings provide evidence that AAT is an important regulator of CD14 expression and release in monocytes and suggest that AAT may be involved in prevention of over-activation of monocytes/macrophages in vivo, that is important in development of chronic lung diseases.