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 bronchitic phenotype, 19 subjects with mixed phenotype of COPD) and 17 healthy subjects (HS) using the flow cytometry analysis.
**P723**

**IL-33 has a nuclear distribution and is not increased in peripheral lung of COPD patients**

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Elena Beljaeva2, Victor Novikov3.

**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 1 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 these 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 FEV1 (r2=0.5) 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).

**Conclusions:**

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.

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**P724**

**Decrease of soluble adhesion molecules levels and bacterial mixt-infection in patients with COPD**

Elena Beljaeva2, Victor Novikov3.

**Methods:**

In the present study, we aimed to investigate the correlation between SP-D and inflammation in COPD pathogenesis. 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.

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**P725**

**Proinflammatory cytokines in induced sputum and COPD phenotype**

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**Results:**

Significant correlation of IL-8, IL-6, IFN-γ and TNF-α did not differ between two groups. The concentration of IFN-γ 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, IL-1β and TNF-α in induced sputum (IS) was measured using flow cytometry with Cytometric Bead Array method. The median concentration of proinflammatory cytokines (IL-8, IL-6, IL-1β and TNF-α) and IL-10 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, IL-1β and TNF-α 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, TNF 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/year, 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.

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**P726**

**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 patients with predominance of chronic bronchitis. The aim of the study was to characterize the inflammatory process in two distinct forms of COPD.**

Elena Beljaeva2, Victor Novikov3.

**Methods:**

In the present study, we aimed to investigate the correlation between SP-D and inflammation in COPD pathogenesis. 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.

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**P727**

**Relationship between % neutrophils in induced sputum and IL-6 and TNF-α in COPD**

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**Methods:**

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/m2), dyspnea according to the modified Medical Research Council scale (MRC), oxygen saturation (SatO2), sputometry before and after 400 μg inhaled salbutamol, lung volumes and carbon monoxide transfer factor coefficient (KCO) measurements were measured. TNF-α and IL-6 were measured by RIA.

**Results:**

The sputum neutrophil% was statistically significant 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 with the FEV1% and the% airflow limitation and with the levels of IL-6 and TNF-α.

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P728
Expression of the phosphorylated myristoylated alanine-rich C kinase substrate (MARKCS) in COPD bronchial glands
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Myristoylated alanine-rich C kinase substrate (MARKCS) is a central regulatory molecule involved in mucin granule release by normal human bronchial epithelial cells in vitro. Upon stimulation, activated PKC phosphorylates MARKCS (p-MARKCS), causing translocation of p-MARKCS 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 p-MARKCS 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-MARKCS+ cells. The number of p-MARKCS+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-MARKCS in the bronchial mucous glands between the 3 groups of subjects. This data suggests that the p-MARKCS 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 remodeling there was conducted an electron microscopy of bronchial mucosa bioptic materials that had been conducted at the bifurcation of the proximal bronchi during exacerbation. The content of type IV collagen in 43 patients with stage 2 COPD before and after 1, 3 and 6 months of treatment with tiotropium bromide was investigated. 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-MARKCS in the bronchial mucous glands between the 3 groups of subjects. This data suggests that the p-MARKCS pathway is not critical for mucin secretion in the bronchial glands of COPD patients.

P730
Differences in biomarkers of systemic inflammation in two phenotypes of COPD patients
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Introduction: Classification of chronic obstructive pulmonary disease (COPD) is usually based on the severity of airflow limitation, but is not 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 TNFα in plasma, in stable COPD.
Methods: 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 computed tomography (CT): A: Phenotype emphysema (n=19), KCO<70% CT compatible; B: phenotype chronic bronchitis (n=21), KCO>70% CT and without emphysema. Plasma level of fibrinogen, fibrin, PCR and C-reactive protein (CRP) were measured. Differences in biomarkers of systemic inflammation between the two phenotypes was evaluated by performing a two-tailed Student's t-test for variables normally distributed and a Mann-Whitney U-test for variables with a non-normal distribution.
Results: The values of fibrinógeno/PCR did not show significant differences between the two phenotypes. The content of PCR f was higher in Group 3 (healthy smoker) and%52 ± 70% an CT compatible; B: phenotype chronic bronchitis (n=21), KCO>70% CT and without emphysema. Plasma level of fibrinogen, fibrin, PCR and C-reactive protein (CRP) were measured. Differences in biomarkers of systemic inflammation between the two phenotypes was evaluated by performing a two-tailed Student's t-test for variables normally distributed and a Mann-Whitney U-test for variables with a non-normal distribution.
Conclusions: Due to the morphological remodeling there is great activity of neo collagenesis during exacerbation of COPD, evidenced by significantly increased production type IV of collagen in bronchial submucosal fluid.

P731
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±4 yrs; 13% male; all smokers, 71±6 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±229 Pa) (p<0.05) compared to that measured in the RG subset (n=6) from 999±424 to 842±343 Pa. The ADI index was associated with NEUT stiffness on admission in the two subsets of patients (p=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. Supported by SEPAR-2010 and CIBERES

P732
Increase of extracellular IL-1β and anti-microbial peptides in the peripheral airways of smokers
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Among several known cytokine-signalling pathways influencing innate effector cell function, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway is central in regulating inflammation and immunity. NF-kB activation is involved in the pathogenesis of chronic obstructive pulmonary disease (COPD) and is considered to be a potential target for novel anti-inflammatory therapies. We investigated NF-kB activation and the expression of NF-kB-inducible genes in the lungs of smokers and non-smokers using quantitative PCR. We found that smokers had significantly higher levels of NF-kB activation and expression of NF-kB-inducible genes compared to non-smokers, which was associated with increased expression of IL-1β and anti-microbial peptides. These findings indicate that smokers have an increased inflammatory response compared to non-smokers, which may contribute to the development and progression of COPD.
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=17-23/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 [0.3-3.3], 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 in smokers with COPD (24 [4-364], pg/ml). Interleukin-17F, IL-22 and LL-37 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 important risk factor for COPD (BS-COPD), but 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 for neutrophils and inflammatory cells were identified and counted under light-microscope. IL6, IL8, 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 oscillometry (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%, 3% and mean CO diffusion test value: 74.6%, 4% respectively. The results for the serum and urinary markers (Mann-Whitney test) comparison with median and quartiles were the following: CRP (COPD 0.09, controls 0.66, p=0.06), leucocytes (COPD 8500, controls 6800, p=0.001), D-dimer (COPD 249, controls 242, p=0.16), BNP (COPD 536.3, control 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 (PMNs), 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-myo-racetate (PMA, 0.1-3.0 nm) and A. aureus bacteria (1-167 bacteria/neutrophil). ROS production was measured flow cytometrically 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 concentrations 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 bronchial 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 6 (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 bronchioalveolar space (94.5±5 to 65.5±30 nmol/ml, p<0.05), but no change in the alveolar space (87.4±48 to 93.5±55 nmol/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 bronchioalveolar space and suggests less bronchial protein degradation in the acute phase after smoke exposure.

**References:**


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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±14%pred) and 20 non-smokers (NSC) (FEV1=101±8.0%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=0.05). Similarly, in blood, COPD had less Baff+ TRegs (10, 0-22,5) and more Baff+ B lymphocytes (23, 5.6-102) compared to NSC (12, 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.

Is centriflobular 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 bronchiodilatation 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) inhalation and their relation to airway reactivity. Therefore, we quantified trypstase 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 smooth muscle of small airways were significantly higher in CLE (206±41 cells/mm2) than in PLE (104±21 cells/mm2; p=0.011, SNE (107±54 cells/mm2; p=0.05) and NS (105±27 cells/mm2; 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/mm2; PC20>8mg/ml: 89±69 cells/mm2) and COPD patients did not differ in either. 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.