a double blind, controlled safety and efficacy study. Four doses of BIO-11006 or control (half normal saline; HNS) were administered by nebulization for 21 days to 5 cohorts; 75 mg QD (n=38) or BID (n=24), 150 mg QD (n=35), and 125 mg BID (n=25); HNS (n=50). The ratio of active/control was 2:1 and 3:1 for QD and BID dosing, respectively. Trough FEV1 (primary endpoint) was measured on days 0, 3, 7, 14, 21, 28 and 49. A trend towards increased FEV1 with the 75mg BID group was maintained on follow-up days 28 and 49. An FEV1 responder analysis (defined as 5% or more improvement of FEV1 = Responder) revealed the percentages of responders for the 75 mg BID dose were 46, 38, 50 (p=0.014 vs HNS), 42, 54 (p=0.04 vs HNS) and 40% on days 3, 7, 14, 21, 28 and 49, respectively. BIO-11006 was systemically well tolerated with some increase in respiratory adverse events. We conclude that the 75 mg BID dose appeared to be the most efficacious by increasing the proportion of FEV1 responders statistically significantly as compared with HNS. Sputum volume and the St. Georges Respiratory Questionnaire Symptoms Score also trended towards improvement with 75mg BID. Thus, BIO-11006, a dual action molecule that decreases both mucus hypersecretion and inflammation, may represent a new advance in the treatment of COPD.

4902

Discrimination of expanded dendritic cell populations in lung tissues from COPD patients

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Rationale: Dendritic cells (DCs) are highly plastic and their characterization in human tissues has been hampered by lack of standardized immunohistochemical identification strategies. This study validates an immunohistochemical method for masking confounding non-DC cells and characterizes multiple DC populations in COPD-affected lungs.
Methods: Lung specimens were obtained from 27 COPD patients and divided into three levels of severity; GOLD I (n=6), GOLD II-III (n=11) and GOLD IV (n=10). Never-smokers (n=8) and non-COPD smokers (n=6) served as controls. Paraffin sections were double stained for combinations of macrophage and DC markers.
Results: Using the non-soluble DAB as the first detection chromogen it was possible to mask confounding non-DC cells e.g. $CD68^+$ macrophages at a bright microscopic level. Using this approach two populations of $CD68^-CD11c^+$ and $CD68^-CD123^+$ cells were identified, indicative of myeloid and plasmacytoid DCs, respectively. The myeloid DCs, which were foremost $BDCA3^-$, were significantly increased in diseased areas of COPD lungs, in particular in patchy areas of fibrosis and granuloma formation. Both $CD68^-CD11c^+$ and $CD68^-CD123^+$ cells displayed a dendritic morphology and were observed in epithelial and subepithelial compartments of small airways and alveolar walls as well as in lymphoid aggregates amidst $CD21^+$ follicular DCs. Further combination of markers could discriminate intraepithelial myeloid and plasmacytoid DCs from $CD207^+$ and $CD1a^+$ epithelial DCs.
Conclusions: This study demonstrates that masking of confounding non-DC populations improves the identification of lung DC populations and reveals novel aspects of their dynamics and heterogeneity in COPD lungs.

4903

Mechanisms of tertiary lymphoid organ formation during lymphoid neogenesis are involved in lymphoid follicle formation in chronic obstructive pulmonary disease

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Tertiary lymphoid organs (TLOs) are aggregates of B and T cells formed in response to chronic immune responses. TLOs are the result of lymphoid neogenesis and are formed via production of lymphoid-organizing chemokines (CXCL13, CCL19 and CCL21), in response to signaling from lymphotoxin α (LTα) via TNFR1, TNFR2 and LTR. Stromal cells and antigen presenting cells (APCs), i.e. dendritic cells (DCs), secrete lymphoid chemokines, which attract B cells, T cells and DCs via CCR7 (receptor for CCL19/21) and CXCR5 (receptor for CXCL13). Lymphoid follicles are frequently found in the peripheral lungs of patients with Chronic Obstructive Pulmonary Disease (COPD). Whether they are the result of lymphoid neogenesis remains elusive. Here, we have identified 18 patients with COPD and lymphoid follicles and used immunohistochemistry to analyze the expression of LTa and lymphoid chemokines. Flow cytometry was applied to study expression of their receptors. LTα is abundantly expressed by alveolar macrophages and lung stromal cells and CXCL13 is strongly expressed inside the follicles. HLA-DR+ve cells (APCs), but not CD45-ve stromal cells, strongly express TNFR1 (43% of APCs), TNFR2 (47%) and LTR (38%). CXCR5 is expressed by B cells (96% of B cells), DCs (74% of DCs) and T cells (24% of T cells). CCL19, CCL21 and CCR7 are rarely expressed. In conclusion, molecular mechanisms underlying TLO formation might be involved in lymphoid follicle formation in COPD as follows: stromal cells and macrophages secrete LTa, which induces CXCL13 production by lung APCs, driving the accumulation of CXCR5

508. Novel mechanisms in COPD

4901

Results of a phase 2a clinical trial with a peptide inhibitor of MARCKS protein indicate improvement of indices of bronchitis and lung function in patients with COPD

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A peptide inhibitor of MARCKS (BIO-11006) attenuates mucus hypersecretion, inflammatory cell influx and airway obstruction in several in vivo models of asthma and bronchitis, suggesting BIO-11006 as an ideal treatment for COPD. In a Phase 2a study, 172 subjects with stable COPD (GOLD Stage 2, 3) were randomized in

bearing B cells, T cells and DCs to sites of TLO. Funded by the Hellenic Thoracic Society.

4904

Late endothelial progenitor cells are senescent and dysfunctional in COPD due to reduced sirtuin-1 levels

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Introduction: Cardiovascular disease (CVD) is a major cause of death in COPD. Numerous studies describe clinical evidence of endothelial dysfunction in COPD but the molecular pathways which link COPD and CVD remain unclear. Late outgrowth endothelial progenitor cells (EPC) could serve as a research tool to investigate endothelial defects in COPD patients.
Aim and objectives: To examine whether EPC from COPD patients exhibit dysfunctional characteristics, illustrating the underlying molecular process of endothelial dysfunction in COPD.
Methods: EPC were isolated from peripheral blood samples received from 16 healthy non smoking volunteers (age ± SEM, 57±2.7yr), 10 healthy smokers (57±2.6yr) and 16 COPD patients (67±1.6yr). The mononuclear fraction was placed in culture in the presence of endothelial growth factors. Late outgrowth colonies of EPC appeared between day 7 and 24, as characteristic cobblestone monolayers. The cells were grown to confluence in T-25 or T-75 flasks and used at passages 4 to 6 for all experiments. Endothelial senescence was measured by senescence-associated β-galactosidase (SA-β-Gal) activity and sirtuin (SIRT) 1 protein levels by Western blotting.
Results: EPC from healthy smokers and COPD patients displayed significantly increased senescence and reduced SIRT1 protein levels compared to healthy non smoking subjects. SIRT1 protein levels negatively correlated with endothelial senescence.
Conclusions: The results from our study demonstrate that EPC from smokers and COPD patients display epigenetic molecular dysfunctions linked to increased senescence. These defects may contribute to endothelial dysfunction and cardiovascular events.

4905

Cigarette smoke-induced oxidative modification of creatine kinase B (CKB) is involved in the pathogenesis of COPD in terms of acceleration of bronchial epithelial cell senescence

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Introduction: Cigarette smoke accelerates cell senescence, implicated in COPD pathogenesis. Energy status is one of the most crucial determinants for aging and longevity. In COPD patients, muscle type CK (CKM), an essential enzyme in energy homeostasis, is oxidized and lost activities, leading to respiratory muscle dysfunction. In lung tissue, brain type CK (CKB) is the predominant isozyme of CK. Therefore, we hypothesized that CKB might also be oxidized and lost activities by smoking stress, resulting in disease pathogenesis of COPD in terms of acceleration of cell senescence.
Aims: To elucidate the role of CKB in cigarette smoke extract (CSE)-induced cellular senescence in human bronchial epithelial cells (HBEC).
Methods: Primary HBEC and Beas2B cells were used. Cellular senescence was evaluated by SA-β-gal staining and p21 expression and cell cycle analysis. CKB was inhibited by siRNA and cyclocreatine. Interleukin (IL)-8 secretions were measured by ELISA.
Results: CSE induced carbonylation of CKB and decreased CKB protein levels, and this decrease was reversed by the proteasome inhibitor. CSE induced cell senescence, and CKB inhibition further enhanced CSE-induced cell senescence. CSE treatment caused increased amounts of IL-8 secretion, a hallmark of senescent associated secretary phenotype (SASP). IL-8 secretion was further increased by CKB ablation.
Conclusions: CSE induces carbonylation and subsequent proteasomal degradation of CKB, and decrease of CKB is implicated in the regulation of cell senescence with SASP.

4906

Up-regulation of decoy receptor D6 in COPD

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D6 is a "silent" chemokine-scavenging receptor involved in the intracellular degradation of pro-inflammatory chemokines and resolution of acute inflammatory responses in mice. Recently it has been found that D6 is increased in autoimmune diseases and in organ rejection conditions in humans. To investigate whether D6 is involved in the immune response in patients with COPD, we examined its expression by immunohistochemistry in surgically resected lung specimens from 3 groups of subjects: 16 smokers with COPD (FEV1=57±6%pred), 9 smokers with normal lung function (FEV1=102±3%) and 9 non-smoking controls (FEV1=106±6%). D6 was mainly detected in alveolar macrophages, where it was markedly up-regulated in smokers with COPD compared to control smokers and non-smokers [median (range): 78 (11-100%) vs 5 (0-61%) vs 0 (0-10%,) $p<0.0005$ for both]. D6 expression was confirmed at mRNA and protein levels by RT-PCR and FACS in alveolar macrophages, isolated from BAL. By immunofluorescence we observed that D6 co-localised mainly within the lysosome-associated macrophagic marker CD68. D6 expression in all patients was related to CD8 T-lymphocytes in alveolar walls ($p=0.007$,$r=0.65$) and to the degree of airway obstruction ($p=0.0008$,$r= 0.57$). In conclusion, this study shows for the first time that D6 is expressed in alveolar macrophages, is up-regulated in COPD patients and correlates with disease severity and number of CD8 T-lymphocytes. Although up-regulation of D6 may occur as an attempt to tune down inflammation, our results suggest that D6 may be playing an important role in the inflammatory, possibly autoimmune, mechanisms of COPD.

4907

Serum amyloid A augments mucosal immunity by opposing resolving lipoxinA4 signaling in chronic lung disease

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Introduction: Persistent innate immune activation contributes to tissue destruction in chronic obstructive pulmonary disease (COPD). Altered responses to glucocorticosteroids (GC) may further compromise host defense in chronic lung diseases. We previously identified SAA as a GC resistant systemic COPD biomarker and here, characterized its function in orchestrating mucosal inflammatory responses that contribute to neutrophil accumulation.
Methods: Three separate patient cohorts were investigated involving 81 patients.
Results: In a prospective study of competing ALX/FPR2 ligands during COPD exacerbation, circulating SAA levels were markedly and disproportionately increased relative to LXA4. Secreted SAA levels in COPD BAL fluid correlated with interleukin-8 and the neutrophil activation marker, neutrophil elastase. SAA was also detected in COPD lung in close proximity to airway epithelia, and in vitro SAA triggered pro-inflammatory mediator (MCP-1, GM-CSF and IL-8) release by airway epithelial cells in an ALX/FPR2 receptor-dependent manner. Lipoxin A4 (LXA4) blocked SAA initiated epithelial responses via allosteric inhibition at ALX/FPR2 (pA2 13 nM). When administered directly into murine lung, SAA initiated robust acute inflammation that was significantly inhibited by equivalent amounts of 15-epi-LXA4 but not dexamethasone. Tissue macrophages (CD68+) colocalised with lung SAA and the GC dexamethasone markedly increased SAA production by THP-1 macrophages (pEC50 43 nM).
Conclusions: Together, these findings implicate airway SAA production as a mediator of GC resistant lung inflammation that can overwhelm organ protective signaling by lipoxins at ALX/FPR2 receptors.

4908

Insufficient autophagy is involved in accelerated cellular senescence in the pathophysiology of COPD

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Introduction: Accelerated cellular senescence has been implicated in the pathogenesis of COPD. One of the manifestations of cellular senescence is the accumulation of damaged organelles and proteins, which are induced by cigarette smoke via oxidative stress. Autophagy may associate with the regulation of the accelerated cellular senescence via clearance of damaged cellular components.
Aims: The purpose is to elucidate the role of autophagy in cigarette smoke extract (CSE)-induced cellular senescence in human bronchial epithelial cells (HBEC).
Methods: HBEC was isolated from lobectomy specimens. To characterize autophagy,detection of LC3-EGFP dot and western blotting of LC3, p62, and ubiquitin were performed. Autophagy was inhibited by 3-methyladenine (MA), bafilomycin A and knock down of LC3 by siRNA. Rapamycin and torin1 were used to induce autophagy. Senescence associated beta-galactosidase (SA-β-gal) stain-

ing and western blotting of p21 were performed to evaluate cellular senescence. Interleukin (IL)-8 was measured by ELISA.

Results: CSE-induced cellular senescence was accompanied by accumulations of ubiquitinated proteins and p62. Although CSE transiently induced autophagy, it was insufficient to inhibit cellular senescence. Increased autophagy suppressed CSE-induced senescence and accumulations of these proteins. In contrast, inhibition of autophagy enhanced not only senescence but also the senescence-associated secretory phenotype (SASP) of IL-8 expression.

Conclusions: These results suggest a potential regulatory role for autophagy in CSE-induced cellular senescence with SASP by preventing the accumulation of ubiquitinated proteins and p62.