508. Novel mechanisms in COPD

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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 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 Control 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. 

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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 BDCAH', 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.

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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 lymphotixin 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 LTα and lymphoid chemokines. Flow cytometry was applied to study expression of its 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 CXCL13 (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 LTα which induces CXCL13 production by lung APCs, driving the accumulation of CXCR5+ TLOs.
Cigarette smoke-induced oxidative modification of creatine kinase B (CKB) is important for aging and COPD patients display epigenetic molecular dysfunctions linked to increased disease severity. Moreover, CKB might also be oxidized and lost during cellular senescence, illustrating the underlying molecular process of endothelial dysfunction in COPD. CKB inhibition further enhanced CSE-induced cell senescence. CKB inhibition further enhanced CSE-induced cell senescence.

**Methods:** CKB was inhibited by siRNA and calecimycin. Interleukin (IL)-8 secretion was measured by ELISA.

**Results:** CKB inhibition further enhanced CSE-induced cell senescence. CKB inhibition further enhanced CSE-induced cell senescence.

**Conclusions:** CKB inhibition further enhanced CSE-induced cell senescence. CKB inhibition further enhanced CSE-induced cell senescence.
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.