73. Update on neutrophil and macrophage function in COPD

382 Identification of pathogenic macrophage subpopulations in lung disease
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Residential macrophages may exist in discreet subpopulations which sur
 vive specific roles in the maintenance of tissue and immunological homeostasis. In lungs, alveolar macrophages (AMφs) comprise of >95% cells in the alveolar airspace, where they act as the primary sentinel of pathogens. Increased alveolar macrophage numbers are observed in many animal models of COPD and also clinically in COPD patients. Currently, M2 macrophage polarization is thought to be a contributor of lung disease, although this in vitro-derived macrophage paradigm may not completely explain the complex behaviour of AMφs in vivo. Using flow cytometry, we have developed an approach which shows that mouse AMφs instead form distinct subpopulations during acute inflammation and in chronic inflammatory lung disease. During acute inflammation, AMφ subpopu
lations are characterised by differential Mac-1 and CD11c integrin expression rather than M1 or M2 Mac-1 low/neg AMφ signatures ex vivo. Resolution is characterised by restoration to a single population being the proinflammatory (M1) and immunoregulatory (M2) phenotypes char
acterized by surface markers and chemokine profiles. Reprogramming of AM toward a partially M2-polarized phenotype has been suggested to contribute to COPD pathogenesis. We thus sought to characterize the phenotype of human lung macrophages (LM) following stimulation with the Th2-type cytokines IL-4 and IL-13.

Methods: LM were isolated from human resected lung tissues am
osterilized with LPS. TNF-α and IL-10 were measured by

Table 1: LPS-stimulated cytokine release

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>ng/ml</th>
<th>Day 0</th>
<th>Day 1</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td>7.5±2.8</td>
<td>6.9±0.7</td>
<td>6.4±1.4</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.1±0.01</td>
<td>0.1±0.07</td>
<td>0.1±0.02</td>
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</tbody>
</table>

Mac-1 low/neg AMφs persisted, and not respond to the environment. To test this, monocyte-derived m
φs were treated with IL-4 or IL-13 (1-150 ng/mL). Cytokines transcript expression was assessed with RT-qPCR, whereas proteins of M1 (TNF-α, CCL3, CCL4 and CXCL8) and M2 cytokines (CCL13, CCL17 and CCL22) were quantified in supernatants.

Results: Unstimulated LM exhibited a rather unclassified phenotype, with weak M1/M2 cytokine expression. On the other hand, transcriptome analysis of 80 cytokines gene revealed that only four M2-type transcripts levels were increased (4- to 8-fold) following stimulation with IL-13 (CCL13, CCL17, CCL22 and CCL26). M2-type cytokine production at the protein level was also concentration-
dependently increased (3- to 20-fold), whereas M1 cytokines were unaffected. CCL13 and CCL22 increase at 48hrs, while the maximum was reached at 24hrs for CCL17. The results obtained with IL-4 were similar, except that IL-4 potency was greater than IL-13 since the low 10 ng/mL concentration provided submaximal cytokine increases.

Conclusions: Our data demonstrate that IL-4 and IL-13 favours LM polarization toward the immunoregulatory M2 phenotype characterized by the expression of CCL13, CCL17, CCL22 and CCL26.

385 Chemokine characterization in human lung macrophages following stimulation with Th2-type cytokines IL-4 or IL-13
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Background: Macrophages may acquire polarized phenotypes, the two extremes being the proinflammatory (M1) and immunoregulatory (M2) phenotypes char
acterized by surface markers and chemokine profiles. Reprogramming of AM toward a partially M2-polarized phenotype has been suggested to contribute to COPD pathogenesis. We thus sought to characterize the phenotype of human lung macrophages (LM) following stimulation with the Th2-type cytokines IL-4 and IL-13.

Methods: LM were isolated from human resected lung tissues challenged for 24 or 48hrs with IL-4 or IL-13 (1-150 ng/mL). Cytokines transcript expression was assessed with RT-qPCR, whereas proteins of M1 (TNF-α, CCL3, CCL4 and CXCL8) and M2 cytokines (CCL13, CCL17 and CCL22) were quantified in supernatants.

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Conclusions: Our data demonstrate that IL-4 and IL-13 favours LM polarization toward the immunoregulatory M2 phenotype characterized by the expression of CCL13, CCL17, CCL22 and CCL26.

386 Lipids in the lung: Respiratory inflammation in COPD
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Background: There is strong association between COPD and metabolic co
morbidities involving lipids. Abnormalities of lipid metabolism are associated with increased inflammatory responses. The aim of this study was to identify abnormalities of lipid content in the lungs of COPD patients, focusing on lung macrophages.

Methods: Resected lung tissue from patients undergoing surgery for cancer was used; isolated macrophages and macrophages within formalin fixed paraffin em
bedded (FFPE) tissue were examined. Comparisons were made between 5 subject groups, never smokers (NS), current smokers with normal lung function (S), ex-smokers with normal lung function (E), COPD current smokers (COPDS) and COPD ex-smokers (COPDE). The presence of lipid laden macrophages was identified using oil red o stain (ORO) where positive staining is expressed as % mean (SD).

Results: The number of ORO positive macrophages was increased in COPD patients and controls who were current smokers (COPDS and S) compared to the other groups of ex- or never smokers, in both isolated macrophages and FFPE tissue.

Discussion: We have shown an increase in lipid laden macrophages in the lungs of

References:

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COPD is associated with increased numbers of highly-activated lung macrophages (Mφ) with reduced phagocytic ability. Mφ may exist as M1-like (classically
activated) or M2-like (alternatively-activated) states. In COPD, M1-like Mφ may persist, and not respond to the environment. To test this, monocye-derived Mφ from non-smokers (NS), smokers (S) and COPD patients were cultured in GM
CSF (G-mφ, M1-like) or M-CSF (M-mφ, M2-like) for 12d (n=12). Media was switched for 6d and Mφ stimulated with LPS. TNFα and IL-10 were measured by

ELISA (n=23). Beads, H influenzae (HI) or S pneumoniae (SP) phagocytosis was measured fluorometrically (n=10), CD163 and CD16 by FACS (n=3). All G-mφ released similar levels of TNFα and IL-10. Switching to M-CSF, TNFα release decreased in NS and S, but not COPD whereas IL-10 increased in NS but not S and COPD (Table 1). M-mφ from NS and S released less TNFα vs COPD but more IL-10. Switching to GM-CSF, IL-10 decreased in all groups (Table 1).
S and COPDS. Current smoking causes to appear dysfunctional lipid metabolism within lung macrophages that may contribute to respiratory inflammation. Smoking cessation may have benefits in returning lipid metabolism to normal in these cells.

### 386 Effect of neutrophil supernatants on ex vivo small airway contractility in COPD

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Airway neutrophilia is a significant feature of COPD. Neutrophils release a variety of cytotoxic products (e.g. proteases) that degrade components of extracellular matrix. This may result in destruction of the lung and impendence of airflow characteristic of COPD. Small airways are the main site of airflow obstruction in COPD. Our aim was to study the effects of products from activated neutrophils on the contractility of small airways. Rat precision cut slices (PCS) were obtained and videomicroscopy used to assess contractility of small airways. Neutrophils were isolated from whole blood from non-smokers, smokers and COPD patients, stimulated with fMLP (100 nM) and supernatants collected. PCS were incubated overnight in neutrophil supernatants, and the contractility of small airways assessed by addition of increasing concentrations of carbachol. PCS incubated in supernatants from COPD patients caused a significant leftward shift in EC50 compared with untreated controls (13±2 vs 34±8μM, n=6, p<0.01). Conversely, PCS incubated in supernatants from smokers had no effect on EC50, but significantly reduced maximal contraction compared with untreated controls (65±16% vs 88±3%, n=4, p<0.05). PCS incubated in supernatants from non-smokers showed small but significant reductions in both EC50 (16±3 vs 32±8μM, n=6, p<0.05) and maximal contraction (79±4 vs 87±2%, n=6, p<0.05), compared with untreated controls. Elasticase content of supernatants correlated with maximal contraction, but not with EC50. We conclude that neutrophil supernatants from COPD patients increase the sensitivity of small airways to cholinergic stimulation, which may contribute to the airflow limitation characteristic of the disease.

### 387 Reduced phagocytosis of pathogenic bacteria by neutrophils from COPD patients

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Acute exacerbations of COPD are the commonest cause of acute medical admissions in the UK, with ~50% associated with bacterial infection. An acute bacterial insult is usually associated with increased recruitment and activation of neutrophils. COPD is characterized by airway neutrophilia; however, despite increased numbers of these cells, bacterial colonization persists. This study examined whether neutrophil phagocytosis was altered in COPD. Neutrophils were obtained from COPD, smoking and healthy subjects and phagocytosis of fluorescently-labelled polystyrene beads, *Haemophilus influenzae* (HI) or *Streptococcus pneumoniae* (SP) measured by flow cytometry. Neutrophils from all subjects ingested beads and the tissue inhibitor of metalloproteinase-1 (TIMP-1) were evaluated in patient sera. TIMP-1 concentration possibly depends on a feedback loop.

### 388 Short term effects of alpha-1-antitrypsin substitution therapy on the degradation of neutrophil granulocytes

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**Introduction:** Alpha-1-antitrypsin-deficiency (AATD) is a hereditary, co-dominant condition which results in low levels of circulating alpha-1-antitrypsin (A1AT), unbalanced protease activity and frequent development of emphysema. The concept behind substitution therapy with human plasma A1AT is to normalize the concentration in blood and tissue and thereby protecting the lungs from progressive destruction by proteases.

**Aims:** To better understand the influence of weekly augmentation therapy on polymorphonuclear neutrophils (PMNs) function we examined direct effects on chemokine release and degradation of matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO).

**Methods:** PMNs were isolated from peripheral blood of ten AATD-patients (PI-ZZ, mean age 55±8.7, FEV1[%] 38,1±14,33) pre and two hours post substitution infusion. The release of IL-8, MMP-9 and MPO was quantified in neutrophil supernatants via ELISA. Standard inflammation markers as well as MMP-9, MPO and the tissue inhibitor of metalloproteinase-1 (TIMP-1) were evaluated in patient sera.

**Results:** PMN-stimulation experiments displayed no differences on chemokine release or degradation between pre and post substitution infusion. Two hours after substitution we measured increased MMP-9 (p=0.002) and MPO (p=0.0034) concentrations in sera of patients, whereas TIMP-1 decreased drastically (p=0.002). IL-8 levels in sera of these patients were within normal range and no changes were detectable due to substitution.

**Conclusions:** We conclude that elevated MMP-9 and MPO concentrations after substitution depend on rapid degradation of PMNs whereas the decline in TIMP-1 concentration possibly depends on a feedback loop.