47. Macrophages and neutrophils in chronic lung disease

211 COPD lung contains a sub-population of steroid-insensitive macrophages
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In COPD, alveolar macrophages (AM) increase, release more inflammatory mediators but respond poorly to glucocorticosteroids. Different macrophage phenotypes are identified in animals based on density but no definite studies on human lung macrophages exist. Cells were isolated from resected human lung tissue from non-smokers (NS, n=5) smokers (S, n=11) and COPD (n=7) patients. Cells were separated into three viable fractions using Percoll density gradients (A: 30-40%, B: 40-50% C: 50-60%). Responses to budesonide after stimulation with lipopolysaccharide (LPS) were investigated by measuring TNFα, CXCL8 and IL-10 release by ELISA. Baseline and LPS-stimulated release of TNFα, CXCL8, and IL-10 did not differ between cell fractions or subjects. LPS-stimulated TNFα release by fraction A from NS and S were responsive to budesonide (EC50 NS: 0.5±0.04nM vs S: 1.8±1.1nM), with inhibition at 10⁻⁴M being ~80% (NS) and ~60% (S). However, COPD cells were unresponsive. Budesonide (10⁻⁷M) inhibited LPS-stimulated CXCL8 release from fraction A similarly in NS and S cells but less effectively in COPD cells (~30%, p<0.05) with EC50 values 0.6±0.1nM (NS), 1.0±0.3nM (S) and 2.2±0.9nM (COPD) cells. This apparent steroid insensitivity of COPD macrophages from fraction A was selective, as budesonide inhibited LPS-stimulated IL-10 release by ~55% in fractions A-C from S and COPD patients (EC50=2.0±0.71nM vs COPD 1.7±1.1nM) but by 80% (EC50=0.9±0.2nM) in NS cells. TNFα and CXCL8 responses of cells from fractions B and C did not differ between subjects. Fraction A COPD macrophages were less responsive to budesonide and may represent AM. Identifying selective fraction A markers will allow development of directed therapies.

212 LSC 2011 Abstract: Production of alpha-1 antitrypsin (AAT) by pro- and anti-inflammatory macrophages and dendritic cells
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AAT acts as an important neutrophil elastase inhibitor in the lung. Although the hepatocyte is considered as the primary source of AAT, local production by monocytes, macrophages and epithelial cells may contribute to the formation of an anti-elastase screen. Since monocytes can differentiate into a heterogeneous population of macrophages with subpopulations ranging from pro-inflammatory properties (M1) to anti-inflammatory properties (M2), and into dendritic cells (DC), we studied whether lipopolysaccharide (LPS), TNFα and oncostatin M (OSM) enhance AAT production differentially in cultured M1, M2 and DC. Monocytes from healthy blood donors were cultured for 7 days in the presence of GM-CSF, M-CSF or GM-CSF + IL-4 to obtain M1, M2 and immature (i) DC. Cells were isolated from resected human lung tissue from non-smokers (NS, n=5) smokers (S, n=11) and COPD (n=7) patients. Cells were separated into three viable fractions using Percoll density gradients (A: 30-40%, B: 40-50% C: 50-60%). Responses to budesonide after stimulation with lipopolysaccharide (LPS) were investigated by measuring TNFα, CXCL8 and IL-10 release by ELISA. Baseline and LPS-stimulated release of TNFα, CXCL8, and IL-10 did not differ between cell fractions or subjects. LPS-stimulated TNFα release by fraction A from NS and S were responsive to budesonide (EC50 NS: 0.5±0.04nM vs S: 1.8±1.1nM), with inhibition at 10⁻⁴M being ~80% (NS) and ~60% (S). However, COPD cells were unresponsive. Budesonide (10⁻⁷M) inhibited LPS-stimulated CXCL8 release from fraction A similarly in NS and S cells but less effectively in COPD cells (~30%, p<0.05) with EC50 values 0.6±0.1nM (NS), 1.0±0.3nM (S) and 2.2±0.9nM (COPD) cells. This apparent steroid insensitivity of COPD macrophages from fraction A was selective, as budesonide inhibited LPS-stimulated IL-10 release by ~55% in fractions A-C from S and COPD patients (EC50=2.0±0.71nM vs COPD 1.7±1.1nM) but by 80% (EC50=0.9±0.2nM) in NS cells. TNFα and CXCL8 responses of cells from fractions B and C did not differ between subjects. Fraction A COPD macrophages were less responsive to budesonide and may represent AM. Identifying selective fraction A markers will allow development of directed therapies.

213 M2 macrophages produce less type I and III IFNs upon human rhinovirus (HRV) infection and have a higher viral load than M1 macrophages
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We conclude that cultured M1 produce more AAT than M2 and DC, which is partly explained by a high spontaneous release of AAT by M1. This suggests that cellular differentiation is a regulator of local AAT production.
Abstract

**Title:** Inhibiting the JAK/STAT pathway diminishes the decrease in phagocytosis of HI and SP caused by IFNγ and decreases the release of inflammatory cytokines and has potential as a novel target in COPD.

**Aims:** To investigate the effect of IFNγ on human blood neutrophils and to determine whether inhibition of the JAK/STAT pathway improves phagocytosis.

**Methods:** Neutrophils were isolated from smokers with COPD (n=12), non-smoking healthy controls (n=12), and that TNFα that chemokine release by neutrophils is altered in COPD as compared to healthy controls.

**Results:**
- Inhibiting the JAK/STAT pathway improves phagocytosis of bacteria
- IFNγ increased bead phagocytosis through the ligation of CXCR1 and CXCR2
- These data indicate that ECM-derived Ac-PGP results in MMP-9 release from activated PMNs through the ligation of CXCR1 and CXCR2
- Ac-PGP released from type I collagen results in MMP-9 release from activated PMNs
- Inhibiting the JAK/STAT pathway reduces the release of MMP-9
- Ac-PGF generated increased MMP-9 when incubated with intact collagen; this effect was inhibited by ERK1/2 pathway inhibitor

**Conclusions:** Inhibiting the JAK/STAT pathway improves macrophage phagocytosis of bacteria in COPD and may offer new anti-inflammatory strategies in respiratory diseases, including chronic obstructive pulmonary disease (COPD).

**Background:** Previous studies have shown that Ac-PGP is a potent chemoattractant and pro-inflammatory cytokine that is increased in COPD patients compared to non-smoking controls (P<0.05). Inhibiting the JAK/STAT pathway diminishes the decrease in phagocytosis of HI and SP caused by IFNγ and decreases the release of inflammatory cytokines and has potential as a novel target in COPD.
Conclusion: mRNA levels of PDE4 A, B and D are increased in AM from COPD patients. Roflumilast inhibits TNFα production from AM from COPD patients.

218 Cigarette smoking augments toll-like receptor 3 expression and responses in macrophages

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Toll-like receptor 3 (TLR3), which reacts to viral-derived double-stranded RNA, is suggested to be involved in the immune responses during viral infection. However, the role of TLR3-mediated response in the pathophysiology of chronic obstructive pulmonary disease (COPD) is unclear. The expression of TLR3 in alveolar macrophages in human lung tissues was analyzed by immunohistochemistry. Furthermore, the effect of cigarette smoke on the expression and responses of TLR3 in macrophage lineage cells was examined. TLR3-positive alveolar macrophages were significantly increased in smokers and COPD subjects compared with non-smoker control subjects, but there was no difference between smokers and COPD subjects. The values of TLR3-positive macrophages were positively correlated with the smoking history and negatively correlated with the values of corrected carbon monoxide diffusing capacity by alveolar ventilation (DLCO/VA) (p < 0.001, r = -0.56), but not with the values of forced expiratory volume in 1 second (FEV1)% of predicted. Furthermore, cigarette smoke extract potentiated the expression of TLR3 in monocyte-derived macrophages and significantly augmented the release of interleukin-8 (CXCL8) and total matrix metalloprotease-9 activity in TLR3 ligand-treated cells. These data suggest that cigarette smoking potentiates the expression and responses of TLR3 in alveolar macrophages, which might affect the pathogenesis of COPD as well as its exacerbation.