47. Macrophages and neutrophils in chronic lung disease

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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 definitive 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 (EC_{50} NS: 0.5±0.04nM *vs* S: 1.8±1.1nM), with inhibition at 10^{-6}M being ~80% (NS) and ~60% (S). However, COPD cells were unresponsive. Budesonide (10^{-6}M) 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 EC_{50} values 0.6±0.1nM (NS), 1.0±0.3nM (S) and 2.4±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 (EC_{50} S=2.0±0.71nM *vs* COPD 1.7±1.16nM) but by 80% (EC_{50}=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.

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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, respectively. Next, cells were stimulated with LPS, TNFα or OSM and synthesis of AAT was assessed by quantitative RT-PCR and ELISA.
Spontaneous release of AAT was higher in M1 than in M2 and iDC after 24h (187 ng/10^6cells vs 50 ng/10^6c and 86 ng/10^6c, p=0.016). LPS significantly increased AAT production in M1, M2 and DC (302 ng/10^6c vs 97 ng/10^6c and 248 ng/10^6c, p=0.019), whereas TNFα and OSM did not affect AAT secretion. The secretion levels of the related proteinase inhibitor alpha-1 antichymotrypsin (ACT) were below the limits of detection by ELISA. Analysis by quantitative RT-PCR showed that 24h LPS exposure caused a maximal 2.1-fold AAT mRNA increase in M1, a 21-fold increase in M2 and 11-fold increase in DC.
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.

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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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Background: Macrophages (MΦ) are innate immune cells that play a key role in lung antiviral immune responses. A model of two major MΦ types has been developed: M1 (classically activated) and M2 (alternatively activated) MΦ. M2 MΦ are believed to be increased in asthma.
Objective: We hypothesized that upon HRV infection, M2 MΦ produce less type I and III interferon (IFN) and consequently have a higher viral load than M1 and un-polarized MΦ.
Methods: Monocyte-derived MΦ (MDM) obtained from peripheral blood mononuclear cells of healthy donors were polarized to M1 or M2 by treatment with IFN-γ and TNF-α or IL-4, respectively. Un-polarized MDM (uMDM) were treated with media alone. M1, M2 and uMDM were infected with major group HRV16 at MOI 1 and cultured for 72h. Cytokines were measured in the supernatants by ELISA and gene expression in cell lysates by real-time PCR. Supernatants of M1, M2 and uMDM were titrated on Hela-Ohio cells to obtain the HRV16 titre.
Result: HRV16 infection of M1 MΦ induced increased levels IFN-α, β, λ1 and λ2/3 in comparison with HRV16 infection of M2 MΦ and uMDM (for genes – at 8h, 24h, $p<0.001$, for proteins – at 24h, 48h and 72h, $p<0.001$). Also, we found that M1 were characterised by more effective virus clearance compared to M2 and uMDM (at 24h, $p<0.001$).
Conclusion: Our data suggest that in response to HRV infection, M2 MΦ produce decreased levels of antiviral proteins such as IFN-α, β, λ1 and λ2/3. This may explain the enhanced susceptibility to viral infections in asthmatic patients.
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Chemokine release by neutrophils in COPD
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Background: Neutrophils are among the first cells to arrive at the site of injury and chemokines secreted by neutrophils affect the migration not only of other neutrophils but also of other inflammatory cells such as monocytes. It has also been reported that LPS-induced release of interleukin-8 (IL-8, CXCL-8) by neutrophils is amplified by neutrophil derived tumour necrosis factor (TNF)-α. We hypothesize that chemokine release by neutrophils is altered in COPD as compared to healthy controls and that TNF-α might be involved in this change.
Methods: Peripheral blood neutrophils isolated from smokers with COPD (n=12), smokers without COPD (n=12) and healthy non-smokers (n=12) were stimulated with LPS, TNF-α or organic dust. Anti TNF-α antibody (infliximab) was used to study the effect of neutrophil derived TNF-α. Release of CXCL-8, macrophage inflammatory protein-1 α (MIP-1α, CCL-3), monocyte chemotactic protein-1 (MCP-1, CCL-2) and TNF-α was measured.
Results: Neutrophils spontaneously release CXCL-8, CCL-2 and CCL-3. Inhibition of TNF-α reduced the spontaneous release of CXCL-8 and CCL-3. Stimulation with LPS and organic dust increased the release of CXCL-8 and CCL-3, an effect that was inhibited by infliximab. In the COPD group inhibition of TNF-α failed to significantly inhibit the release of LPS induced CXCL-8.
Conclusion: Neutrophil derived TNF-α contributed to the release of chemokines after stimulation with LPS and organic dust as the response was inhibited by infliximab. In the COPD group infliximab did not significantly inhibit the release of CXCL-8 suggesting that the role of TNF-α is somehow altered in COPD.

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A matrikine-mediated pathway of MMP-9 release from neutrophils
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Introduction: Our group has previously characterized N-acetyl-Pro-Gly-Pro (Ac-PGP) as an important neutrophil (PMN) chemoattractant generated through collagen degradation by matrix metalloproteinase-9 (MMP-9). MMP-9 is present in PMN granules but whether Ac-PGP regulates MMP-9 release from these cells is unknown.
Aims: The aim of this study was to determine if Ac-PGP can mediate MMP-9 release from PMNs and to highlight the pathways which regulate this response.
Methods: Primary human blood PMNs were pretreated with and without an ERK1/2 MAPK inhibitor (U0126) or the CXCR1/CXCR2 inhibitor Repertaxin and then incubated with Ac-PGP. The cell supernatants and lysates were analyzed for MMP-9 levels and intracellular pathway activation, respectively. In addition, after stimultating PMNs with C13, N15 labeled Ac-PGP, supernatants were incubated overnight with type I collagen to measure the ongoing generation of Ac-PGP.
Results: We found that Ac-PGP induced significant release of MMP-9 (in a dose- and time-dependent manner) and activated the ERK1/2 MAPK pathway. This MMP-9 release was attenuated by an inhibitor of ERK1/2 and upstream blockade of CXCR1/CXCR2 with Repertaxin led to decreased MMP-9 release and ERK 1/2 activation. Supernatants obtained from PMNs stimulated by C13 N15 labeled Ac-PGP generated increased endogenous Ac-PGP when incubated with intact collagen; this effect was inhibited by an ERK1/2 pathway inhibitor.
Conclusions: These data indicate that ECM-derived Ac-PGP results in MMP-9 release from activated PMNs through the ligation of CXCR1 and CXCR2 and subsequent activation of the ERK1/2 MAPK, demonstrating a new pathway of matrikine-mediated protease regulation and subsequent "feed-forward" Ac-PGP production.

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JAK/STAT inhibition improves macrophage phagocytosis of bacteria
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In COPD, the lower airways are colonised with bacteria that contribute to exacerbations. This may be due to defective macrophage phagocytosis. IFNγ is elevated in COPD and can suppress macrophage phagocytosis. IFNγ activates the JAK/STAT pathway, thus we hypothesise that inhibiting this pathway will ablate the IFNγ effect on macrophage phagocytosis.
Human lung macrophages were treated with two structurally distinct JAK/STAT inhibitors (PF95 & PF13, 10^{-4}-10^{-10}M), stimulated with 10ng/ml IFNγ for 18h. Phagocytosis was measured after 4h exposure to beads, *H. influenzae (HI)* or *S. pneumoniae (SP)*. CXCL10 and IL-6 release after IFNγ or IFNγ+TNFα (10ng/ml) stimulation for 24h was measured by ELISA n=5. Cell viability was assessed by MTT.
IFNγ increased bead phagocytosis by 30±6% which was not altered by PF95 or PF13. However IFNγ significantly inhibited uptake of *HI* by 24±9% and *SP* by 29±9%. Both PF95 and PF13 reversed these effects in a concentration-dependent manner [Table 1]. PF13, but not PF95, was cytotoxic at concentrations ≥10μM. Both PF95 and PF13 decreased CXCL10 release after IFNγ stimulation (EC_{50} 0.31±0.17μM & 15±9.5nM respectively) and IFNγ+TNFα (EC_{50} 0.57±0.2μM & 19±8.2nM respectively). Only PF13 decreased IL-6 after IFNγ or IFNγ+TNFα stimulation (EC_{50} 0.45±0.72μM and 2.3±2.1μM respectively).

Table 1. Effect of JAK/STAT inhibitors on IFNγ suppression of bacterial phagocytosis

Drug		HI	SP
PF95	EC_{50} μM	0.27±0.26	0.21±0.19
	% increase	39±16	62±15
PF13	EC_{50} nM	2.5±2.4	12±15
	% increase	36±17	37±12

Date are mean±SEM, n=5-6

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.

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Increased expression of phosphodiesterase 4 (PDE4) A, B and D in alveolar macrophages from chronic obstructive pulmonary disease (COPD) patients
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Background: Phosphodiesterase-4 (PDE4) inhibitors, such as roflumilast, may offer novel anti-inflammatory strategies in respiratory diseases, including chronic obstructive pulmonary disease (COPD). Although it is widely accepted that PDE4 is expressed in macrophages, there is a lack of knowledge regarding the expression levels of PDE4 subtypes in alveolar macrophages (AM) from COPD patients. We examined the mRNA levels of PDE4 A, B and D in AM from patients with COPD and the modulatory effects of roflumilast on LPS-evoked cytokine release.
Methods: mRNA levels of PDE4 A, B and D were analysed in AM from 11 COPD patients, 8 smokers and 8 non-smokers by real-time PCR. AM from 11 COPD patients were stimulated with LPS following preincubation with roflumilast. After 24h, the supernatants were analysed for TNFα, IL-8 and IL-6.
Results: Expression of PDE4, A, B and D, are significantly augmented in AM form COPD patients compared to non-smoking controls ($P<0.05$). Roflumilast significantly ($P<0.01$) reduced LPS induced TNFα production in a concentration-dependent fashion (0.05-5μM) while had no significant effect on IL-6 or IL-8 release.

Table 1

	NS	S	COPD
PDE4 A	1	1.4	1.8*#
PDE4 B	1	0.8	3.3***
PDE4 D	1	1.4	4.3**

Relative expression of PDE4 A, B and D in AM from 8 non-smokers (NS), 8 smokers (S) and 11 COPD patients. Values are mRNA expression as a ratio of NS expression. Target genes normalised to GAPDH. Mann-Whitney Test: COPD compared to NS *$P<0.05$, **$P<0.01$, ***$P<0.001$, COPD compared to S #$P<0.05$.

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.

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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) ($p < 0.001$, $r_s = -0.56$), 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.