Macrophages in the Lung

• Host Defence
  Maintain sterility of the airways

• Clearance:
  Inhaled particles
  Bacterial pathogens
  Apoptotic cells
  - Phagocytosis
  - Efferocytosis
Macrophage Polarization

**M1**

- Membrane receptors
  - TLR2, TLR4
  - FcγRI, II, III
  - CD16, CD32, CD64
  - CD80, CD86, CD40

- Cytokines
  - IFNγ/LPS
  - TNF-α
  - IL-1
  - IL-6
  - IL-12

- Cytokine receptors
  - IL-1R type 1

- Chemokines
  - CXCL9, CXCL10, CXCL11
  - CCL2, CCL3, CCL4, CCL5
  - CXCL8

- Chemokine receptors
  - CCR7

- Effector molecules
  - NOS-2
  - ROI

**M2**

- Scavenger receptors A + B
  - CD163
  - Mannose receptor
  - CD14

- Cytokines
  - IL-4/IL-13
  - TGFβ, IL-10

- Cytokine receptors
  - Decoy IL-1R type II

- Chemokines
  - CCL-17, CCL-22
  - CCL-24
  - CCL-18
  - CCL-16

- Chemokine receptors
  - CCR2
  - CXCR1, CXCR2

- Effector molecules
  - arginase

Comparison of Differential Gene Regulation between Humans (MDM) and Mice (BMDM) (GM-CSF vs MCSF)


530 in same direction
M1/M2 Differential Gene Expression in Human MDM

Colour-Wheel of Macrophage Activation

- **M1**
- **M2a** Wound healing
- **M2b**
- **M2c** Tumour associated
- **Regulatory**

**Classically activated**
- CXCL8
- TNFα
- CD40

**Tumour associated**
- IL-10
- CD163
- CD206

Adapted from Mosser et al. 2008 Nat. Rev Immunol.
Different Types of Pulmonary Macrophages

Interstitial Mφ

8 μm

Alveolar Mφ

16 μm

Macrophage Phagocytosis
What types of macrophages in the lung?

- MDM, n=7
- AM, n=4
- TM, n=6

Fluorescent beads (x10^6/ml)

2 μm diameter

RFU x 10^3

Fluorescent beads (x10^6/ml)
## Changes in Lung Inflammatory Cells

<table>
<thead>
<tr>
<th>Inflammatory cells</th>
<th>Asthma</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CD3+</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>CD4+</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CD8+</td>
<td>2</td>
<td>8</td>
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<tr>
<td>Macrophages</td>
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<td>9</td>
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<tr>
<td>Neutrophils</td>
<td>-2</td>
<td>2</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>93</td>
<td>4</td>
</tr>
</tbody>
</table>

Fold change in number of cells vs healthy controls

Chronic Obstructive Pulmonary Disease

• COPD is an inflammatory respiratory disease

  Chronic bronchitis
  Small airway disease
  Emphysema

• Alveolar macrophages drive pulmonary inflammation in COPD

• Exacerbations of COPD are the major cause of acute hospitalizations

  In hospital mortality 8%, with 15% dead within 90 days
  • 1/3 readmitted in 30 days (1 in 10 die)
  • 20% survival at 5 years
Macrophages in COPD

Neutrophil

CXCL8

LTB4

CXCL1

CXCL9

CXCL10

CXCL11

CCL2

MMP-9

Cathepsins

Granzyme B

Perforin

CD8+

Emphysema

Bronchiolitis

Chronic bronchitis

Elastase

Neutrophil

Monocyte

Glucocorticosteroid Insensitive
COPD and Phagocytosis

**E. coli**
- NS
- S
- COPD

Phagocytosis index
- NS
- S
- COPD

**NTHI** (14P13H5)
- NS
- S
- COPD

% Phagocytosis
- NS
- COPD

Apoptotic cells
- NS
- COPD

Finney-Hayward et al., ATS (2005)
Berenson et al., J Infect Dis (2006)
Are COPD Macrophages a Distinct Population?

• More M1-like macrophages?
  ↓ phagocytosis, ↑ mediator release, ↓ CD163

• Fewer M2-like macrophages?
  ↓ phagocytosis, ↓ anti-inflammatory cytokines

• Different phenotype?
Macrophage Separation by Density

Human lung tissue

Percoll Gradient

10%
20%
30%
40%
50%
60%

X

Older

Younger
Release of Active MMP-9

**30-40%**
- Non-smokers n=5
- Smokers n=4-5
- COPD n=4-5

**40-50%**
- Non-smokers n=5
- Smokers n=4-5
- COPD n=4-5

**50-60%**
- Non-smokers n=5
- Smokers n=4-5
- COPD n=4-5
Cytokine Release

- No difference in basal release of TNFα, CXCL8 or IL-10 between cell fractions or subjects
- No difference in basal release of TNFα, CXCL8 or IL-10 between cell fractions or subjects
- Glucocorticosteroid effects?
Effect of budesonide on LPS-stimulated cytokine release (30-40%)
Effect of budesonide on LPS-stimulated cytokine release (40-50%)
Effect of budesonide on LPS-stimulated cytokine release (50-60%)
Flow Cytometry: Strategy

No difference in expression of CD40, CD163, CD206, CD16, CD14 between cell fractions
Flow Cytometry: Non-smokers

HLADR^+ >99%

CD14^+CD16^- >80%
CD14^-CD16^- CD163^- CD40^- >90%
CD163^- CD40^- >90%
CD14^+CD16^+ >75%
CD163^+ CD40^- >90%

All cells CD206 positive
Flow Cytometry: COPD

HLADR⁺

- 33%
  - CD14⁺CD16⁻
    - 85%
      - CD163⁻
      - CD163⁺
    - 75%
      - CD163⁻
      - CD163⁺
  - 40%
    - CD14⁻CD16⁻
    - 50%
      - CD163⁻
      - CD163⁺

- 26%
  - CD14⁺CD16⁺

> 99%

All cells CD40 negative

All cells CD206 positive
Cell Surface Marker Expression

- Significantly lower levels of expression of CD206, CD163 and CD40 in COPD cells

- Does local environment alter COPD macrophages phenotype?

- Is this due to different monocyte precursors?
Uptake of Inert Particles by MDM

No beads

0.2 µm

10 µm

30 µm
Effect of Uptake of Inert Particles by MDM on Phagocytosis

Non-smokers n=5  Smokers n=9  COPD n=10
Confocal Image of MDM treated with DEP (100µg/ml)
Effect of DEP on MDM Phagocytosis

Non-smokers n=13  Smokers n=16  COPD n=29
Effect of 0.2µm, 10µm and 30µm particles on MDM Cytokine Release

(30µm n=10, 10µm n=10, 0.2µm n=10)

Below Limit of Detection
Effect of DEP on MDM Mediator Release
(Non-Smokers n=11, Smokers n=12, COPD n=14)

DEP: TNFα and IL-6 NOT detectable
Summary

• Pulmonary macrophages are a mixed phenotype and in COPD this is functionally altered
  \[ \uparrow \text{MMP-9} \]
  Glucocorticosteroid insensitive
  \[ \downarrow \text{Cell surface marker expression} \]

• MDM models maybe useful

• Particle size alone does not alter macrophage function
Conclusions

• Evidence of pulmonary macrophage heterogeneity in non-smokers and disease

• Does not correlate with M1/M2 polarisation

• May derive from differing monocyte populations
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