Breathing in Trouble - When Particles Strike

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Background - IPF

- Pulmonary fibrosis affects millions of people worldwide\(^1,2\)
- Average life expectancy of 3-5 years after diagnosis for IPF\(^3\)
- Only 20% of patients survive 5 years after diagnosis\(^3\)
- An estimated 40,000 lives are lost each year in the U.S. from IPF\(^1,4\)
- Lung macrophages have a decisive role in fibrotic remodeling of the injured lung
  - Monocyte-derived macrophages contribute to the pathogenesis of pulmonary fibrosis\(^5-8\)

\(^7\)He et al. (2019) JCI Insight.
Recruited MDMs are increased in IPF lung

MDM = monocyte-derived macrophages: CD11b+HLA-DR++CD206++CD169+CD163+

RAM = residential alveolar macrophages: CD11b+HLA-DR++CD206++CD169+CD163++

Larson-Casey et al. (2020) PLOS ONE.
Background - Acute Exacerbations of IPF

• Sudden acceleration of the disease that leads to a significant decline in lung function
• Up to 85% mortality rate during or immediately after AE-IPF\(^1\)
• Median survival after AE-IPF is ~3-4 months\(^2\)

AE-IPF may be triggered by:
  • Viral infection
  • Lung procedures/operations
  • Aspiration
  • Pulmonary toxicity from medication
  • Environment: air pollution

\(^1\)Song et al. (2011) Eur. Respir. J.
\(^2\)Kishaba et al. (2014) Lung.
Air Pollution & AE-IPF

- Over 120 million Americans live in communities where air pollution exceeds air quality standards\(^1\).
- Adverse effects of air pollution exposure are well-established in patients with COPD and asthma, however; the mechanism(s) that air pollution induces AE-IPF is not known\(^2,3\).
- Air pollution exposure is associated with increased rate of decline in lung function, number of AE, and mortality in IPF\(^4,5,6,7\).
- Increased PM\(_{2.5}\) and PM\(_{10}\) exposures are risk factors for mortality in patients with IPF\(^4,8\).
- PM\(_{2.5}\), PM\(_{10}\) and NO\(_2\) exposure are associated with accelerated lung function decline in IPF patients\(^5\).

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\(^3\) Fann et al. (2012) Risk Anal.
\(^6\) Tahara et al. (2021) Respir. Res.
\(^7\) Tomos et al. (2021) Environ. Health.
\(^8\) Johannson et al. (2014) Eur. Respir. J.
**PM$_{2.5}$ increases number of cells in BAL**

Saline or Bleomycin
1.75 U/kg

Daily PM$_{2.5}$ (i.t) injections at day 21

BAL at Day 28

Cell count/differential

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Larson-Casey *et al.* under review.
Monocyte/macrophage phenotype

- Monocyte/macrophage function varies depending on location and environment
- Monocyte/macrophages develop mixed phenotypes in complex pathological conditions
- In the IPF lung, macrophages polarize to an anti-inflammatory phenotype
- Monocyte/macrophage phenotype in PM$_{2.5}$-mediated AE-IPF is not known
PM$_{2.5}$ mediates anti-inflammatory & pro-inflammatory phenotype

Saline or Bleomycin 1.75 U/kg

Daily PM$_{2.5}$ (i.t) injections at day 21

BAL at Day 28

mRNA analysis

Saline or Bleomycin 1.75 U/kg Daily PM$_{2.5}$ (i.t) injections at day 21 BAL at Day 28 mRNA analysis

PM$_{2.5}$ mediates anti-inflammatory & pro-inflammatory phenotype

Saline or Bleomycin 1.75 U/kg Daily PM$_{2.5}$ (i.t) injections at day 21 BAL at Day 28 mRNA analysis

Saline or Bleomycin 1.75 U/kg Daily PM$_{2.5}$ (i.t) injections at day 21 BAL at Day 28 mRNA analysis

Larson-Casey et al. under review.
Exposure to PM$_{2.5}$ regulates recruitment of monocytes that display a mixed phenotype to promote fibrotic progression.
**PM$_{2.5}$ does not increase MDM recruitment**

- **Saline** or **Bleomycin**
- **PM$_{2.5}$**
- Flow cytometry

**Graph:**
- **Y-axis:** Macrophage ($\times 10^5$)
- **X-axis:** Saline, Bleo, Saline, Bleo, Vehicle, PM$_{2.5}$

**Legend:**
- **RAM** = residential alveolar macrophages: CD45$^+$CD11b$^+$/Ly6G$^-$/CD64$^+$Ly6C$^-$Siglec F$^\text{lo}$
- **MDM** = monocyte-derived macrophages: CD45$^+$CD11b$^+$/Ly6G$^-$/CD64$^+$Ly6C$^-$Siglec F$^\text{hi}$

**Procedure:**
- Daily PM$_{2.5}$ (i.t) injections at day 21
- BAL at Day 28
- Flow cytometry
**PM$_{2.5}$ increases CCL2 in BAL fluid**

- Saline or Bleomycin 1.75 U/kg
- Daily PM$_{2.5}$ (i.t) injections at day 21
- BAL at Day 28
- ELISA analysis on BAL fluid

**Graphs:**
- BAL Cells ($\times 10^6$) vs. Treatment
- CCL2 (pg/mg protein) vs. Treatment

Larson-Casey et al. under review.
Monocyte recruitment during tissue injury in mice

Ma et al. (2018) Transl Res.
PM$_{2.5}$ recruits Ly6C expressing monocytes

Saline or Bleomycin 1.75 U/kg

Daily PM$_{2.5}$ (i.t) injections at day 21

BAL at Day 28

Flow cytometry

Saline

Bleomycin

PM$_{2.5}$

Vehicle

Ly6C$^{LO}$

Ly6C$^{HI}$

Monocytes (x 10$^5$)

Ly6C$^{high}$: CD45$^+$CD11b$^+$Ly6G$^-$CD64$^{+/−}$MHC II$^+$Siglec F$^+$Ly6C$^{hi}$

Ly6C$^{low}$: CD45$^+$CD11b$^+$Ly6G$^-$CD64$^{+/−}$MHC II$^+$Siglec F$^+$Ly6C$^{lo}$
PM$_{2.5}$ exposure recruits anti-inflammatory Ly6C$^{LO}$ monocytes & pro-inflammatory Ly6C$^{HI}$ monocytes

Saline or Bleomycin 1.75 U/kg

Daily PM$_{2.5}$ (i.t.) injections at day 21

BAL at Day 28

Flow cytometry

mRNA analysis

Larson-Casey et al. under review.
**PM$_{2.5}$ promotes epithelial and endothelial injury**

- Daily PM$_{2.5}$ (i.t.) injections at day 21
- BAL at Day 28
- Isolate lung tissue
- ELISA analysis on BAL fluid
- Confocal analysis

**Graph:**
- Albumin content (µg/mg protein) in BALF
- Statistical significance:
  - **P < 0.001**

**Images:**
- Confocal analysis of lung tissue with ZO-1 and DAPI staining
- Comparison between Saline and Bleomycin treatments with and without PM$_{2.5}$ exposure.
PM$_{2.5}$ mediates progression of pulmonary fibrosis

Saline or Bleomycin 1.75 U/kg
Daily PM$_{2.5}$ (i.t) injections at day 21
BAL at Day 28
Isolate lung tissue
Masson’s trichrome staining

Hydroxyproline (µg/mg dry weight)

Larson-Casey et al. under revision.
PM$_{2.5}$ mediates progression of pulmonary fibrosis

Saline or Bleomycin 1.75 U/kg
Daily PM$_{2.5}$ (i.t) injections at day 21
BAL at Day 28
Micro-CT imaging

Larson-Casey et al. under review.
Ly6C<sup>hi</sup> monocytes drive fibrotic progression

Bleomycin 1.75 U/kg

Ly6C<sup>hi</sup> monocyte adoptive transfer (i.t) at day 21

BAL at Day 28

Masson’s trichrome staining

Larson-Casey et al. under review.
Anti-CCR2 monoclonal antibody inhibits Ly6C^{HI} monocyte recruitment

Saline or Bleomycin 1.75 U/kg

Daily PM_{2.5} (i.t) injections at day 21

Daily anti-CCR2 (i.p) injections at day 23

BAL at Day 28

Flow cytometry

Larson-Casey et al. under review.
Anti-CCR2 treatment inhibits the pro-inflammatory phenotype

Saline or Bleomycin
1.75 U/kg

Daily PM$_{2.5}$ (i.t) injections at day 21

Daily anti-CCR2 (i.p) injections at day 23

BAL at Day 28

mRNA analysis

Anti-CCR2 treatment inhibits the pro-inflammatory phenotype.
Anti-CCR2 treatment inhibits PM$_{2.5}$ mediated fibrotic progression.

Larson-Casey et al. under review.
**Anti-CCR2 treatment improves lung function**

- **Saline** or **Bleomycin 1.75 U/kg** daily
- **Daily PM₂.₅ (i.t) injections** at day 21
- **Daily anti-CCR2 (i.p) injections** at day 23
- **BAL at Day 28**
- **mRNA analysis**

**Compliance (ml/cmH₂O)**

- **Saline** or **Bleomycin**
- **IgG** or **anti-CCR2**

**Graph**

- **Vehicle**
- **PM₂.₅**

Larson-Casey et al. under review.
Conclusion
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