Metabolic Adaptation of Macrophages as Mechanism of Defense against Crystalline Silica

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Silica

• Crystalline silica (Silicon Dioxide - SiO$_2$) is a common mineral found in the earth's crust.
• Materials like sand, stone, concrete, cement and mortar contain crystalline silica.
• 2.3 million workers might be exposed to respirable crystalline silica in the United States, in operation like cutting, sawing, grinding, drilling, and crushing stone.
• Workers exposed to respirable crystalline silica are at increased risk of developing:
  • Silicosis;
  • Tuberculosis;
  • Lung cancer.
Silicosis: Number of deaths, crude and age-adjusted death rates, U.S. residents age 15 and over, 1968–2014
Silicosis: Age-adjusted death rates by state, U.S. residents age 15 and over, 1996-2005
Pathogenesis of silicosis

The initial response to silica in the lung is mediated by the innate immunity, represented by macrophages.

Two-stage process:
1. Epithelial disruption, injury to the conducting epithelium (broncho-alveolar duct junction), and phagocytosis of silica particles by macrophages.
2. Oxidative stress, release of reactive oxygen species (ROS), and the transcription and release of inflammatory cytokines such as IL-1β, TNF-α, Interferons.
Silica induces macrophages activation.

The inflammatory response and subsequent development of pulmonary fibrosis after inhalation of silica is dependent on the NLRP3 inflammasome. (Cassel, 2008)

1. Cells exposed to silica alone without LPS priming do not develop inflammation (no IL-1β release).

2. NLRP3 activation does not explain the massive secretion of TNFα during silicosis.
Macrophages immune-metabolic response

- LPS-activated BMDM showed the ‘Warburg effect’ of aerobic glycolysis → decreased mitochondrial respiration and increased glycolysis, responsible for stabilization of HIF-1α and consequent secretion of IL-1β. Inhibition of glycolysis blocks IL-1β, but not TNFα release. (Tannahill, 2013)

- Succinate pro-inflammatory signal acting via SDH and Reverse Electron Transport in complex I in macrophages. (Mills, 2016)

- Remodeling of mitochondrial electron-transport chain (ETC) complexes (↓Complex I and ↑Complex II activity) in macrophages is required for optimal responses to bacterial infection. (Garaude, 2016)

- Silica nanoparticles induce metabolic reprogramming in RAW 264.7 macrophages → increased glycolytic activity, altered TCA cycle, reduced ATP generation, and increased TNFα production. (Saborano, 2017)
AIM:
To determine the immune-metabolic responses induced by silica in macrophages that lead to the secretion of cytokines and perpetuation of inflammation.
Crystalline silica and low-dose LPS enhance glycolysis, without affecting macrophage viability
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Silica remodels ETC-complexes activity.

A. Oxygen Flux stimulation with Succinate

B. Oxygen Flux with Rotenone-Succinate

C. Complex I activity

D. Complex II activity
Evolutionarily Conserved Signaling Intermediate In Toll Pathway (ECSIT) is a complex I–assembly factor.

- ECSIT deletion leads to a metabolic shift toward glycolysis in macrophages, accompanied by a complete loss of CI function, strong impairment of OXPHOS and an increase in baseline mROS production.
- ECSIT-deleted macrophages exhibit increased mitochondrial mass, suggesting a defect in the mitochondrial quality control pathway involving selective autophagy of damaged mitochondria, or mitophagy. (Carneiro et al, 2018)
Silica inhibits CI activity in part by reducing ECSIT expression.
Silica remodels ETC-complexes activity.
The importance of mitochondrial Complex II activity on macrophage survival in response to silica.
Silica and LPS exert similar effects on glucose uptake and glycolysis in macrophages

**Table G: Conversion Ratios**

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>LPS</th>
<th>SILICA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYRUVATE/HEXOSE</td>
<td>0.594</td>
<td>0.775</td>
<td>0.687</td>
</tr>
<tr>
<td>LACTATE/PYRUVATE</td>
<td>0.291</td>
<td>0.388</td>
<td>0.331</td>
</tr>
<tr>
<td>LACTATE/HEXOSE</td>
<td>0.173</td>
<td>0.301</td>
<td>0.227</td>
</tr>
<tr>
<td>SUCCINATE/GLUTAMATE</td>
<td>ND</td>
<td>0.594</td>
<td>ND</td>
</tr>
</tbody>
</table>
Silica and LPS differ in the effects on the TCA cycle in macrophages.
Silica and LPS differ in the effects on the TCA cycle in macrophages.
LPS, but not silica exposure, induces stabilization of HIF-1α, activation of NLRP3 inflammasome, and release of IL-1β.
Malonylation of GAPDH correlates with TNF-α production in LPS, but not in Silica-exposed macrophages.
Decreased itaconate levels correlate with decreased IFN-β in silica-exposed macrophages
Respirable, sterile, crystalline silica alone is capable of inducing an innate immune response without requiring previous macrophages activation by LPS.

Silica and LPS activates macrophages differently since they affect differently the Complex II of ETC.

Complex II plays a crucial role in macrophages survival and inflammatory response.
CONCLUSION

Exposure of macrophages to respirable silica alone is sufficient to stimulate an immune response sustained by a metabolic reprogramming of ETC: increased uptake of glucose and glycolysis and increased Complex II activity, are necessary for macrophages survival and for cytokine production associated with the pro-inflammatory phenotype (IL-1β, TNF-α and IFNβ).
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QUESTIONS?