



Skaggs School of Pharmacy
and Pharmaceutical Sciences

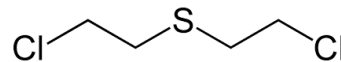
UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS

Role of Mast Cell Activation in Pulmonary Toxicity of Nitrogen Mustard

Jared Brown, PhD

Sulfur Mustard (SM)

- ▶ Chemical warfare agent (blistering or vesicating agent)
- ▶ Recently used in Syria and ISIS attacks
- ▶ Military personnel:
 - Over 50% exposed have respiratory effects
- ▶ Associated with Gulf War Illness /Chronic Multisymptom Disease



Army Apologizes to Troops Exposed to US-Designed Chemical Weapons in Iraq

Mar 30, 2015



US official: 'IS making and using chemical weapons in Iraq and Syria ...

<https://www.bbc.com/news/world-us-canada-34211838>

Sep 11, 2015 - There is a growing belief within the US government that the Islamic State militant group is making and using crude chemical weapons in Iraq ...

US says Syria making new chemical weapons despite 2013 deal

<https://www.apnews.com/.../US-says-Syria-making-new-chemical-weapons-despite-20...>

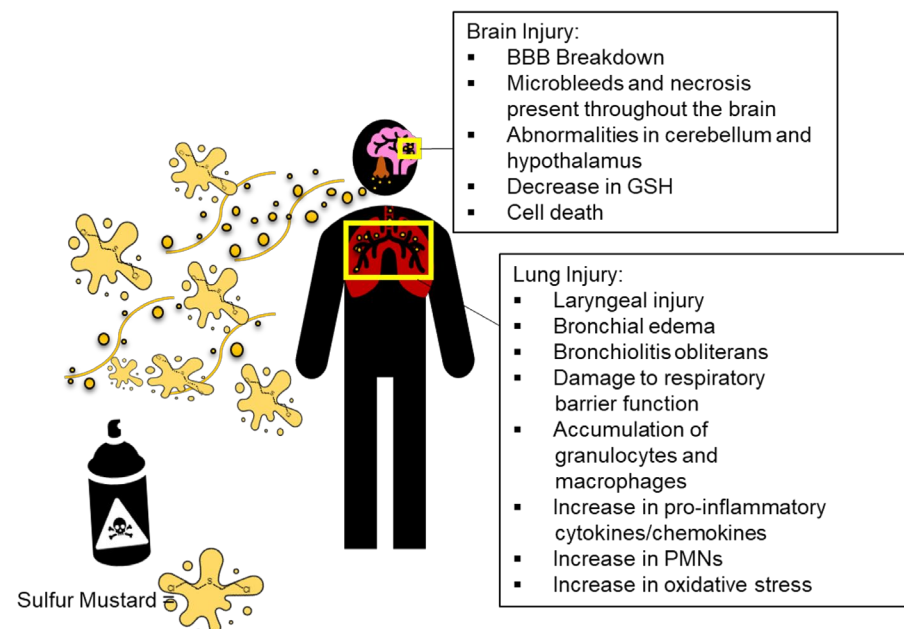
Feb 1, 2018 - US says Syria making new chemical weapons despite 2013 deal ... of Syria or Iraq, the officials said the extremist group continues to use sulfur ...

ISIS suspected of mustard attack against US and Iraqi troops

By Barbara Starr, CNN Pentagon Correspondent
Updated 12:26 PM ET, Tue September 27, 2016

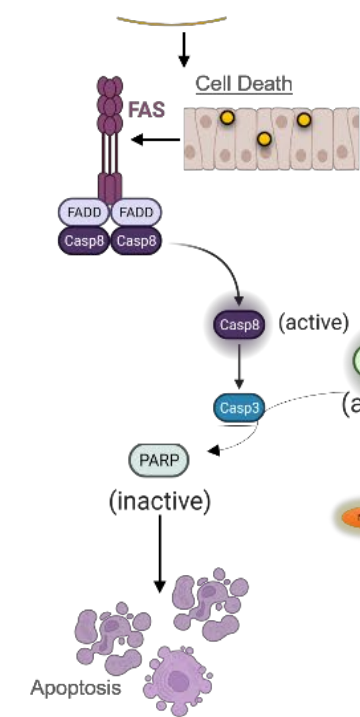


SM Inhalation Exposure

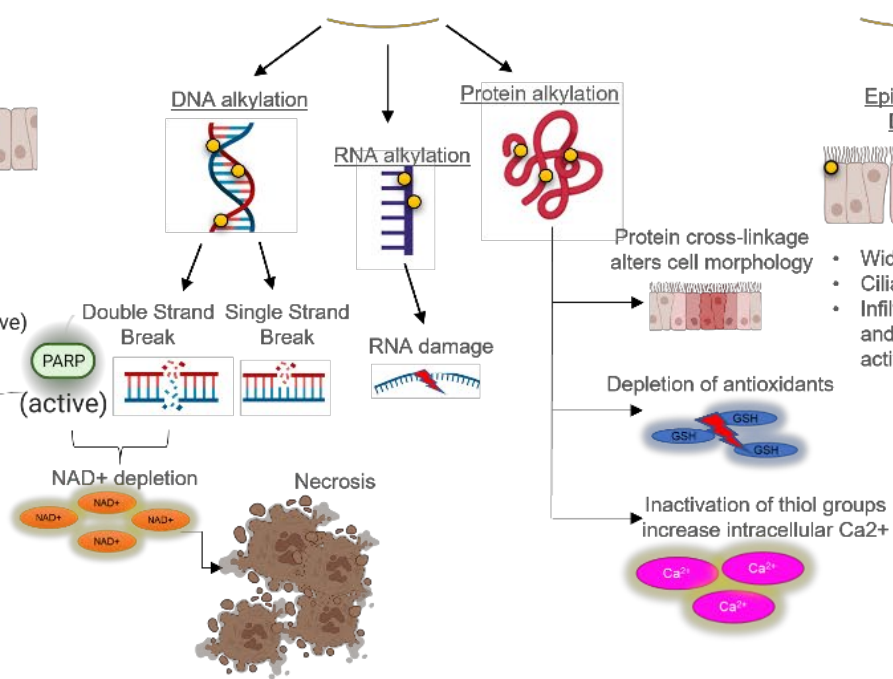


Proposed Mechanisms of Toxicity For Sulfur Mustard

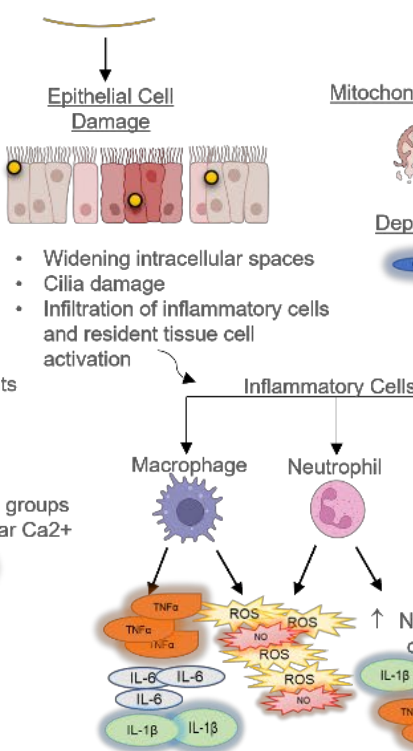
Programmed Cell Death



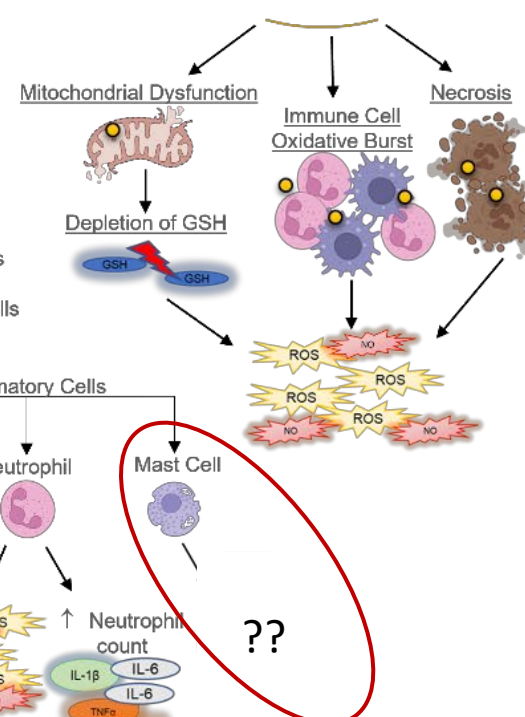
Alkylation Events



Inflammation

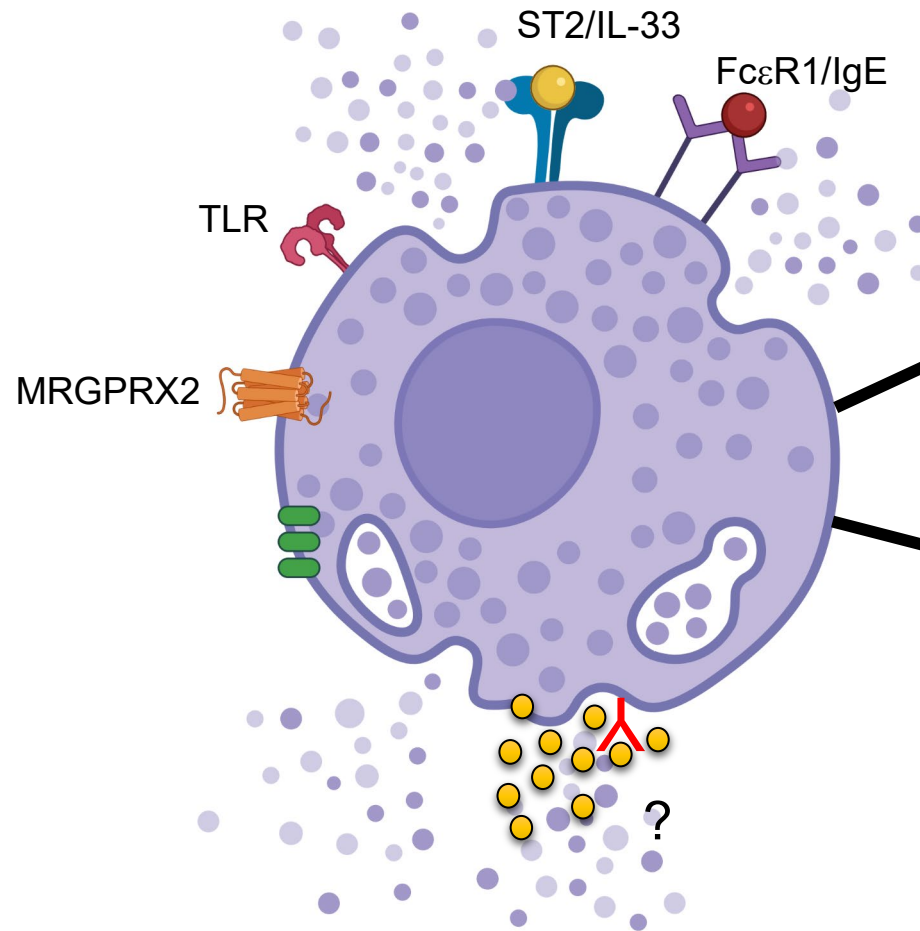


Oxidative Stress



Mast Cells

- ▶ Derived from stem cells in the bone marrow
- ▶ Resident tissue cell
 - Skin, lung, GI, eyes
- ▶ Activation
 - IgE – Allergic Responses
 - Non-IgE
 - SM unknown
- ▶ Recruitment and activation



Early Phase Activation:

Degranulation (seconds)

Lysosomal Enzymes and Proteases

B-hexosaminidase
 Trypsase
 Chymase
 Cathepsin B,C,D,E,L

Biogenic Amines

Histamine
 Serotonin

Cytokines and Growth Factors

TNF α
 IL-4,5,6

Late Phase Activation:

(minutes – hours)

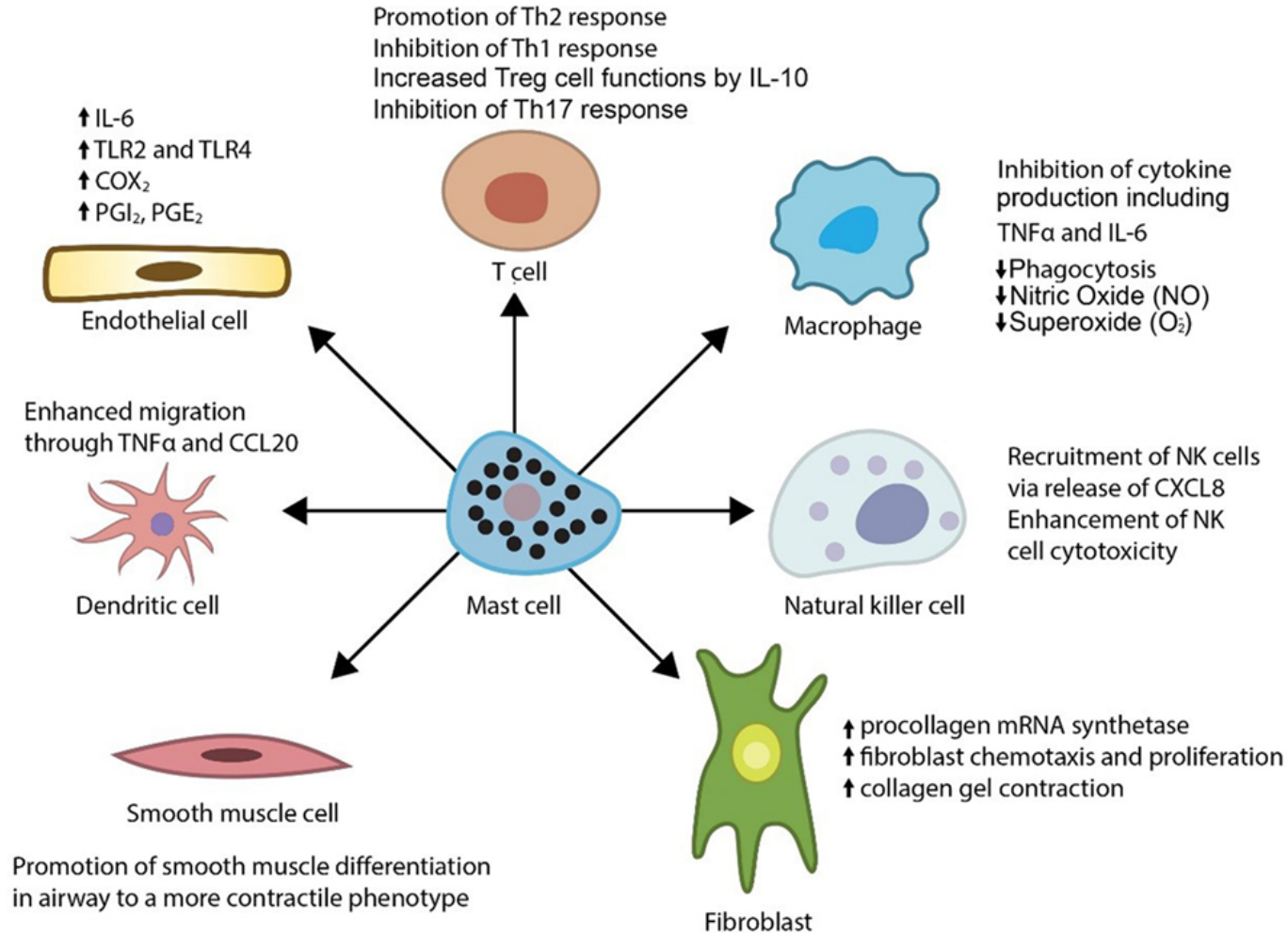
Eicosanoid Formation (De novo synthesis)

Leukotrienes (LTB₄, LTE₄)
 Prostaglandins (PGD₂, PGE₂)

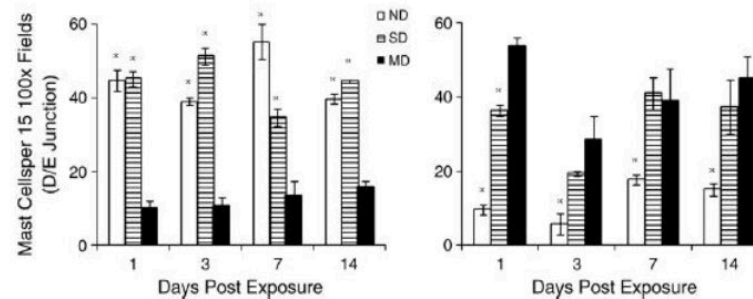
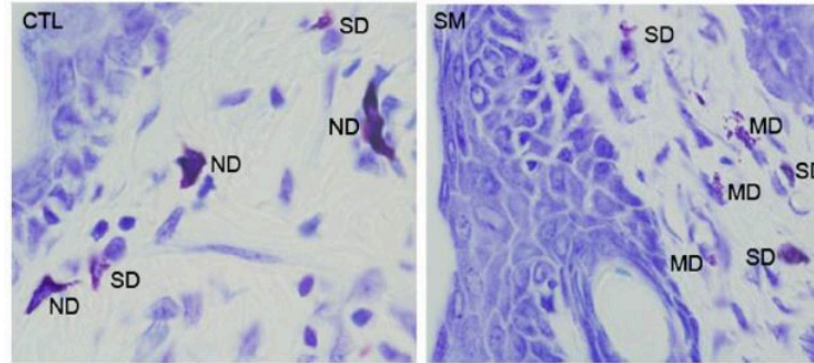
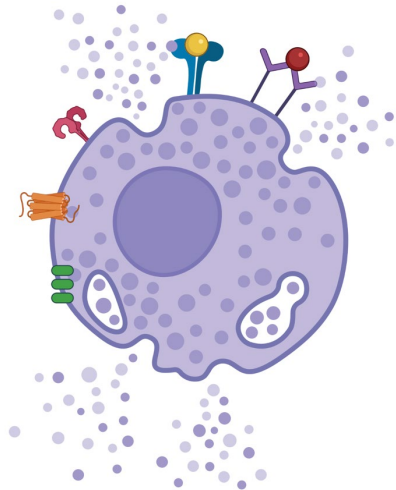
Cytokines, Chemokines, and Growth Factors

TNF α
 IL-4,5,6,13,17
 bFGF
 VEGF
 TGF β

The Importance Of Mast Cell Mediators

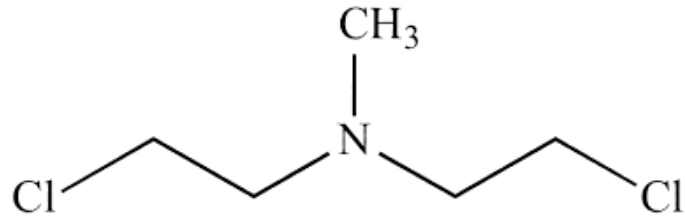


Rationale: Why the mast cell in SM exposure?



- ▶ SM exposure is reported to induce mast cell degranulation

Nitrogen Mustard (NM): A Surrogate for SM

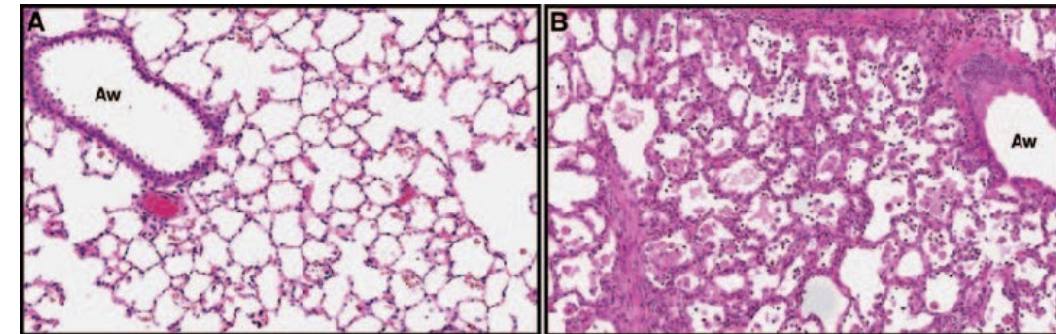


- ▶ NM is commonly used as a surrogate for SM
- ▶ DNA alkylation inducing damage
- ▶ Similar pathology & immune response in the lung
- ▶ Been used as a chemotherapeutic

Sulfur Mustard

Control

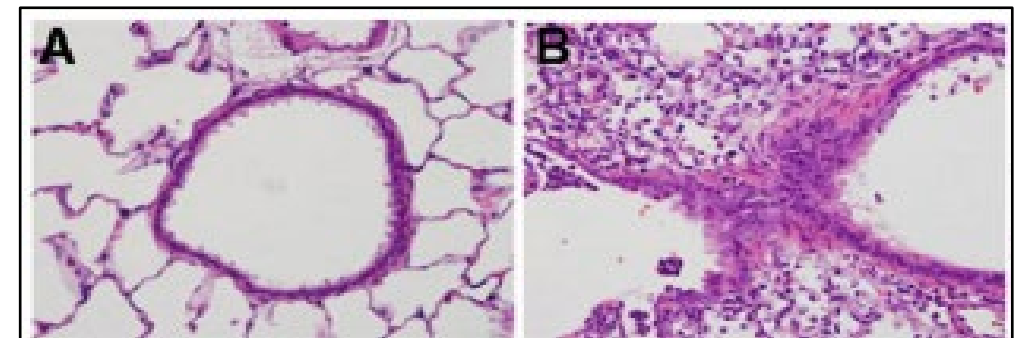
Treated



Nitrogen Mustard

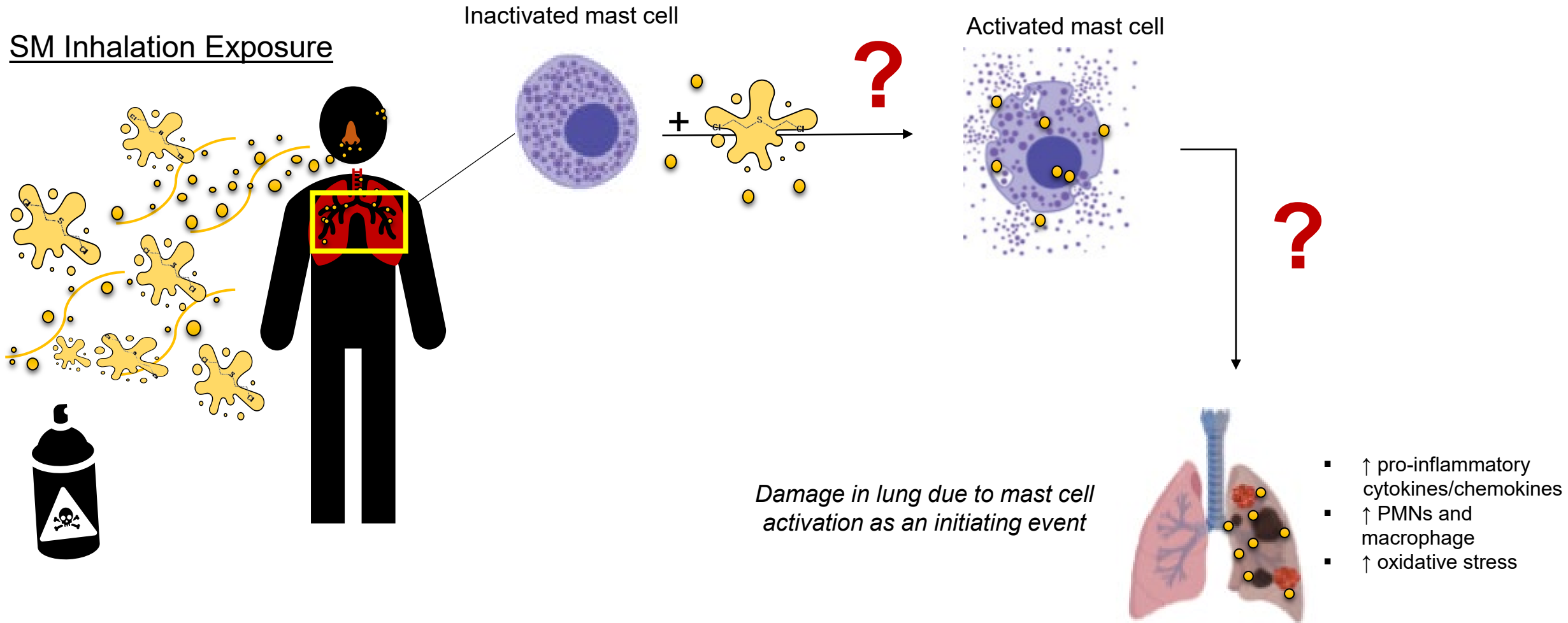
Control

Treated



Hypothesis

SM Inhalation Exposure



Acute NM Exposure: *In Vivo* Methodology

Mice Strains

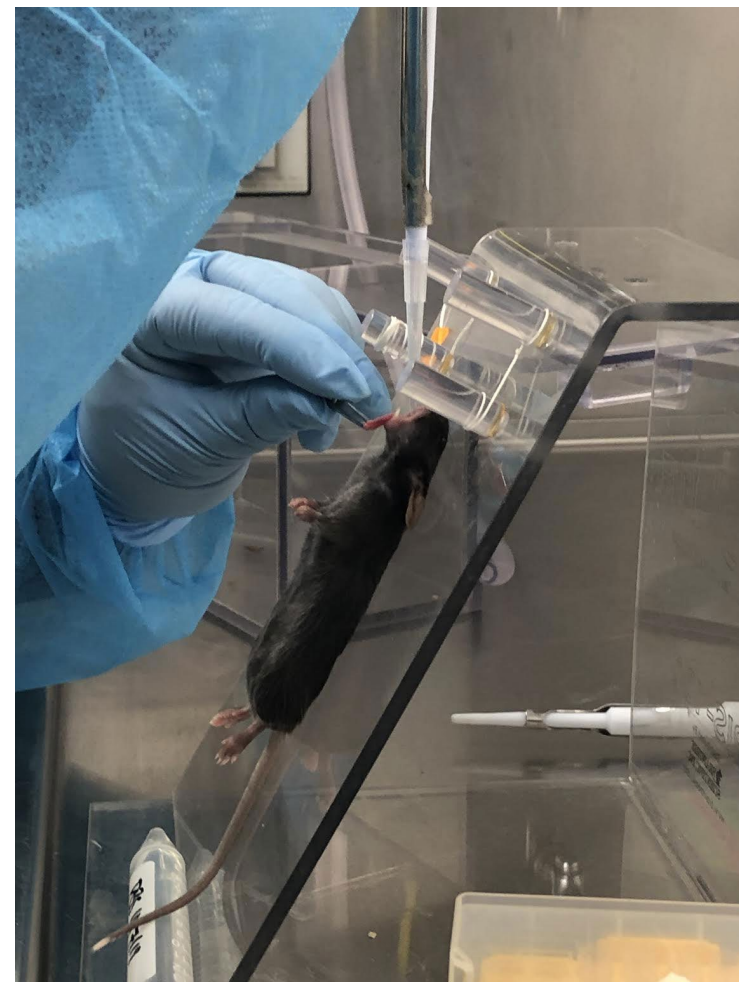
- Wild Type Mice (WT)
- Mast Cell Deficient Mice (MC Deficient)

Dosage

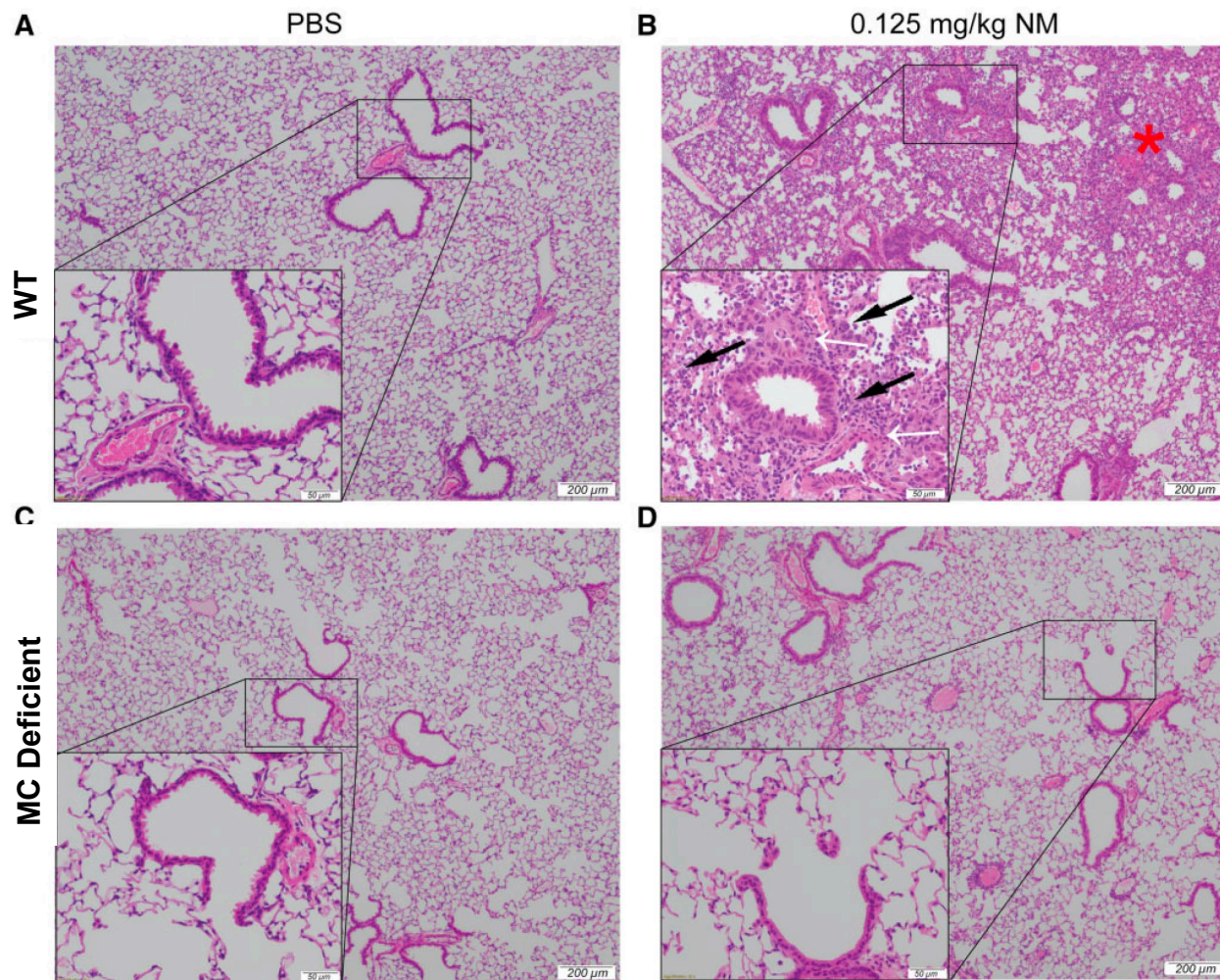
- Control: PBS
- Dose: 0.125mg/kg NM

Time point

- 24-72 h

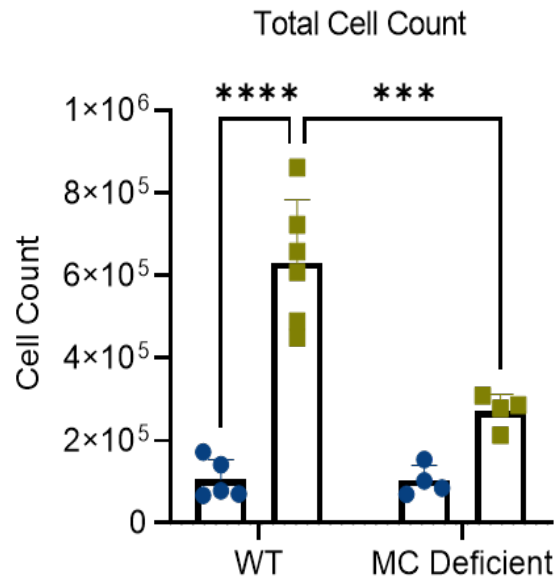


Pulmonary Damage Characterized by H&E

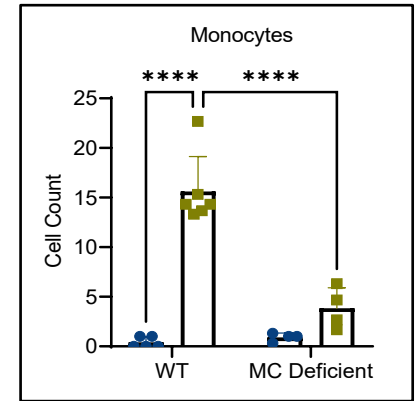
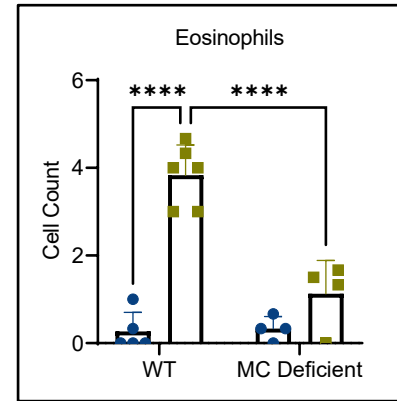
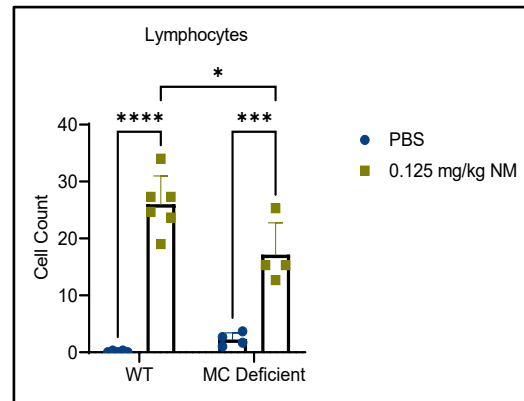
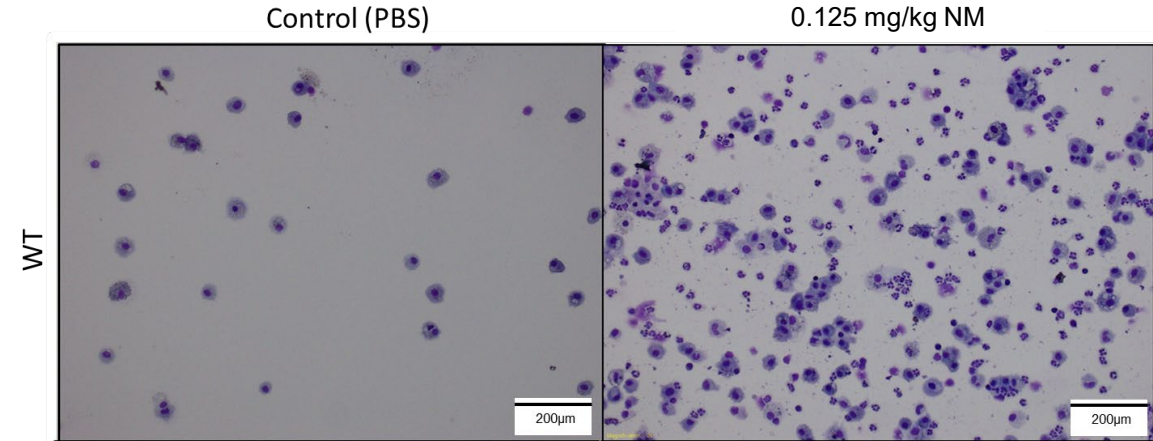


- ▶ *Significant lung injury is observed in wild type that is largely absent in mast cell deficient mice*

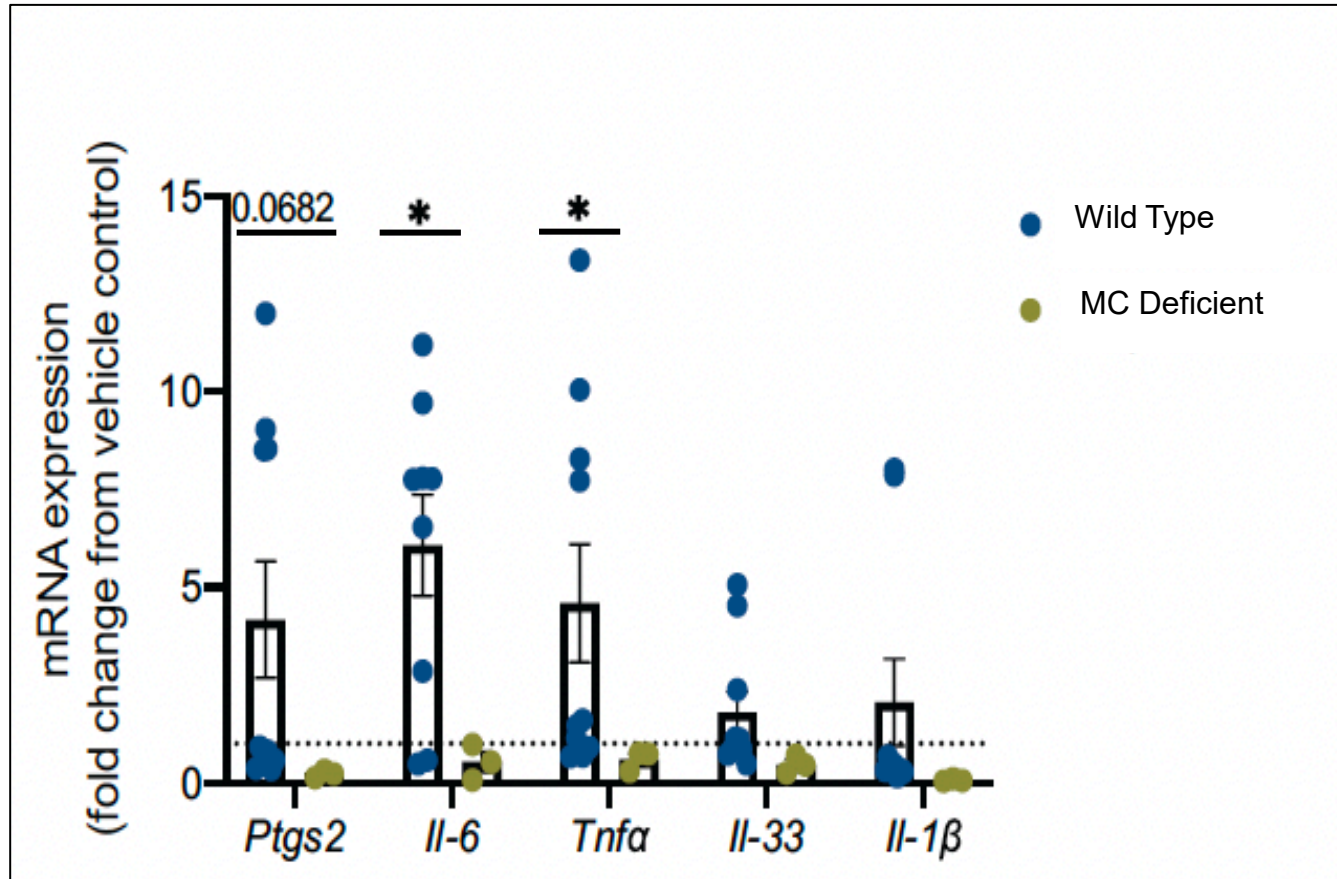
Characterization of Lung Lavage Fluid



● PBS
■ 0.125 mg/kg NM



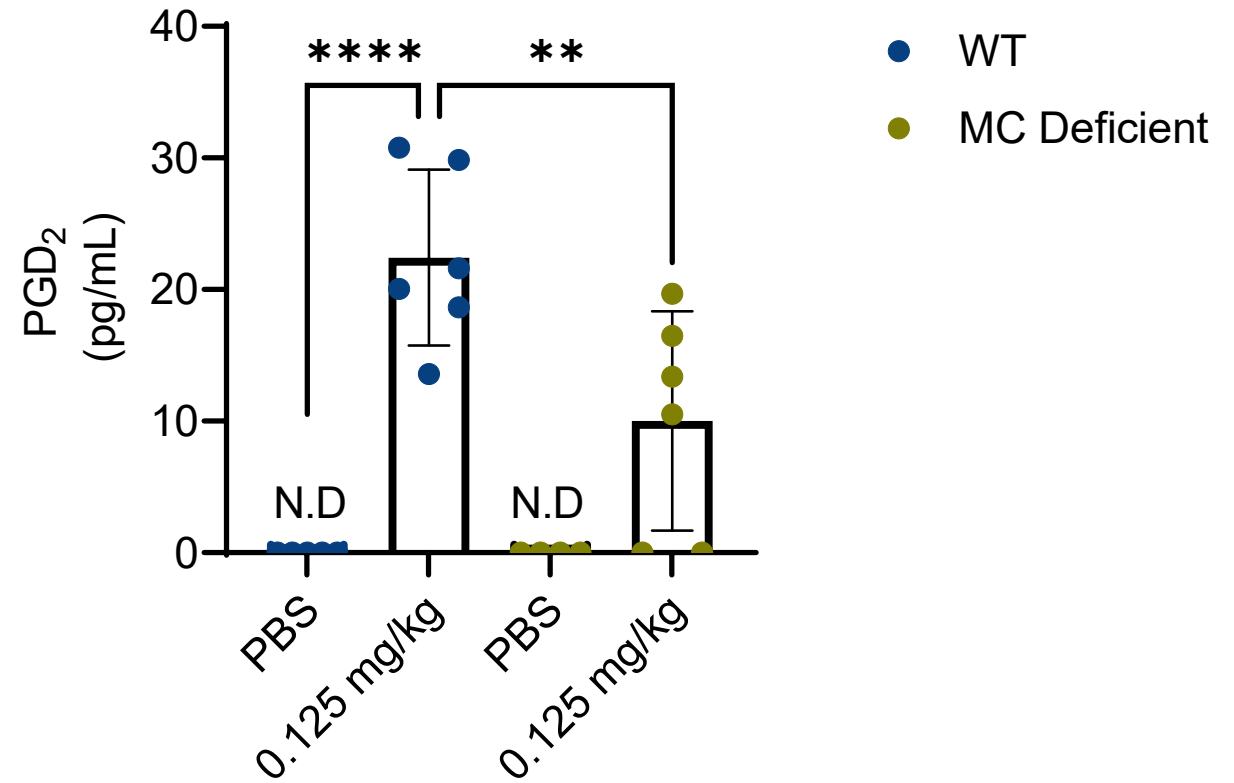
Proinflammatory Gene Expression



- ▶ *Persistent upregulation in ptgs2, il-6, and tnfa*
- ▶ *Macrophages and monocytes produce these but in the absence of mast cells upregulation in these are not observed*

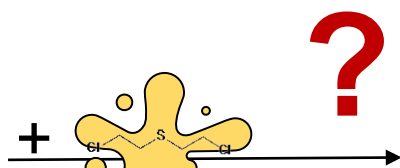
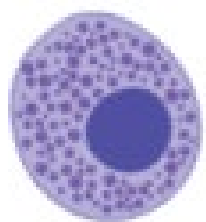
Prostaglandin D₂

- ▶ *In the lung, PGD₂ is primarily produced by activated mast cells, and to a smaller degree by a subset of T cells (T helper cell 2: Th2) and dendritic cells*
- ▶ *Crucial for the initiation and progression of lung inflammation*
 - *Bronchoconstriction and asthma pathogenesis*



Does NM Activate Mast Cells *In Vitro*?

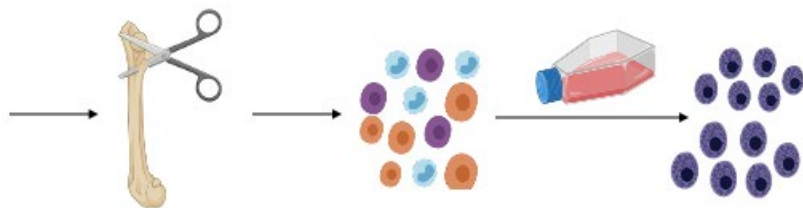
Inactivated mast cell



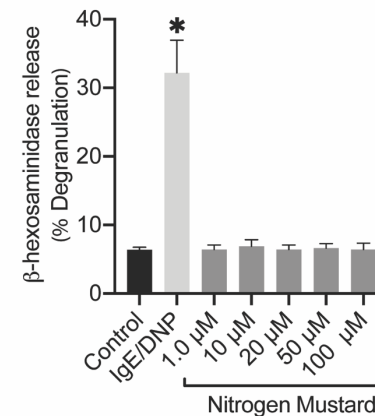
Activated mast cell



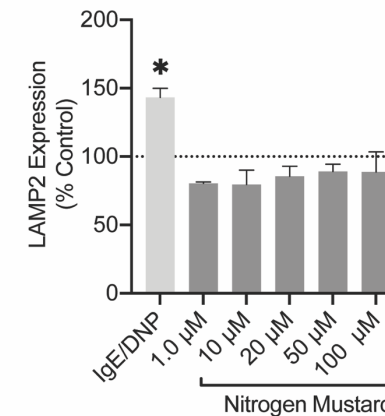
C57BL/6J



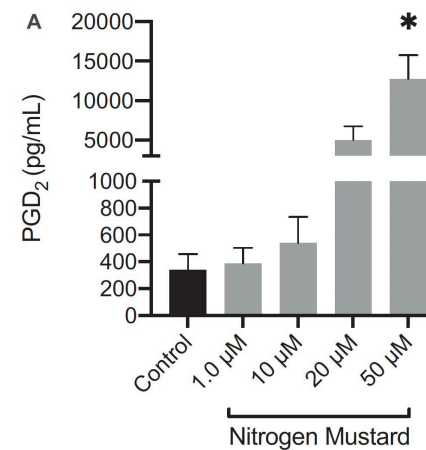
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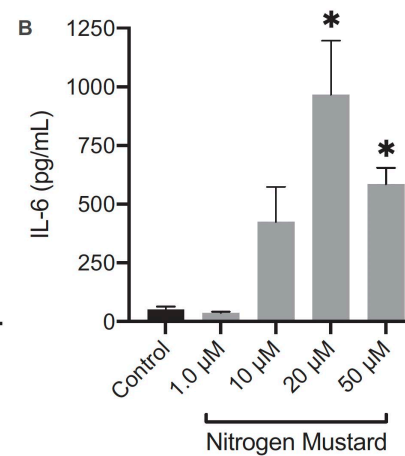
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