

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:

Skaggs School of Pharmacy

and Pharmaceutical Sciences

ANSCHUTZ MEDICAL CAMPUS

- 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 @wr 38.2015



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

G 💙 🚭

SM Inhalation Exposure









Biochemical and Cellular-Molecular Mechanisms of Injury From Mustard Gas; Ghanei et al., 2016 A Review of Chemical Warfare Agents Linked to Respiratory and Neurological Effects Experienced in GWI: Cruz-Hernandez et al., 2022

Mast Cells

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





Mast Cells and Influenza A Virus: Association with Allergic Responses and Beyond; AC Graham 2015 Mast Cell: A Multifunctional Mast Cell; Krystel-Whittemore et al., 2016

The Importance Of Mast Cell Mediators





Protective Role of Mast Cells in Primary Systemic Vasculitis: A Perspective; Springer et al., 2017

Rationale: Why the mast cell in SM exposure?





SM exposure is reported to induce mast cell degranulation



Inhalation of Sulfur Mustard Causes Long-term T Cell Dependent Inflammation: Possible role of Th17 cells in chronic lung pathology; Mishra et al., 2012 Sulfur mustard induced mast cell degranulation in mouse skin is inhibited by a novel anti-inflammatory and anticholinergic bifunctional prodrug; Joseph et al 2019

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









Functional and inflammatory alterations in the lung following exposure of rats to nitrogen mustard; Sunil et al., 2014 Chemical warfare agent and biological toxin-induced pulmonary toxicity: Could stem cells provide potential therapies?; Angelini et al., 2013



SM Inhalation Exposure





Activated mast cell

Damage in lung due to mast cell activation as an initiating event

- ↑ pro-inflammatory cytokines/chemokines
- ↑ PMNs and macrophage
- ↑ oxidative stress



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

Skaggs School of Pharmacy and Pharmaceutical Sciences UNIVERSITY OF COLCRADO ANSCHUTZ MEDICAL CAMPUS

Mast Cells Promote Nitrogen Mustard-Mediated Toxicity in the Lung Associated with Proinflammatory Cytokine and Bioactive Lipid Mediator Production; Cruz-Hernandez A. et al., 2021 Images were obtained using an Olympus light microscope at a 4x objective (indicated by 200 μ m scale) and a 20x objective (indicated by 50 μ m scale) (N=5-9/group).

Characterization of Lung Lavage Fluid



Total Cell Count

PBS
0.125 mg/kg NM





Skaggs School of Pharmacy and Pharmaceutical Sciences UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Mast Cells Promote Nitrogen Mustard-Mediated Toxicity in the Lung Associated with Proinflammatory Cytokine and Bioactive Lipid Mediator Production; Cruz-Hernandez A. et al., 2021 Data is presented as the mean \pm SD (n=4-6 animals/ group), compared to fold change from control (normalized to 1); Two - way ANOVA with Tukey's *post hoc* test, * $p \le 0.05$, ** $p \le 0.01$, **** $p \le 0.001$, **** $p \le 0.001$

Proinflammatory Gene Expression



Persistent upregulation in ptgs2, il-6, and tnfα

Macrophages and monocytes produce these but in the absence of mast cells upregulation in these are not observed

Skaggs School of Pharmacy and Pharmaceutical Sciences UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Mast Cells Promote Nitrogen Mustard-Mediated Toxicity in the Lung Associated with Proinflammatory Cytokine and Bioactive Lipid Mediator Production; Cruz-Hernandez A. et al., 2021

Data is presented as the mean ± SD (n=3-8 animals/ group), compared to fold change from control (normalized to 1); Two - way ANOVA with Tukey's post hoc test, *p \leq 0.05, ** p \leq 0.01, **** p \leq 0.001, **** p \leq 0.001

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



WTMC Deficient

Skaggs School of Pharmacy and Pharmaceutical Sciences Mast Cells Promote Nitrogen Mustard-Mediated Toxicity in the Lung Associated with Proinflammatory Cytokine and Bioactive Lipid Mediator Production; Cruz-Hernandez A. et al., 2021 Data is presented as the mean \pm SD (n=4-6 animals/ group); Two - way ANOVA with Tukey's post hoc test, *p \leq 0.05, ** p \leq 0.01, *** p \leq 0.001, **** p \leq 0.001

Does NM Activate Mast Cells In Vitro?







Acknowledgements

• Angela Cruz-Hernandez, PhD



Collaborators

- Alison Bauer, PhD
- Nichole Reisdorph, PhD

Funding Sources

Department of Defense: W81XWH-18-1-0169, DOD-CDMRP

NIEHS R01 ES019311

NIEHS T32 T32ES029074



National Institute of Environmental Health Sciences



