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TITLE: Mast Cells in Sulfur Mustard Exposure: Novel Targets for Modulation to Develop Therapies Against the Long-Term Health Effects in Gulf War Veterans

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**14. ABSTRACT**

**This Tier 1 Discovery award is focused on identifying a novel immune mechanism of Sulfur mustard [mustard gas; bis(2-chloroethyl) sulfide); SM] pathophysiological effects that could contribute to Gulf War Illness (GWI) mediated through mast cells. Mast cells are well known to contribute to allergic inflammatory diseases, but also have wide ranging effects on many physiological systems that are affected in GWI including pulmonary, dermal, gastrointestinal and nervous systems when activated (e.g. degranulation). Importantly, a role for mast cells has been suggested in the mechanism of vesicating chemical agents-induced inflammatory response, changes in immune parameters and tissue damage.**

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## 1. INTRODUCTION

This Tier 1 Discovery award is focused on identifying a novel immune mechanism of Sulfur mustard [mustard gas; bis(2-chloroethyl) sulfide); SM] pathophysiological effects that could contribute to Gulf War Illness (GWI) mediated through mast cells. Mast cells are well known to contribute to allergic inflammatory diseases, but also have wide ranging effects on many physiological systems that are affected in GWI including pulmonary, dermal, gastrointestinal and nervous systems when activated (e.g. degranulation). Importantly, a role for mast cells has been suggested in the mechanism of vesicating chemical agents-induced inflammatory response, changes in immune parameters and tissue damage. A key role of mast cells in GWI is further supported by reported changes in immune parameters in GW veterans, similar to SM exposure. Mast cell driven pathologies such as hypotension, edema, bronchoconstriction, and skin abnormalities as well as neuropathic pain and gastrointestinal symptoms parallel a majority of the health disorders observed in GWI where SM exposure alone or in combination to other environmental factors could play an important role. Also, there are clinical therapeutics targeted towards mast cells and their products that could be beneficial in GWI patients, therefore, we aim to uncover a novel mechanism of SM toxicity that is mediated by mast cells and which could also be applicable to other chemical exposures in GWI. For Tier 1 Discovery studies under this proposal to obtain pilot data, we will embark on understanding the role of mast cell-induced immune responses in vesicant inhalation and skin exposures using nitrogen mustard [NM; bis(2-chloroethyl) methylamine] as a surrogate for SM.
Overall, the proposed studies will have a strong potential to aid in understanding the mechanism of the inflammatory process and immune response following low exposures to mustard warfare agent SM and identification of applicable and effective markers for therapeutic approaches with a strong translational impact to treat some problems of GWI.

## 2. KEYWORDS

Gulf war illness, sulfur mustard, mast cells, pulmonary injury, dermal injury, mechanisms, inflammation, biomarkers, therapeutic approaches.

## 3. ACCOMPLISHMENTS

### i) Major project Goals and Objectives:

**Specific Aim 1. *In vitro* studies to elucidate mast cell-induced immune responses following mustard vesicating agent exposure.**

Under this aim, we will employ rodent mast cells (grown from femoral bone marrow of C57BL/6 mice with IL-3) and LUVA human mast cells to examine the effects of sulfur mustard (SM) surrogate nitrogen mustard (NM) on mast cell degranulation and cytokine production *in vitro*.
**Following NM exposures (**0.5h, 1h, 6h, and 24h)**, under Aim 1a we will examine following:**
NM-mediated mast cell degranulation by measuring β-hexosaminidase, histamine, serotonin and LAMP2 expression as published.

a) Effects of NM on IgE-mediated degranulation to determine if NM may exacerbate mast cell degranulation by an allergen. For these experiments, mast cells will be sensitized with IgE anti-2,4-dinitrophenol (DNP) overnight, exposed to NM and DNP (allergen), and followed by measurement of mast cell degranulation at 30 min.
b) Prostaglandin, leukotriene and cytokine production by mast cells exposed to NM will be measured using quantitative PCR and ELISAs.

**Under Aim 1b, we will examine the effectiveness of several mast cell inhibitors in preventing NM-mediated degranulation:**

a) Cromolyn sodium, a mast cell stabilizer drug currently used for severe cases of asthma and
b) BLT-2 (2-(2-butoxyethyl)-1-cyclopentanone thiosemicarbazone), a small molecule inhibitor of scavenger receptor class B1 that inhibits mast cell degranulation to several toxicants such as silver nanoparticles and crystalline silica

**Specific Aim 2. *In vivo* studies to confirm the role of mast cells in mustard-induced inflammation.**

For studies under this aim, we will use mast cell deficient (B6.Cg-KitW-sh/HNihrJaeBsmJ) and wild-type (C57BL/6) mice to confirm a role for mast cells in NM-induced lung pathology, NM dermal pathology and systemic effects related to immune system activation.

**ii) Accomplishments under these goals: Major Activities, Methods and Results**

1. Aim 1-In vitro Studies: most of the studies have been carried out in mast cells from mouse

Methods

*Primary Mast Cells; Bone marrow-derived mast cells (BMMC) isolation:* BMMCs were isolated as hematopoietic progenitor cells from femurs of C57BL/6 mice. Cells were cultured for 4-6 weeks in supplemented media containing IL-3 for mast cell differentiation. Maturity of BMMCs was tested by measuring expression of surface receptors c-KIT and FcεR1.

*Cell Culture:* Cells were cultured in RPMI 1640 medium (Corning, Manassas, VA), supplemented with 10% FBS (Corning, Manassas, VA), 25 mM HEPES (Corning, Manassas, VA), 1.0 mM sodium pyruvate (Sigma–Aldrich, St. Louis, MO), 1.0 mM non-essential amino acids (Sigma–Aldrich, St. Louis, MO), 100 U/ml penicillin, 100 µg/ml streptomycin (Life Technologies, Carlsbad, CA), 100 µg/ ml Primocin$^{TM}$ (Invitrogen, San Diego, CA), 0.0035% β-mercaptoethanol and 30 ng/ml recombinant murine IL-3 (Biolegend, San Diego, CA).

*BMMC Maturity Assay:* Maturity was tested at 4 weeks. $5x10^4$ cells are transferred to 3 separate tubes for not treated, isotype control, and antibody stain (c-KIT and FcεR1). Control only contained stain buffer, isotype control contained Rat IgG2b kappa isotype control (APC) and Armenian Hamster IgG isotype control (PE) diluted 1:200 (Invitrogen, San Diego, CA) and antibody stain contained anti-Mo CD117 (c-kit) (APC) and anti-Mo FcεR1alpha (PE) diluted 1:200 (Invitrogen, San Diego, CA). After incubation, signal was read on flow cytometer (BD AccuriTM C6, BD Biosciences, San Jose, CA).

*Viability Assay:* To assess cell viability, annexin V (BD Biosciences, San, Jose, CA)/ propidium iodide (PI) (Invitrogen, San Diego, CA) stain was used. BMMCs were plated at 1 x $10^5$ cells well and then exposed to NM at varying concentrations (1 µM - 50 µM) for 6 and 24 hrs. Hydrogen peroxide was used as positive control (10 µM for 1 hr). After exposure, cells were

washed 2x with PBS and resuspended in propidium iodide (PI)-containing stain buffer at a final concentration of 3 μM. 2.0 μL of Annexin-V was added directly to each sample and gently vortexed. Samples were incubated in the dark at room temperature for 15 minutes before signal was measured on BD Accuri flow cytometer.

*Degranulation Assay:* Mast cell degranulation was measured by the release of β-hexosaminidase. Cells were plated in HEPES biological buffer at 5 x $10^4$ cells per well in a 96-well plate. Cells were exposed to NM, at varying concentrations (1 μM - 50 μM) for 1, 6 and 24 hr time points for BMMCs. A subset of mast cells were sensitized with IgE anti-dinitrophenylated (anti- DNP) (100 ng/ml) (Sigma–Aldrich, St. Louis, MO) for a minimum of 2 hrs followed by addition of DNP human serum albumin (DNP-HSA) 100 ng/ml) (Sigma–Aldrich, St. Louis, MO) to generate a positive control IgE pathway mediated response. After exposures, the plate was centrifuged at 300 g for 5 min to pellet cells. 50 μL of supernatant was removed without disturbing cell pellet and was transferred to new 96-well plate. 150 μL of Triton-X was added to plate with cell pellet to lyse cells. 50 μL of this cell lysate was transferred into new 96-well plate. 100 μL of p-nitrophenyl-N-acetyl-b-D-glucopyranoside (PNAG) (Sigma–Aldrich, St. Louis, MO), a chromogenic substrate, was added to supernatant and lysed cells and incubated at 37°C for 90 min. To stop the reaction, 100 μL of glycine was added to all wells and optical density was read at 405 nm on uv vis spectrophotometer (Biotek Synergy HT microplate reader, Agilent, Winooski, VT). Percentage of degranulation was calculated as follows: [(supernatant×2)/(lysate×4)]×100.

*Cytokine release by enzyme-linked immunosorbent assay (ELISA) in vitro:* IL-6 was measured in BMMCs. Cells were plated in 24 well plate at 1x $10^6$ cells/ well and then exposed to NM at varying concentrations for 24 hrs. A subset of mast cells were sensitized with IgE anti-dinitrophenylated (anti- DNP) (100 ng/ml) (Sigma–Aldrich, St. Louis, MO) for a minimum of 2 hrs followed by addition of DNP human serum albumin (DNP-HSA) 100 ng/ml) (Sigma–Aldrich, St. Louis, MO) to generate a positive control IgE pathway mediated response. After exposure, cells were centrifuged at 800 *g* at 4°C for 5 min and supernatants were collected and stored at -80°C until further processing. Cytokine release was measured with DuoSet® ELISA Development Systems (R&D Systems, Minneapolis, MN) according to the manufacturer instructions for IL-6.

*mRNA production in vivo and in vitro:* Gene expression was measured through the amplification of mRNA transcripts using real time PCR. Cells were plated in a 24 well plate at 1x $10^6$ cells/ well and then exposed to NM at varying concentrations for 1 and 6 hrs. Post exposure cells were centrifuged and supernatants were discarded. Pellet was reconstituted in 400uL of TRI reagent and then RNA was isolated using the Direct-20L™ RNA MiniPrep kit. mRNA was then transcribed into cDNA using an iScript cDNA Synthesis kit and thermocylcer. SYBR green supermix and primers: COX-2, TNFα, IL-6, and IL-1β (Qiagen, Germantown, MD) were added to cDNA and real time PCR was performed. Gene expression was quantified using $\Delta\Delta C_T$ method, relative to GAPDH (housekeeping gene). For animal tissues 20-30 mg was removed from lung was homogenized using Fast prep 5G homogenizer in 700 μL of TRI reagent. Supernatant was collected and processed for read time PCR as described above.

*Statistical analysis:* Data are presented as mean ± standard error mean (SEM). One-way ANOVA with Dunnett's and Turkey's post-hoc testing was utilized to test for significant

differences between multiple treatment groups. Significant differences are at $p<0.05$. Each flask of cells pooled from two separate mice was considered a biological replicate (N).

## Results

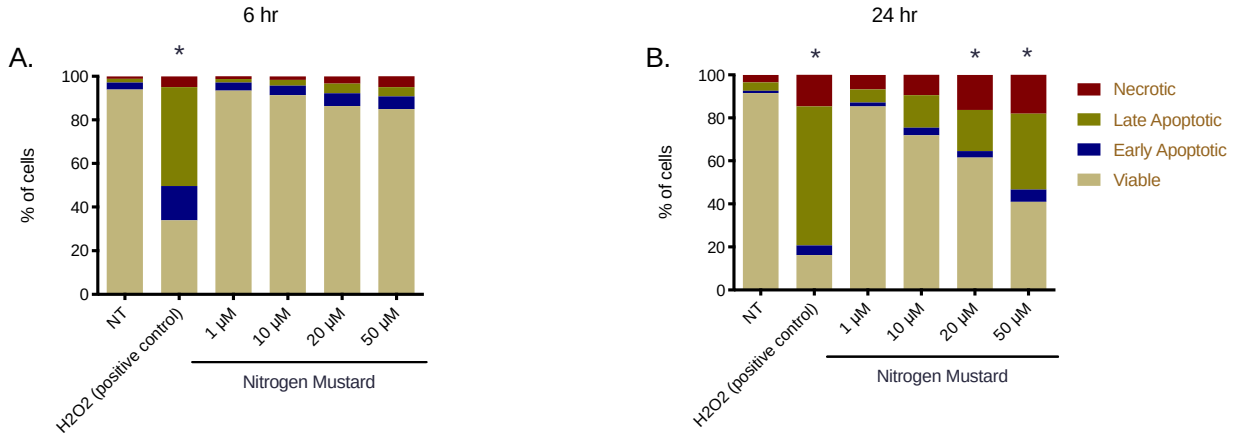


**Figure 1. Annexin V/PI cell viability.** Cell viability in BMMCs at A) 6 hrs and B) 24 hrs following exposure to NM at concentrations of 1.0µM- 50µM. Data is presented as the mean; one way ANOVA with Dunnett's *post hoc* test ($p<0.05$), *signifies significant difference in viable cells from NT viable cells. n=3

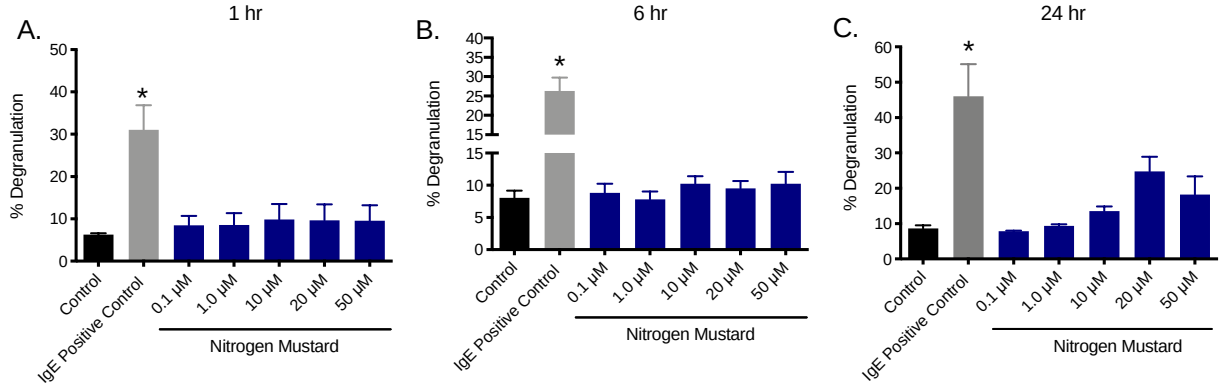


**Figure 2. BMMC degranulation in response to NM.** Percent degranulation (β-hexosaminidase release) from BMMCs following NM exposure concentrations of 1µM - 50µM at A) 1 hr, B) 6 hrs and C) 24 hrs. Data is presented as the mean ± SEM; one way ANOVA with Dunnett's post hoc test ($p < .05$), *signifies significantly difference from control at same time point. n=3.

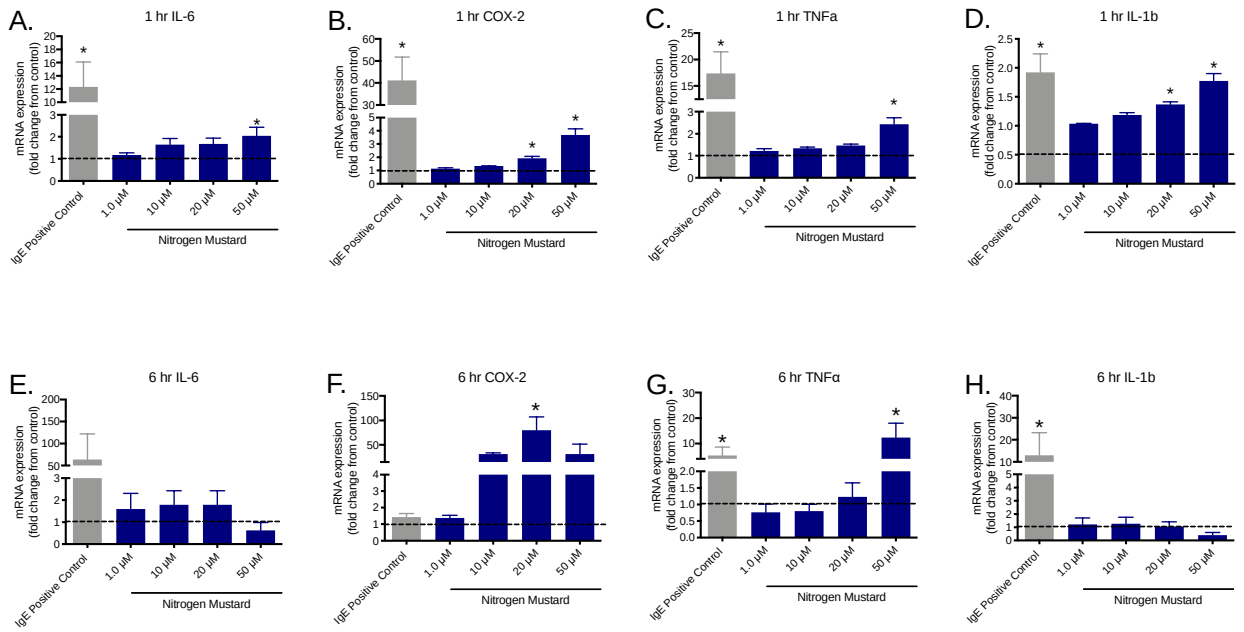


**Figure 3. Cytokine mRNA production at 1 and 6 hr of NM exposure.** mRNA gene expression from BMMCs following NM exposure concentrations of 1μM - 50μM for A) IL-6, B) COX-2, C) TNF α, D) IL-1β at 1 hr and E) IL-6, F) COX-2, G) TNF α, H) IL-1β at 6 hrs. Data is presented as the mean ± SEM; one way ANOVA with Dunnett's post hoc test ($p < .05$), *signifies significantly difference from control at same time point. n=3.

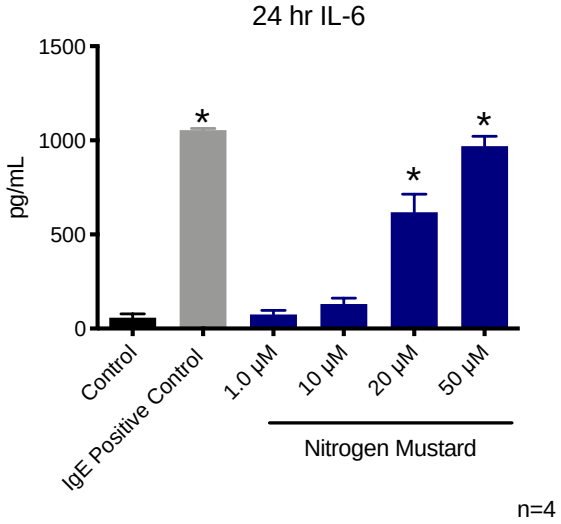


**Figure 4. Late phase cytokine formation of IL-6 at 24 hr post NM exposure**. IL-6 protein was quantified (ELISA) at 24 hr of NM exposure (1μM - 50μM). Data is presented as the mean ± SEM; one way ANOVA with Dunnett's post hoc test ($p < .05$), *signifies significantly difference from control at same time point. n=4.

**<u>Aim 1 a is accomplished in mast cells isolated from mice and studies under aim 1 b are being carried out.</u>**

Aim2-Preliminary data *in vivo* in mouse lungs

*Animals:* C57BL/6J and B6.Cg-KitW-sh/HNihrJaeBsmJ (mast cell deficient) mice were purchased from Jackson Laboratories at 4 weeks old. Mice were allowed to acclimate to vivarium for 3 weeks prior to the start of the experiment. Mice were kept in temperature controlled environment with 12-hr light-dark cycles. Mice were provided standard chow and water ad libitum. All animals utilized for this study complied with the guidelines approved by University of Colorado's Institutional Animal Care and Use Committee.

*Oropharyngeal Aspiration:* Mice were anesthetized using 3% isoflurane for 2 mins. Once anesthetized, mice were hung by teeth at 90 degree incline on small plastic platform designed for the technique. The tongue of the mouse was pulled out and held down with blunt forceps. Using a pipette, mice were dosed to nitrogen mustard (NM), diluted in phosphate-buffered saline (PBS), at a low dose (0.063 mg/kg) or high dose (0.125 mg/kg). A group of mice was dosed to PBS solely as a vehicle control. Once dosed, 25 seconds was allotted for fluid to be absorbed before tongue was released. Animals were then placed in a dry box to make sure liquid was not spit up. Mice were placed back in cage after fully awake. Animals were dosed once and sacrificed 24 or 72 hrs post-exposure.

*Animal Lavage and bronchoalveolar lavage fluid analysis*: Tracheostomies were performed on mice and female luer stubs were inserted into the trachea. The lungs were rinsed with Hanks' Balanced Salt solution (HBSS) at an initial volume of 700μL and bronchoalveolar lavage fluid (BALF) was collected. This procedure was repeated 3 times with a total volume of 3mL of BALF placed in 15 mL conical tube. BALF was centrifugated at 1200 g for 5 minutes to isolate cells. Protein concentration of BALF was analyzed with bicinchoninic acid (BCA) colormetric assay (Thermo Scientific, Rockford, IL) and BALF cytotoxicity measured with LDH-GLO™, a bioluminescent assay used to quantify lactate dehydrogenase (LDH) release (Promega, Madison, WI).

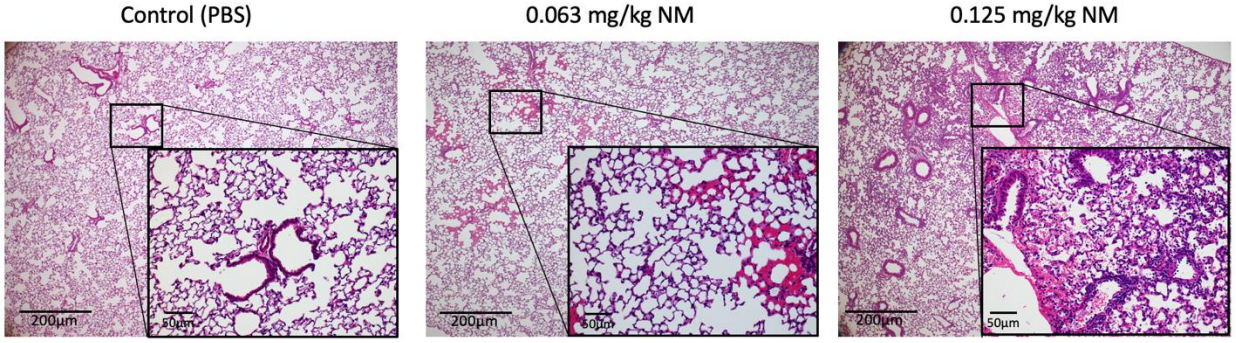


**Figure 5. C57BL/6J (WT) exposed to NM at 24 hr causes significant lung injury.** 24 hrs exposure to low dose (0.063 mg/kg) of NM shows vessel leakage and exposure to high dose (0.125 mg/kg) shows significant damage and immune cell infiltration.

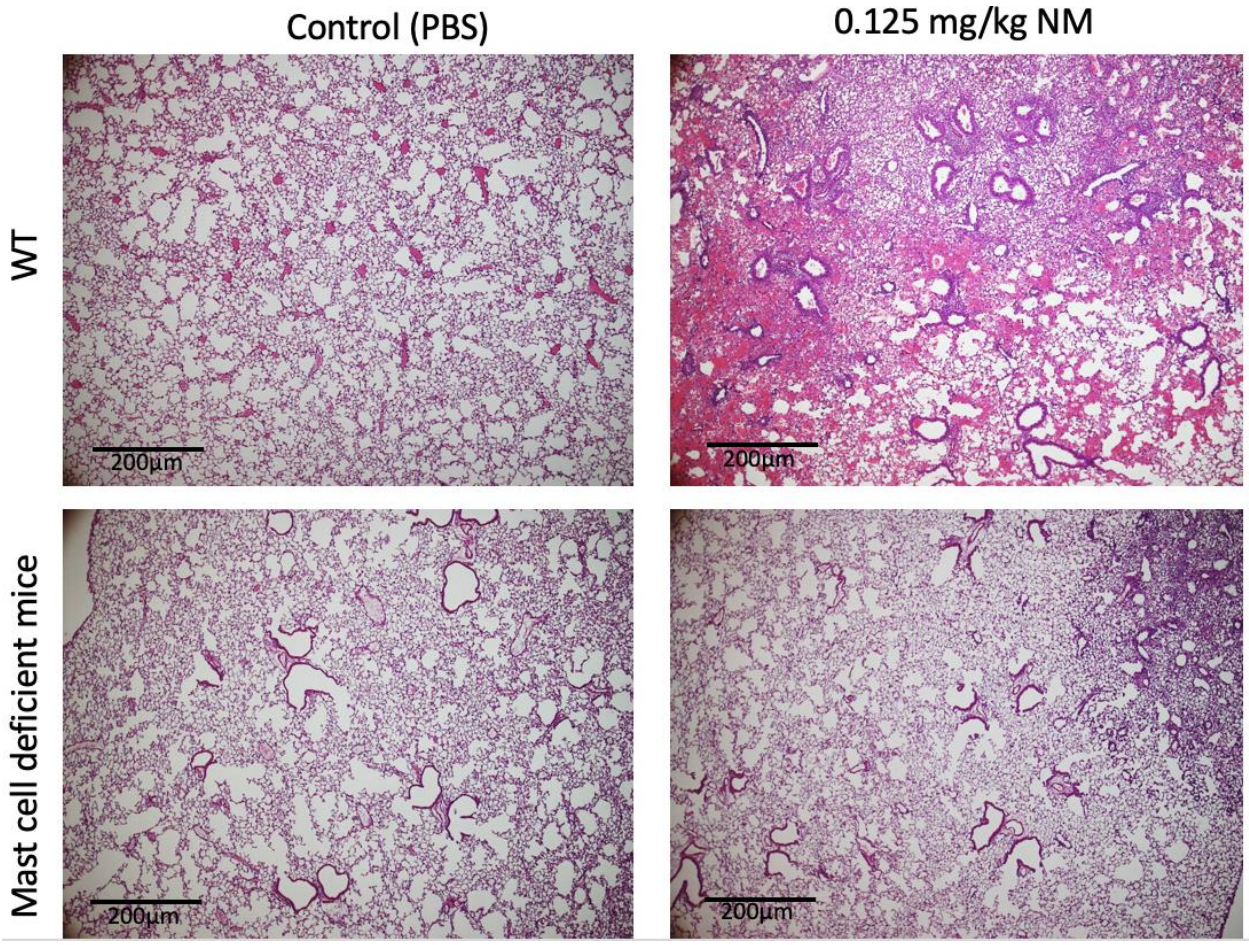


**Figure 6. C57BL/6J (WT) vs B6.Cg-KitWsh/HNihrJaeBsmJ (Mast cell deficient) mice exposed to NM at 72 hr displays significant lung injury in WT and not MC deficient.** 24 hr exposure of high dose of NM shows vessel leakage and severe immune cell infiltration that is not present in mast cell deficient mice.

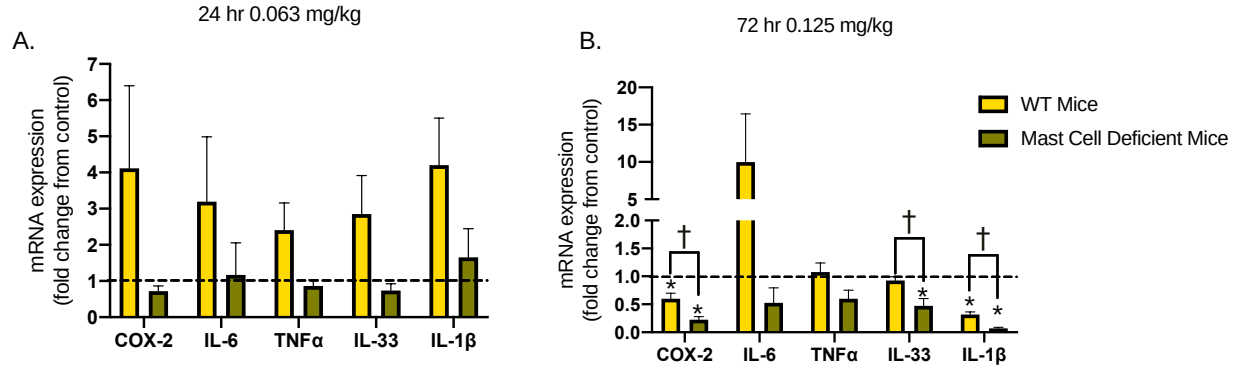


**Figure 7. Eicosanoid and cytokine mRNA production in wild type and mast cell deficient mice exposed to NM for 24 hrs and 72 hrs**. mRNA gene expression is shown for post exposure of A) low dose at 24 hrs and B) high dose at 72 hrs for COX-2, IL-6, TNFα, IL-33, and IL-1β.

Data is presented as the mean ± SEM; one way ANOVA with Tukey's multiple comparisons test ($p < .05$), *signifies significant difference from control. n=3-8.

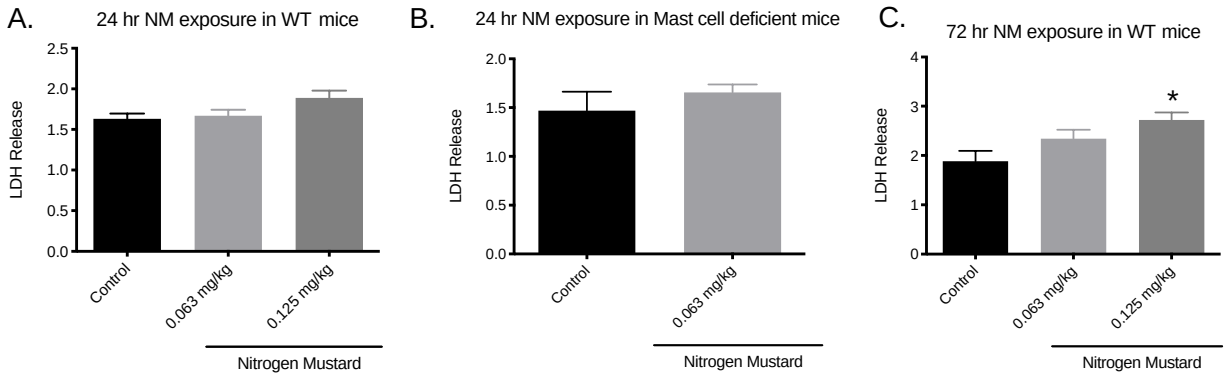


**Figure 8. LDH release from BALF at 24 and 72 hrs post NM exposure in wild type and mast cell deficient mice**. LDH release is shown at 24 hr for A) WT and B) Mast cell deficient mice and C) 72 hr for WT mice at low and high doses. Data is presented as the mean ± SEM; one way ANOVA with Dunnett's post hoc test ($p < .05$), *signifies significantly difference from control. n=5-8.

**<u>We have obtained some very promising data in lung studies in animals and hope to continue these studies as well as skin studies in mice in this year.</u>**

**Results Data Summary**

Mast cells play a prominent role in the inflammatory response induced by NM in the lungs. Although early NM-induced activation is not present (Fig.2), NM does induce both intermediate and late phase activation as demonstrated by the increase of mRNA production of cyclooxygenase-2 (COX-2) and various pro-inflammatory cytokines (Fig.3). In addition to increased mRNA expression, there was an increase in production and release of the inflammatory cytokine IL-6 (Fig. 4).

In vivo, WT mice exhibited a dose dependent increase in LDH release (Fig.8) at 24 and 72 hrs post NM exposure, which is strongly indicative of lung damage. At 24 hrs, WT mice exhibited an increase in mRNA expression of various cytokines that was not present in mast cell deficient mice at 24 hours, implying an important mast cell role in this pathology (Fig. 7A). At 72 hrs, transcription of many proinflammatory cytokines was significantly inhibited in mast cell deficient mice compared to WT (Fig. 7B). We can conclude that there is significant lung injury observed in WT mice that was significantly less in mast cell deficient mice following NM exposure at 0.125 mg/kg in 72 hrs. The data thus far is suggestive that mast cells play a role in the injury induced by NM exposure. Being able to understand the role mast cells play in the immune response modulated by NM exposure will help us better understand the mechanism in which sulfur mustard maybe acting. This will help with diagnosis as well as effective treatment strategies against sulfur mustard exposure in veterans. Understanding this mechanism will further our knowledge in determining if this exposure plays an active role in the symptoms seen in veterans that have been diagnosed with Gulf War Illness.

**iii)** O**pportunities for training and professional development**
Training-A graduate student Agela Cruz Hernandez is being trained under this project

**iv) Results disseminated to communities of interest**
Nothing to Report.

**v) Plan to do during the next reporting period to accomplish the goals**

SPECIFIC AIM 1. In vitro studies to elucidate mast cell-induced immune responses following mustard vesicating agent exposure.
Under this aim 1a, we plan to employ the LUVA human mast cells to examine the effects of nitrogen mustard (NM) on mast cell degranulation and cytokine production in vitro.
Also, we further plan to Under Aim 1b, to examine the effectiveness of several mast cell inhibitors in preventing NM-mediated degranulation.

SPECIFIC AIM 2. In vivo studies to confirm the role of mast cells in mustard-induced inflammation. We aim to accomplish studies proposed under this aim using mast cell deficient (B6.Cg-KitW-sh/HNihrJaeBsmJ) and wild-type (C57BL/6) mice to confirm a role for mast cells in NM-induced lung pathology, NM dermal pathology and systemic effects related to immune system activation.

In the January of 2019, I (PI: Neera Tewari-Singh) accepted a position at Michigan State University and therefore the grant transfer process from University of Colorado Denver is still underway and has slowed down the research progress. We plan to speed up work on the proposed studies and further ask for project extension, if required to accomplish the proposed goals.

## 4. IMPACT

**i) Impact on the development of the principal discipline(s) of the project**

Mast cells are well known to contribute to allergic inflammatory diseases, but also have wide ranging effects on many physiological systems that are affected in GWI including pulmonary, dermal, gastrointestinal and nervous systems when activated (e.g. degranulation). Importantly, a role for mast cells has been suggested in the mechanism of vesicating chemical agents like SM-induced inflammatory response and tissue damage. The cell culture and preliminary in vivo data obtained in our study ssuggest that mast cells play a prominent role in the inflammatory response induced by nitrogen mustard (NM). Moving forward in our studies, we aim to uncover a novel mechanism of NM and sulfur mustard (SM) toxicity that is mediated by mast cells and which could also be applicable to other chemical exposures in GWI. It is reported that during the Gulf War (GW; 1990-1991) U.S. troops could have been exposed to a number of chemicals including the low level chemical warfare agents (CWAs) like SM released by the destruction of Iraqi facilities.
Identification of mast cell activation can significantly contribute to GWI by serving as an important biomarker and therapeutic target in vesicating agents-related GWI pathogenesis. In addition, the other biomarkers related to mast cell activation could also be important in GWI and help in this area of research by identifying targeted therapies to effectively treat GWI

**ii) Impact on other disciplines**

-Novel mechanism of NM and sulfur mustard (SM) toxicity that is mediated by mast cells could also be applicable to other chemical exposures in GWI.
-Development of vesicating chemical agent NM–induced animal model of GWI (chronic injury) will not only contribute in elucidating the possible molecular and physiological mechanisms underlying GWI but also can be used for efficacy studies to identify effective therapies by us and other research groups.

**iii) Impact on technology transfer**

- Nothing to Report.

**iv) Impact on society beyond science and technology**

-Nothing to Report.

**5. CHANGES/PROBLEMS:**

**i) Actual or anticipated problems or delays and actions or plans to resolve them**

-In the January of 2019, I (PI: Neera Tewari-Singh) accepted a position at Michigan State University and therefore the grant transfer process from University of Colorado Denver is still underway and has delayed the research progress. We are hiring a staff for studies at Michigan State University to speed up work on the proposed studies and further ask for project extension, if required to accomplish the proposed goals.

**ii) Changes that had a significant impact on expenditures**

-Since the PI moved to a new Institution since January 2019, the grant transfer, new staff hiring has taken longer and hence the proposed expenditure (year 1) to obtain the proposed goals will be carried out in the year 2 of the project.

**6. PRODUCTS**

**i) Other publications, conference papers, and presentations**

1. Effects of Nitrogen Mustard Gas on Mast Cell Activation
Poster Presentation, Mountain West SOT 2018; Phoenix, Arizona

2. Effects of Nitrogen Mustard Gas on Mast Cell Activation Potentially Contributing to Gulf War Illness
Poster Presentation, Skaggs School of Pharmacy Student Winter Symposium 2019; Aurora, Colorado

3. Effects of Nitrogen Mustard on Mast Cell Activation
Poster Presentation, Society of Toxicology Annual Meeting 2019; Baltimore, Maryland

4. Investigating the Role of Mast Cell Activation by Nitrogen Mustard in Gulf War Illness"
Poster Presentation, Skaggs School of Pharmacy Annual Retreat 2019; Breckenridge, Colorado

5. Investigating the Role of Mast Cell Activation by Nitrogen Mustard in Lung Toxicity

Oral presentation, Mountain West SOT 2019; Fort Collins, Colorado

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### i) Individuals that have worked on the project

1. PI: Neera Tewari-Singh, MS, PhD (Institution change)
   Assistant Professor
   Department of Pharmacology & Toxicology
   Michigan State University
   B307 Life Sciences, 1355 Bogue Street
   East Lansing, MI 48824
   Phone (Office): 517-884-0252
   Cell: 970-988-3671
   Fax:517-353-8915
   Email: tewarisi@msu.edu

2. CO-I: Jared Brown, PhD (No Change)

3. Graduate student: Angela Cruz Hernandez (no Change)

4. Dinesh G Goswami (worked in this project at UCDenver from August 2018-December 2018)

### ii) Funding Support:

**Change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period**

**PI: Tewari-Singh, Neera**
**Changes: Projects no longer active**
**Title:** Therapeutic strategy for corneal healing in diabetic and non-diabetic dry eye models
**Time Commitments:** 1.20 calendar
**Supporting Agency:** CCTSI
**Address:** University of Colorado Anschutz Medical Center
**Contracting/Grants Officer:** N/A
**Performance Period:** 6/13/2017-6/12/2018
**Level of funding:** $30,000
**Project Goals:** The major goal of this proposal is to obtain an effective treatment strategy for non-diabetic and diabetic dry eye-related corneal damage using an aldose reductase inhibitor and doxycycline treatment
**Specific Aims:**

1. Develop non-diabetic and diabetic dry eye models in rats, and examine the efficacy of AR inhibitor sorbinil, antibiotic DC, and their combination treatments against dry eye-related corneal damage.
2. Evaluate clinical, histopathological and immunohistochemical changes in the rat corneal tissue to determine the efficacy of the treatments (Aim 1) in non-diabetic and diabetic dry eye.

**Overlap:** N/A

**Title:** Elucidating the role of aryl hydrocarbon receptor in polycyclic aromatic hydrocarbons-induced skin inflammatory diseases for targeted treatment strategies
**Time Commitments:** 2.40 calendar
**Supporting Agency:** Skaggs Foundation
**Address:** 101 Skaggs Rd STE 404, Branson Mo 65616
**Contracting/Grants Officer:** Lou Diamond
**Performance Period:** 07/01/2017-06/30/2019
**Level of funding:** $150,000
**Project Goals:** The objective of the current proposal is to investigate the metabolic signature in mouse skin following polycyclic aromatic hydrocarbon (PAH) exposure and explore AhR-related signaling pathways in in skin inflammatory diseases like psoriasis and atopic dermatitis which can exacerbate from PAH exposure.
**Specific Aims:**
1. To investigate the metabolic consequences of PAH-induced skin inflammatory response in the mouse skin.
2. To evaluate the role of AhR in PAH-induced changes in metabolic profile in mouse skin inflammatory response.

**Overlap:** N/A

**Changes: Project with change in performance dates (grant award)**

**Title:** Phosgene Oxime Cutaneous Toxicity and Mechanisms to Identify Therapeutic Targets
**Time Commitments:** 3.10 academic and 1 summer month
**Supporting Agency:** NIH/NIAMS-CounterACT, 7 R21 AR073544-03
**Address:** NIAMS, 1 AMS Circle, Bethesda, MD 20892-3675
**Contracting/Grants Officer:** Simmons, Sheila
**Performance Period:** 07/23/19-08/31/2020
**Level of funding:** $153,825 (Direct); $240,736 (Total)
**Project Goals:** The goal of this proposal is to develop a relevant cutaneous CX exposure mouse injury model to elucidate mechanisms of skin damage by CX and examine its systemic toxic effects with the objective of identifying novel targets for therapeutic intervention and drug development.
**Specific Aims:**
1. Characterize and establish CX-induced in vivo rodent skin injury model following its cutaneous exposure, and identify associated mechanism/s of action.
2. Examine systemic toxic effects and related mortality from CX cutaneous exposure.

**Overlap:** N/A

**Changes: New Project (project now active)**

**Title:** Targeted Therapeutic Approaches to Counteract Toxicity from Phosgene Oxime Skin Exposure
**Time Commitments:** 4.8 academic and 1 summer month

**Supporting Agency:** NIH/NIAMS-CounterACT, 1 U01 AR075470-01
**Address:** NIAMS, 1 AMS Circle, Bethesda, MD 20892-3675
**Contracting/Grants Officer:** Simmons, Sheila
**Performance Period:** 09/10/19-08/31/2022
**Level of funding:** $893,851 (Direct); $1,422,277 (Total)
**Project Goals:** To validate mast cells as key players and molecular targets in phosgene oxime (CX) toxicity and investigate whether blocking these targets in established mouse toxicity models, will assist to mitigate CX induced skin morbidity and mortality resulting from its cutaneous exposure. In addition, we anticipate identifying an FDA approved therapeutic strategy that can target mast cell activation and release of histamine
to mitigate CX-induced skin injury including urticaria and mortality from CX cutaneous exposure.
**Specific Aims:**

1. To further establish mast cells as key players and molecular targets in CX toxicity by employing mast cell deficient mice.
2. To test the efficacy of FDA approved therapies that can counteract CX-induced morbidity and
   mortality from cutaneous exposure in mice, mainly by targeting mast cell activation and release of
   histamine.

**Overlap:** N/A

**iii) What other organizations were involved as partners?**
Organization Name: University of Colorado Denver (Academic).
Location of Organization: Aurora, Colorado, USA
Partner's contribution to the project: Collaboration (e.g., partner's staff work with project staff on the project).

**8. SPECIAL REPORTING REQUIREMENTS**
**None.**

**QUAD CHARTS.**
**Not applicable**

**9. APPENDICES.**
**None.**