A Rodent Model of Sulfur Mustard Hematologic Toxicity for the Efficacy Evaluation of Candidate Medical Countermeasures

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ABSTRACT

Introduction:
While exposure to sulfur mustard (SM) is commonly associated with the production of vesicating dermal, ocular, and respiratory injuries, systemic damage to bone marrow and lymphatic tissue can decrease critical immune cell populations leading to higher susceptibility to life-threatening infection and septicemia. There are currently no approved medical countermeasures for SM-induced myelosuppression. An intravenous SM challenge model was developed in adult rats as a preliminary proof-of-principle platform to evaluate the efficacy of candidate immunostimulants.

Materials and Methods:
Adult male and female Sprague Dawley rats were exposed to SM through tail vein injection. Toxicity progression was monitored through clinical observations, body weights, body temperatures, hematology, serum clinical chemistry, and flow cytometry of blood and bone marrow samples.

Results:
Following SM exposure, overt toxicity progression was characterized by weight loss, changes in body temperature, and manifestation of toxic clinical signs (diarrhea, lethargy, hunched posture, rough hair coat, respiratory distress, and death). Drastic alterations in complete blood cell profiles included an early-onset lymphopenia followed by a delayed-onset neutropenia and thrombocytopenia. Only transient changes in serum clinical chemistry parameters were observed. Flow cytometry analysis of circulating blood revealed that B-cells were more predominantly affected by SM exposure than T-cells. Challenge with SM resulted in loss of hematopoietic and mesenchymal stem cell populations in the bone marrow.

Conclusions:
The small animal model developed in this study replicates many key aspects of human SM exposures and should serve as a relevant, rapid, and cost-effective platform to screen candidate medical countermeasures for SM-induced hematologic toxicity.

INTRODUCTION
Sulfur mustard (SM) toxicity is exerted through the production of highly reactive intermediates that can produce permanent alkylation and oxidative stress.1 Dermal exposure to SM can result in powerful vesicant effects, producing large fluid-filled blisters at contacted areas of the skin.2,3 Ocular exposure to SM can damage the cornea, leading to temporary blindness and long-term visual deficits.4 SM inhalation can cause pulmonary edema and compromise respiratory function.5 Individuals exposed to SM rarely manifest symptoms immediately resulting in an asymptomatic latency period where exposure may unknowingly continue.

Beyond the skin-, lung-, or eye-specific injuries after SM exposure, immune system function may also be compromised.6–9 SM-induced alkylation of cells in the bone marrow, spleen, and lymphatic tissue can decrease circulating lymphocyte and neutrophil counts, a clinical condition termed lymphopenia and neutropenia, respectively.6–8 Following SM exposure, reductions in these critical cell populations can lead to increased susceptibility to potentially life-threatening infection and septicemia.9–12 Currently, there are no approved medical countermeasures (MCMs) for the treatment of SM-induced immunosuppression.

Immunostimulant therapies may be effective in overcoming the myelosuppressive effects of SM. For example, granulocyte colony-stimulating factor (filgrastim), which increases neutrophil production from the bone marrow,13 has been used to successfully treat neutropenia induced by exposure to myelosuppressive anticancer drugs and ionizing radiation (hematopoietic syndrome of acute radiation). However, beyond the initial efficacy demonstrated in a pilot study with African green monkeys,14 it remains unclear whether
filgrastim, other similar growth factors, and/or cytokine treat-
ments are viable MCM options for hematologic complications
induced by SM exposure.

Unfortunately, to evaluate the efficacy of a potentially large
and diverse cadre of candidate MCMs to specifically miti-
gate the immunosuppressive effects of SM, an appropriate,
economical, and reproducible high-throughput small animal
model is required. We report here the development of an in vivo
intravenous (IV) SM challenged rat model of myelo-
suppression that could subsequently be utilized to screen and
characterize initial proof-of-principle efficacy of candidate
MCMs. While IV SM intoxication is not a realistic scenario
of exposure, this route was intentionally selected to specifi-
cally isolate the myelosuppressive consequences of SM from
potentially confounding factors that may arise from skin, oph-
thalmic, and/or respiratory injuries that are more likely to
occur via the dermal, ocular, or inhalation routes. Further-
more, the volume and concentration of SM necessary in a
dermal or inhalation exposure model can present significant
occupational hazards for laboratory personnel in addition to
higher costs associated with the need for more sophisticated
exposure system (in the case of an inhalation exposure route).
Consequently, this IV-based approach affords several logis-
tic benefits, specifically cost-effectiveness, personnel safety,
throughput, and improved accuracy of delivering consistent
systemic doses.

Model development was conducted in three phases. First,
using an up/down lethality study design, the 14-day median
lethal dose (LD₅₀) was estimated in male and female Sprague
Dawley rats by administration of varying SM levels via tail-
vein injection and monitoring mortality outcomes in addition
to the spectrum, severity, and duration of toxic clinical signs.
Second, dose optimization was performed where longitudi-
nal monitoring of complete blood count (CBC) profiles was
performed in animals that were administered varying LD₅₀
levels (0.6×, 0.8×, and 1.0×) of SM. Lastly, using a fixed
IV challenge dose of SM (1.0× LD₅₀), a comprehensive
35-day natural history of toxicity study was performed in SM-
exposed rats. Systemic SM-induced injury progression was
characterized through clinical observations, body weights,
body temperatures, CBC profiles, serum clinical chemistry,
flow cytometry of cell lineage and activation on blood and
bone marrow samples, gross necropsy, and bacteremia deter-
mination.

**MATERIALS AND METHODS**

**Animals**

Male and female Sprague Dawley rats were obtained from
Taconic Biosciences, Inc. (Rensselaer, NY) with surgically
implanted jugular vein catheters. Both males (~400-500 g)
and females (~250-350 g) were approximately 14 to 16
weeks of age when used on study. During quarantine, animals
were implanted with programmable temperature transpon-
ders (IPTT-300, Bio Medic Data System (BMDS)) posterior
to the jugular vein catheter. Animals were sedated with a
CO₂/O₂ combination for the implantation. Animals were sin-
gle housed, maintained on a 12-hour light/dark cycle with
no twilight with air temperature maintained within a 16°C to
27°C range and relative humidity maintained between 30% and
70%. Food and water were provided ad libitum. All ani-
mal were maintained under the Battelle animal care and use
program accredited by the Association for Assessment and
Accreditation for Laboratory Animal Care International. This
care and use program was in accordance with the guidelines
set forth in the “Guide for the Care and Use of Laboratory
Animals,” National Research Council, and/or with the regula-
tions and standards promulgated by the Agricultural Research
Service, U.S. Department of Agriculture, pursuant to the Lab-
oratory Animal Welfare Act of August 24, 1966, as amended.
The Battelle Institutional Animal Care and Use Committee at
Battelle, Columbus, OH, approved the experimental protocol.

**SM Exposure**

Distilled SM (HD) was acquired through the U.S. Army Edge-
wood Chemical and Biological Center (Aberdeen Proving
Ground, MD) as part of an Interagency Agreement between
the NIH and the DoD. Before the study, the purity of neat
SM was confirmed at Battelle to be 98.4% using a gas
cromatography-flame ionization detector. To prepare SM
dosing solutions, neat distilled SM was diluted in absolute
ethanol to a stock concentration of 9.52 mg/mL. Final SM
dosing solutions (1.5 mg/mL) were prepared from the
9.52 mg/mL SM stock through dilution with propylene gly-
col. Vehicle control solutions were prepared using absolute
ethanol and propylene glycol to match the equivalent com-
position of the 1.50 mg/mL SM dosing solution. Final SM
dosing solutions were prepared fresh, maintained at room
temperature, and administered to animals within 60 minutes
of preparation. New SM dosing solutions were prepared for
each 60-minute interval of challenge. Samples from each SM
dosing solution were pulled for predose and postdose analy-
yses of SM dosing solution concentrations. Challenge material
was administered as a slow IV push into the tail vein. After
IV delivery of SM (or vehicle), a saline flush was performed
to ensure complete delivery of the challenge material.

**Clinical Observations, Body Weights, and Body
Temperatures**

Twice-daily AM/PM clinical observations were recorded dur-
ing quarantine through the scheduled terminal time point. To
limit unnecessary distress to animals, preestablished crite-
ria for early euthanasia were in place for animals that were
observed with (1) significant respiratory distress, an ability
right itself, and a weight loss of 25.0% to 29.9%
or (2) a weight loss of ≥30% regardless of toxic manifesta-
tions. All scheduled euthanasia performed on study was
performed according to American Veterinary Medical Asso-
ciation guidelines. Animals were weighed during quarantine

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(for randomization), on day-1 (for dosing calculations), and daily (or every other day) postchallenge starting on day 1 through the scheduled terminal time point. Body temperatures from the IPTT-300, BMDS, were recorded daily in the AM starting on day-3 through the scheduled terminal time point.

**Hematology and Serum Clinical Chemistry**

From unanesthetized animals, whole blood samples were collected from the jugular vein catheter or directly from the jugular vein (if the catheter was not functioning). For hematology, blood was transferred into K$_3$EDTA tubes and retained at room temperature until same-day hematology analysis. Collected blood samples were analyzed using the Advia 120 Hematology Analyzer. For serum clinical chemistry, blood was collected in serum separator tubes (SSTs), processed to serum, and frozen at $-70^\circ$C until batched analysis was performed at later date. Collected serum samples were analyzed using the Advia 1200 Chemistry Analyzer. In all cases, prechallenge bloods were collected from all animal to provide subject-specific baselines for hematology and serum clinical chemistry. Samples were not collected from animals that were found dead.

**Flow Cytometry**

Collected blood and bone marrow (femur) samples were analyzed using a BD LSR FORTESSA flow cytometer. Analysis was performed using FlowJo software (v. 10.0, Tree Star, Inc.) and compensation was verified at the time of acquisition using FACSDiva software (v. 8.0, BD Biosciences). A complete listing of antibodies, manufacturers, lot numbers, and dilutions is provided in Supplementary Table S1. Blood and bone marrow samples for flow cytometry were not collected from animals that were found dead.

**Statistical Analyses**

Statistical analysis was performed using GraphPad Prism (version 7.0.3.). For binary comparisons between SM- and vehicle-challenged animals at distinct postchallenge time points, multiple $t$-tests were performed. Statistical significance was determined using the Holm-Sidak method with alpha $= .05$. For comparisons among groups in which terminal bone marrow samples were analyzed by flow cytometry, statistical analysis was performed as an ANOVA with the vehicle group (day 35) as the control group for the Dunnett’s posttest. For hematology data plotted on a Log y-axis, data reported as 0 were changed to 0.001 (an order of magnitude below the lowest nonzero value on the analyzer) to allow for all collected data to be plotted. When appropriate, individual cell count outliers were removed by a Grubbs’s test ($P < .05$). Body weights were normalized to each animal’s individual baseline weight and expressed as a percentage. For survival analysis, Kaplan-Meier estimates were plotted for each group.

**RESULTS**

**Initial Estimation of the 14-day LD$_{50}$ following IV SM Challenge**

Before determining SM challenge levels for model development, a small pilot study was first conducted to define the 14-day LD$_{50}$ of IV administered SM in rats. SM was administered via tail vein injection to male ($n = 16$) and female ($n = 16$) Sprague Dawley rats at eight distinct SM doses ranging from 0.1 to 7.5 mg/kg ($n = 2$ animals per gender per dose). Although all animals challenged with 0.1 and 0.42 mg/kg of SM were observed as clinically normal, and only half of the animals challenged at 1.78 mg/kg displayed a mild clinical sign (diarrhea), all animals challenged at doses equal to or greater than 3.6 mg/kg displayed a spectrum of clinical signs including lacrimation, nasal discharge, swollen eyes, lethargy, weakness, roughed hair, hunched posture, and respiratory distress. The average time to death for all lethal SM doses (3.6, 4.3, 5.2, 6.2, and 7.5 mg/kg) ranged from 4 to 6 days postchallenge. Analysis of mortality outcomes estimated a 14-day LD$_{50}$ of 2.53 mg/kg (87.5% CI, 1.78-3.60 mg/kg), a value generally consistent with previously reported LD$_{50}$ estimates (0.70-3.83 mg/kg) in other rat strains using other IV routes of SM administration.$^{15,16}$ As such, the challenge level of 2.53 mg/kg of SM was selected as an anchor point for a follow-on dose optimization study.

**Optimization of an IV SM Dose That Induces Hematologic Toxicity**

Before comprehensive model characterization, a SM dose-ranging study was performed to determine appropriate IV SM challenge levels in Sprague Dawley rats that leads to broad-spectrum hematologic toxicity. Using the initial 14-day LD$_{50}$ estimate, SM challenge levels selected for further study included 1.52, 2.02, and 2.53 mg/kg of SM, corresponding to 0.6×, 0.8×, and 1.0× LD$_{50}$, respectively. Studies were conducted in both male ($n = 48$) and female ($n = 48$) rats. Blood collections at individual time points for each group were evenly split among animals from the following number of total replicates per gender per group: $n = 12$ for vehicle group, $n = 4$ for 1.52 mg/kg SM group, $n = 12$ for 2.02 mg/kg SM group, and $n = 20$ for 2.53 mg/kg SM group. To reduce overall animal numbers, the number of replicates per SM dose were varied because of a stage-wise study design where results from previous challenge days were collected and analyzed before rationale dose selection on subsequent challenge days. To monitor the hematologic consequences of SM exposure, blood samples were collected before and after challenge and analyzed for CBC profiles. Relative to the day of challenge (day 0), sample collections and hematology analysis was performed on the following days: $-1, 1, 2, 3, 4, 5, 6, 7, 9, 12, and 15$.

Animals challenged with SM showed a dose-dependent increase in red blood cell counts (Fig. 1A) and hemoglobin

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**Rat Sulfur Mustard Model of Hematologic Toxicity**

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Natural History of Toxicity Progression following IV SM Exposure

Using the revised LD$_{50}$ estimates for IV SM-challenged male (2.06 mg/kg) and female (2.30 mg/kg) Sprague Dawley rats, a 35-day natural history of toxicity study was performed in male and female animals challenged with SM at their respective 1.0 × LD$_{50}$ estimates ($n$ = 36 males, $n$ = 36 females). Blood collections at individual time points for each group were evenly split among animals from the following number of total replicates per gender per group: $n$ = 4 for the vehicle group, $n$ = 8 for 2.06 mg/kg for SM group. Blood collections at individual time points for each assay (hematology, serum clinical chemistry, and flow cytometry) for each group for each gender were evenly split among animals from the following number of total replicates per gender per group: $n$ = 4 for the vehicle group, $n$ = 8 for SM group. Relative to the day of challenge (day 0), for vehicle groups, blood collections for hematology, serum clinical chemistry, and flow cytometry analysis were performed on the following days: −1, 5, 7, 14, 21, 28, and 35. For SM groups, blood collections for hematology, serum clinical chemistry, and flow cytometry analysis were performed on the following days: −1, 3, 5, 7, 10, 14, 21, 28, and 35. Scheduled termination for vehicle groups was day 35, and scheduled terminal for SM group was performed on both days 10 and 35. At termination, bone marrow was collected from the femur for flow cytometry analysis.

Although all animals challenged with vehicle (12 out of 12 males, 12 out of 12 females) survived to the prescribed study termination point (day 35), 8 out of 24 males and 6 out of 24 females died before the scheduled euthanasia time point (day 10 or 35). Mortality was observed between 5 and 9 days postchallenge with an average time to death of 5.5 days for SM-challenged males and 7 days for SM-challenged females. Although vehicle-challenged animals exhibited little-to-no body weight loss, SM-challenged animals exhibited significant weight loss, peaking at ~20% loss for males and ~17% for females on day 5 (Fig. 2B). SM-challenged males increased in body weight but remained statistically distinct from vehicle controls until day 21. In contrast, SM-challenged females recovered body weight more rapidly than males and were statistically indistinct from vehicle controls by day 9. Compared with baseline body temperatures, vehicle-challenged animals exhibited little-to-no fluctuations in body temperature. In contrast, SM-challenged males displayed an initial increase in body temperature on day 1, followed by a general decline in body temperature on days 4 to 5 before a rapid secondary increase on days 6 to 7 before returning to temperatures that were statistically indistinguishable from controls on days 8 to 35 (Fig. 2C). Compared with males, females displayed fewer fluctuations in body temperature following SM challenge.

Mean absolute lymphocyte count values between days 3 and 5 (Fig. 1E) were greater in males than females. This finding was consistent with the increased sensitivity of male rats to the lethal effects of SM challenge, where combined analysis with the previous SM dose-ranging study (0.1–7.5 mg/kg SM) indicated a significant difference in LD$_{50}$ values between males and females at day 12. Revised 12-day LD$_{50}$ estimates were 2.06 mg/kg for males (95% CI, 1.91–2.24 mg/kg) and 2.30 mg/kg for females (95% CI, 2.11–2.56 mg/kg), with a P-value of .0003 between the two sexes.
FIGURE 1. Dose-dependent response in hematologic toxicity following intravenous sulfur mustard (SM) challenge. Sulfur mustard or vehicle was administered intravenously at the doses shown (1.52, 2.02, and 2.53 mg/kg) to male and female Sprague Dawley rats, and blood was collected at the indicated time points for complete blood count (CBC) analysis. Owing to complete lethality, data are not available in rats challenged at 2.53 mg/kg SM beyond 7 days postchallenge. Each point represents the group mean ± SEM. Blood collections at individual time points per gender for each group were evenly split among animals from the following number of total replicates per gender per group: \( n = 12 \) for vehicle group, \( n = 4 \) for 1.52 mg/kg SM, \( n = 12 \) for 2.02 mg/kg SM, and \( n = 20 \) for 2.53 mg/kg SM. The following CBC parameters are presented: (A) red blood cell counts, (B) red cell distribution width, (C) platelet counts, (D) white blood cell counts, (E) lymphocyte counts, and (F) neutrophil counts.

Compared with vehicle controls, SM-challenged animals exhibited notable variations in CBC profiles indicating bone marrow toxicity, including the loss of reticulocytes (Fig. 2D), white blood cells (Fig. 2E), and platelets (Fig. 2F). Further supporting the broad-spectrum nature of hematologic toxicity following SM challenge, changes were also observed in red blood cell counts (Figure S2A), hemoglobin (Figure S2B), red cell distribution width (Figure S2C), mean platelet volume (Figure S2D), lymphocyte counts (Figure S2E), neutrophil counts (Figure S2F), as well as other cell populations (monocytes, eosinophils, basophils, and large unstained cells; data not shown). Save for a transient increase on day 5, a time point coinciding with the onset of toxic signs, few alterations in serum clinical chemistry values were observed in animals challenged with SM (Figure S3). In most cases, SM-induced alterations to all CBC parameters resolved by day 21. In general, SM-induced hematologic toxicity was to a lesser degree in females than males, despite females receiving a greater SM challenge dose (2.30 vs. 2.06 mg/kg, respectively).

Flow cytometry analysis of blood revealed significant alterations in lymphocyte populations following SM exposure. In SM-challenged animals, total B-cells (Fig. 3A) and RT1B\(^+\) activated B-cells (Fig. 3B) were observed to be reduced by day 3 and remain depressed through day 21. B-cell
percentage was observed to recover to vehicle-treated levels on days 28 and 35. Although not statistically significant, SM exposure was found to alter T-cell populations (Fig. 3C) and appeared to decrease the percentage of CD4\(^+\) helper T-cells (data not shown) and increase the percentage of CD8\(^+\) cytotoxic T-cells at days 5 to 7 postchallenge (Fig. 3D). Through day 35, CD4\(^+\) helper T-cells remained suppressed, whereas the percentage of CD8\(^+\) cytotoxic T-cells in blood remained elevated (although to a lesser degree in females).

Compared with vehicle-control bone marrow samples obtained at 35 days, significant alterations in cell percentage populations were apparent in animals challenged with SM, particularly at 10 days. In SM-challenged animals, decreases in total B-cells (Fig. 3E) and RT1B\(^+\) activated B-cells (Fig. 3F) were observed. At 10 days post-SM exposure, significant decreases in bone marrow CD8\(^+\) cytotoxic T-cells were also observed (Fig. 3G) in both males and females, and a significant increases in bone marrow CD4\(^+\) helper T-cells were observed in males only (Fig. 3H). Bone marrow samples obtained at 10 days after SM exposure were found to generally contain a lower percentage of stem cells. Although not statistically significant, CD34\(^+\) immature hematopoietic stem cell populations appeared to be decreased at 10 days following SM challenge (Fig. 4A), whereas mesenchymal stem cells were significantly decreased at 10 days (Fig. 4B). At 35 days, both stem cell populations recovered to levels similar to that of vehicle-challenged animals, indicating likely reversibility of SM-induced toxicity to bone marrow stem cells.
FIGURE 3. Blood and bone marrow analysis of lymphocyte populations following intravenous sulfur mustard (SM) challenge. Sulfur mustard (2.06 mg/kg for males, 2.30 mg/kg for females) or vehicle was administered intravenously to male and female Sprague Dawley rats and blood and bone marrow was collected at the indicated time points for flow cytometry analysis of B- and T-lymphocyte populations. Each point represents the group mean ± SEM. Owing to blood volume withdrawal limits, blood collections at individual time points for each group were evenly split among animals from the following number of total replicates per gender per group: n = 4 for vehicle group, n = 8 for SM group. Representative flow cytometry acquisitions (in males) at 5 days postchallenge and quantitation are presented for: (A) B-cells, (B) RTB1+ B-cells, (C) T-cells, and (D) CD8+ cytotoxic T-Cells. For bone marrow analysis, terminal samples were collected and analyzed from the following number of animals at the indicated time points: vehicle (35 days; n = 4), SM (10 days; n = 4), SM (35 days; n = 4). Representative flow cytometry acquisitions of (E) B-cells and quantitation of (F) RTB1+ B-cells, (G) CD4+ helper T-cells, and (H) CD8+ cytotoxic T-cells are presented below. * indicates P < .05, ** indicates P < .01.

DISCUSSION
The robust changes in hematology from SM-challenged rats determined in this study are consistent with those observed in SM-challenged nonhuman primates and human victims of SM exposure. Although the temporal progression of hematologic toxicity following exposure appears to be consistent across species, such that early onset lymphopenia is followed by a delayed onset neutropenia and thrombocytopenia, notable differences in recovery were observed. Though Anderson et al. reported a prolonged decrease in
lymphocyte and neutrophil counts for weeks following SM exposure, rats in this study appeared to recover on an accelerated timescale of days. Regardless of duration, the temporal onset of hematologic toxicity following SM exposure generally appears consistent with chemotherapy-induced lymphopenia and radiation-induced lymphopenia, which is a common early accompaniment in cancer therapy. In human patients of SM exposure, common early hematologic indicators of exposure include neutrophilia and lymphopenia. These data collectively suggest that decremented lymphocyte counts (or elevated neutrophil counts) could be useful as an early diagnosis of systemic SM exposure in humans, particularly when SM exposure levels are unknown or during the asymptomatic latency period after exposure. Evidence of lymphopenia in SM-exposed populations may likely serve as the earliest “trigger” to initiate postexposure growth-factor like treatment to boost immune cell counts in an effort to address potential immunosuppression. Additionally, while observed to occur after lymphopenia onset, the decrease in reticulocyte counts, which is a clinically relevant biomarker for decreased bone marrow activity, may also provide value in confirming systemic SM-induced toxicity.

Immunophenotyping of blood samples from SM-challenged animals revealed alterations in cell populations that were consistent with the collected hematology data. In agreement with a previous report published in mice exposed to SM through intraperitoneal injection, B-cells were relatively more affected than T-cells following SM challenge. In SM-challenged animals, the percentage of CD4+ helper T-cells and CD8+ cytotoxic T-cells was found to be down- and upregulated, respectively. Interestingly, even 20 years after exposure, severely affected SM victims of the Iran–Iraq war were found to have lower CD4+ helper T-cell levels and higher CD8+ cytotoxic T-cell levels, a finding that is consistent with the data produced in this study.

The depressive effects of SM on bone marrow are well described. Consistent with published reports of bone marrow toxicity, flow cytometry analysis of terminal bone marrow samples collected from SM-challenged animals revealed decreases in mesenchymal stem cells and hematopoietic stem cells. Although the bone marrow is extremely sensitive to SM-induced alkylation because of its high proliferation rates, the effects of SM observed on this study were not permanent as stem cell percentage levels recovered to that of vehicle controls at 35 days postchallenge. Consistent with these data, interstrand crosslinks in the DNA of bone marrow tissue collected from SM-exposed rats were found capable of repair.

Lastly, it is worthwhile to note the observed sex-specific differences in robustness of response. It is possible that the estrus cycle of female animals and resulting fluctuations in
hormonal milieu remain a potential study confounder, as studies have reported of the benefit of estrogen in regard to overall immune system function.\textsuperscript{25} As such, given the more robust response of males to SM challenge compared with females, restricting efficacy screening initially to a single sex would ease cost, throughput, and data interpretation.

CONCLUSIONS

The goal of this study was to develop an economical, reproducible, high-throughput animal model of SM intoxication that specifically focuses on (and mitigating) the hematologic deficits. Collectively, the data produced in this study provide a solid foundational framework in which different hematologic toxicity endpoints could be employed as a screening tool to identify efficacious MCMs. Depending on the putative mechanism of action of the candidate MCMs, the desired endpoint(s) can then be utilized to monitor improvements in the depth and/or length of a cell count nadir to provide the most specific readout of therapeutic efficacy. Although hematologic analysis of collected blood is unquestionably required to determine an improvement in the cell profiles of countermeasure-treated animals, additional endpoints such as clinical observations, body weights, and flow cytometry analysis of bone marrow samples would provide added value.

Although an IV model of SM intoxication is not representative of a real-world exposure scenario, this study supports the systemic toxicologic relevance of this approach by replicating key aspects of published clinical reports in human SM victims. Most notably, the post-SM exposure temporal progression of hematologic toxicity observed in IV challenged rats aligns with clinical findings, characterized by an early-onset lymphopenia followed by a delayed-onset neutropenia and thrombocytopenia.\textsuperscript{8} These key aspects should provide an appropriate small animal platform for initial proof-of-concept efficacy testing of candidate MCMs, particularly for countermeasures aimed at increasing immune cell populations after SM exposure. Although it remains unclear if explicitly boosting a single cell population to treat SM-induced myelosuppression will provide sufficient protection from either serious morbidity or lethality after a realistic scenario of SM exposure, it is more likely that mitigation of the hemopoietic deficits must accompany therapies specifically prescribed to treat the primary dermal, ocular, or pulmonary injuries.

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SUPPLEMENTARY MATERIAL

Supplementary material is available at Military Medicine online.

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CONFLICT OF INTEREST STATEMENT

The author’s declare no conflicts of interest.

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