Procedure Type: Compound Administration/Drug Administration Procedure Title: Thioglycollate Induction of Activated Macrophages Species: Mouse Pain/Distress Category: D

Background Information:

Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

General compound administration guidelines: Doses will comply with ACUC Guidelines for "Dosing Techniques and Limits." If doses will not comply with ACUC Guidelines, insert variation with justification below in the section: "How does this procedure fit into or address your overall research goals?"

Provide a list of all compounds that will be administered, dosage, route(s) of administration, and any known toxicities in the Other Agents Utilized tab.

Procedure Description Tab:

Procedure Description: (select all that apply):

Procedure	Procedural Description			
Thioglycollate				
Induction of	Procedural Steps:			
Activated	1. Following the steps outlined below, inject mice			
Macrophages	intraperitoneally with \leq 3ml of sterile thioglycollate medium (3%			
	w/v of an autoclaved stock prepared from dehydrated			
	thioglycollate medium and sterile saline water). Dose volume for			
	mice ≥ 2 months of age is 2-3 ml and is 1-1.5 ml for mice that are <			
	2 months.			
	IP Procedural Steps:			
	a. Animals will be restrained manually or placed in a plastic			
	decapicone bag to facilitate restraint. Restraint will be < 5			
	minutes.			
	b. The injection is made using a 22-27 gauge needle in the left or			
	right lower quadrant of the abdominal cavity, in between the			
	midline and medial side of the hind leg to avoid the liver and the			
	bladder. The needle should be angled 20-30 degrees relative to			
	the animal to avoid penetration of the abdominal organs.			
	c. Prior to injecting the compound, pull back gently on the			
	plunger to confirm placement. If fluid (e.g., blood, urine or			
	intestinal contents) is aspirated, discard needle, syringe, and			
	solution due to contamination, and repeat with fresh supplies.			

Once proper placement has been confirmed, slowly inject solution. d. If bleeding occurs at the injection site, apply pressure until
hemostasis is achieved.
e. Return mouse to its cage.
Note: To reduce discomfort, use a new needle for each animal, bring solutions for injection to 37°C, and avoid injecting material with a high or low pH.
2. Mice will be euthanized for recovery of peritoneal inflammatory cells at various time points following injection. Leukocytes will accumulate rapidly in normal mice in response to the injection (e.g., large numbers of cells can be recovered after 24-48 hours). Some strains may require up to 4 days to produce adequate numbers of cells, so the study will last 1-4 days post-injection, after which animals will be euthanized as outlined in the Animal Disposition section.
Potential Adverse Events: Lethargy, weight loss, perforated bowel or bladder, peritonitis, damage to internal organs.

How does this procedure fit into or address your overall research goals? (Insert protocol-specific rationale here.)

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.

Mice will be examined immediately following compound administration and daily thereafter. Mice will be assessed for general appearance and activity level, as well as the potential adverse events listed above.

Treated mice will be monitored on a daily basis for signs of discomfort, including hunched posture, ruffled fur and lack of movement around the cage. Body condition score (BCS) index will also be used to evaluate the welfare of mice. If the animals show signs of discomfort or a BCS \leq 2, they will be euthanized.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If animals are moribund or if any other abnormal signs are noted, an OLAC veterinarian will be contacted or the mouse will be euthanized immediately.

Anesthetic Regimen Tab:

Not applicable

Peri procedure Care/Analgesics Tab:

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Recovery Location Building Name: (Insert protocol-specific information here.) Room Number: (Insert protocol-specific information here.) Responsible Personnel: (Insert protocol-specific information here.)

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

Treated mice will be monitored on a daily basis for signs of discomfort, including hunched posture, ruffled fur and lack of movement around the cage. Body condition score index will also be used to evaluate the welfare of the mice.

Monitoring Duration

Mice will be examined immediately following injection(s) as well as the following day. Mice will be monitored for general appearance and activity level as well as the potential adverse events listed under the Procedure Description.

Monitoring Frequency

Mice will be observed with sufficient frequency to ensure that they are euthanized according to established endpoints.

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be consulted or the mouse will be euthanized.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened food may be provided on the cage floor to stimulate appetite.

Describe record keeping/documentation methods for post-procedure monitoring:

A record of the compound administered, the date, and the animal's ID will be kept in the laboratory notebook.

Other Agents Utilized Tab:

Agent Name Dosage (in mg/kg if possible) and volume Route Describe timing, frequency and duration of administration.	Age	Agent Name		Route	frequency and duration of
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	Dose volume for mice ≥2 months of age is 2-3 ml and is 1-1.5 ml for mice that are < 2 months.	Intraperitoneal (IP)	Administered once.

Literature Search for Alternatives:

Suggested Keywords Thioglycollate, macrophage collection, mouse, alternatives, refinement