PROCEDURE PROTOCOL FOR USE OF ANIMALS

LIPID MAPS Protocol ID PP0000001100
Version 1, 11-7-05

Vertebrate Animals

The care and use of laboratory animals at ((Your Institution)) is in accordance with the principles and standards set forth in the Principles For Use Of Animals (NIH Guide for Grants and Contracts), The Guide for the Care and Use of Laboratory Animals (DHEW, PHS, NIH Publ. No. 85-23, Rev. 1978) and the provisions of the Animal Welfare Acts (P.L. 89-544 and its amendments), and other applicable laws and regulations.

1. Proposed use of animals

The proposed studies will use mice to study the lipid metabolism and its regulation in primary peritoneal macrophages. Macrophages will be isolated from euthanized mice and studied in vitro. Two minor procedures will be performed; removal of the distal two thirds of the tail for genotype determination and injection of thioglycolate or bio-gel beads into the peritoneal cavities of adult mice to increase the numbers of macrophages that can be obtained from a single mouse. The number of mice used will be approximately 200 per year. Several strains will be used, including DBA, B6D2, and C57BL6/J. Both males and females will be used, ranging in age from birth to one year.

2. Justification

Mice are extensively used for studies of macrophages and currently represent the most powerful animal model for the study of gene expression. The ability to selectively disrupt individual genes allows the study of gene function both in vivo and in vitro using cells obtained from genetically engineered animals. Studies in mice form the basis for subsequent clinical investigation in humans. The strains listed correspond to the available strains that have been engineered to contain deletions in genes encoding proteins involved in lipid sensing and metabolism. The number of mice has been estimated based on the requirements for macrophages to be for mass spectrometry analysis of various lipid species. Each experiment will generally contain eight to ten experimental groups and experiments are performed at least three times to assure reproducibility.

3. Veterinary Care

All mice are housed in special facilities in vivariums of ((Your Institution)) Veterinary care is under the direction of ((Your Institution Veterinarian)). The Office of Animal Resources is staffed by three full-time and two part-time veterinarians, 24 hours a day, 7 days a week. The facilities have AAALC and U.S. Department of Agriculture approval. Experimental protocols involving animals are reviewed by the campus veterinarian and by the ((Your Institution)) Animal Core Committee.

4. Procedures to minimize pain and discomfort

To genotype mice, two-thirds of the tails will be removed. Anesthesia will be by Methoxyfluorane at up to 3%, and bleeding controlled by cauterization. To obtain large numbers of murine macrophages for lipid analysis, the peritoneal cavity will be injected with thioglycollate or Bio-Gel spheres. Thioglycollate broth will be prepared according to manufacturer instructions. Two and one half ml of thioglycollate or one ml of 2% bio-gel will be injected per mature mouse intra-peritoneally. The cell infiltrate will be harvested by peritoneal lavage three to five days later.

Bio-Gel will be used to obtain largely non-activated macrophages. One ml of a 2% (w/v) suspension of Bio-Gel P100 fine (45 to 90 μm, Biorad Richmond, CA) will be prepared in PBS, autoclaved to sterilize and de-gas, and injected intra-peritoneally. Exudate cells will be collected by lavage four to five days post-injection. Peritoneal cells are harvested following asphyxiation with CO_2 by lavaging the peritoneal cavity with PBS or balanced salt solutions.

5. Euthanasia

Macrophages will be collected from mice immediately following euthanasia. Euthanasia of mice will be accomplished by either cervical dislocation or the use of carbon dioxide according to the approved procedures of the American Veterinary Medical Association Panel on Euthanasia.

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