

The ACC would like to thank Dr. Linda Shapiro for allowing us to use an excerpt from one of her protocols as an example for the search for alternatives section of the animal care and use protocol form. Dr. Shapiro acknowledged that this was written with help from Dr. Adam Adler.

Alternatives to Painful Procedures:

- a. For D and E category proposals: Provide a written statement of the methods and sources used to determine that alternatives to potentially painful/distressful procedures are not available. The following areas must be addressed: **reduction** of animal numbers— the number of animals used should be the minimum necessary to achieve scientific goals; **refinement** of procedures to eliminate or minimize pain and distress must be considered— procedures should be used that have the least amount of potential pain, discomfort, distress, or morbidity; and **replacement** of animals with non-animal alternatives— when objectives can be achieved using reasonably available non-animal models, the alternative(s) should be used. If alternatives to painful or distressful procedures exist, but were not chosen, explain the reasons for not using the alternatives. If literature searches are used a method for alternatives search, please provide: 1. the date of the literature search; 2. the years searched; 3. name of the databases searched (must be at least 2); and 4. the search strategy used. Each potentially painful/distressful procedure must be addressed.

**Searches**

We have performed MEDLINE and CRISP queries (1/10/07) covering the years 1996 – 2006 to determine that there are no less painful/distressful alternatives to the procedures described in this protocol. The keywords used were:

Inflammation, leukocyte trafficking, leukocyte migration, adhesion molecules, neutrophil adhesion, monocyte adhesion, leukocyte adhesion, primary monocyte production and isolation, aminopeptidase, CD13/APN, PSMA, clinical trials, mouse models of inflammation, peritonitis, intravital imaging.

In addition, the appropriateness of these protocols and animal numbers are based on conversations with experts in the field and information gained regarding state of the art techniques from scientific meetings.

**Refinement** The current protocol has inherent procedures to limit pain and discomfort in the animals. Injections of thioglycollate and antibodies will be performed under anesthesia and will only be performed if the mice are deep under narcosis. The mice used in the peritonitis model are expected to develop acute peritonitis. As outlined, the procedures will induce adverse health and animals will be carefully monitored according to the distress guidelines outlined in the "**Guide for the Care and Use of Laboratory Animals**"

(<http://www.nap.edu/readingroom/books/labrats/>). However, any and all animals exhibiting signs of severe illness (such as weight loss, extreme loss of motility or moribund state, open wounds, urinary dysfunction) will be humanely euthanized immediately. Animals, food, and water will be inspected daily and cages and bedding will be changed periodically.

**Reduction** The number of animals required for the outlined experiments has been carefully considered to obtain statistically significant results using the least number of animals based on published studies using these

protocols and considering the variability inherent in any disease model. Multiple measurements will be taken from a single mouse whenever possible in an attempt to minimize the number of mice required for these studies.

**Replacement-** The nature of our investigations (the role of cell surface peptidases in *in vivo* inflammation) precludes the use of alternative, non-animal models because *in vitro* models cannot reproduce the living animal.