

POLICY ON ASCITES PRODUCTION IN MICE AND RATS

Introduction

In November, 1997, the NIH/OPRR (now OLAW *) issued a report directing IACUCs to “... critically evaluate the proposed use of the mouse ascites method. ... IACUCs must determine that (1) the proposed use is scientifically justified; (2) methods that avoid or minimize discomfort, distress, and pain (including *in vitro* methods) have been considered; and (3) the latter have been found unsuitable” (<http://grants2.nih.gov/grants/olaw/references/dc98-01.htm>). A 1999 report commissioned by the NIH summarizes the findings of the National Academy of Sciences concerning scientific justifications for the production of monoclonal antibodies by ascites, including ways to minimize any pain or distress that might be associated with ascites production (<http://grants1.nih.gov/grants/policy/antibodies.pdf>).

* Effective March 2, 2000, the Division of Animal Welfare, Office for Protection from Research Risks (OPRR) became the NIH Office of Laboratory Animal Welfare (OLAW) -- <http://grants.nih.gov/grants/olaw/olaw.htm>.

ACUC Guidelines for Antibody Production by the Ascites Method

In vitro methods of monoclonal antibody production must be employed when 100 mg or less total antibody is required, unless scientific justification can be provided for the use of *in vivo* methods. When more than 100 mg of antibody is required, *in vitro* methods must be considered. In some cases, *in vitro* production of monoclonal antibody may not meet the scientific aims of the project. The demonstration that a hybridoma cell line does not adapt well to *in vitro* conditions, for example, or that ascites is essential to the experimental design could provide necessary scientific justification for the use of *in vivo* methods.

Pristane Priming:

The volume of pristane (2,6,10,14- tetramethylpentadecane, a branch-chain component of mineral oil) injected intraperitoneally into each animal should not exceed 0.5 ml. Based upon the literature and experience the preferred priming agent is pristane. Alternative priming agents, such as Freund's Incomplete Adjuvant, may be used if a scientific justification is provided.

Hybridoma Inoculation:

Hybridoma cells should be in log phase growth, in isotonic PBS or culture medium (without serum), and injected intraperitoneally 10 to 14 days following pristane priming.

Abdominal Paracentesis:

Animals should be monitored daily for signs of ascites production. Ascites pressure should be relieved by paracentesis when visible abdominal distension becomes apparent. A 20 to 19 gauge needle should be used for ascites collection. The maximum number of abdominal “taps” is limited to four (4). The final tap should be performed after the animal has been euthanized. Animals that develop marked abdominal distention with associated clinical signs of pain or distress should be euthanized. Post-paracentesis mice should be monitored for 30 minutes for the development of circulatory shock. The presence of roughened fur, hunched posture, inactivity, pallor of the ears and eyes, dehydration, weight loss, inactivity, difficulty in ambulation, tachypnea and dyspnea at any time during the procedure are signs of distress and warrant euthanasia of the animal.

Additional information about antibody production and monoclonal antibody production by ascites is available on-line at:

<http://www.nal.usda.gov/awic/newsletters/v8n3/8n3mcard.htm>.

<http://oacu.od.nih.gov/ARAC/ascites.pdf>.

<http://www.ccac.ca/english/gdlines/antibody/antibody.pdf>.

<http://www.nal.usda.gov/awic/newsletters/v8n3/8n3oprr.htm>.

***In vitro* methods:**

Information on a simple, laboratory scale, single use *in vitro* bioreactor for monoclonal antibody production can be found on line at: <http://www.biovectra.com/BioChem/Products/3525.htm>.

The University of Virginia **Lymphocyte Culture Center**, a core research support facility of the School of Medicine, is expert in the use of these bioreactors and can provide *in vitro* monoclonal antibody production and purification on a fee for service basis.