Monoclonal Antibody Production in Mice via Ascites

Purpose
These guidelines address the importance of using in vitro methods of antibody production, the scientific justification required if animals are proposed for use, as well as the care and use of mice bearing ascites tumors in instances when the in vivo method is scientifically justified.

Background
Monoclonal antibodies are exceptionally powerful research tools and have potential clinical uses. There is increased availability and use of tissue-culture systems for the generation of monoclonal antibodies. The Guide for the Care and Use of Laboratory Animals and the PHS Policy on the Humane Care of Laboratory Animals requires that in vitro methods be considered prior to in vivo methods. The Institute of Laboratory Animal Research Executive Summary recommendations include the following statements:

1. The routine use of in vitro methods is preferred;
2. The use of the mouse ascites method should not be banned; and
3. When the mouse ascites method is used efforts are made to minimize pain and distress.

Policy
Alternative methods, rather than in vivo production, must be considered before any in vivo methods are approved. The use of in vivo methods (i.e. mouse ascites) requires scientific justification, which may include, for example:

1. Failure of a cell line to adapt to in vitro culture.
2. Purification methods lead to denaturation or decreased antibody activity.
3. Contamination and resulting loss of utility of the cell line.
4. Inability of the cell line to maintain production of monoclonal antibodies.

If an investigator is unable to provide adequate justification for in vivo use, the IACUC may permit simultaneous evaluation of in vivo and in vitro methods for a period of approximately 12 months. At this time, the Principal Investigator must provide the IACUC with convincing documentation that the production of the desired antibody using in vitro methods will not be feasible.

If a researcher prefers to use an off-campus organization for the production of custom antibodies in animals, that organization must have an Animal Welfare Assurance on file with NIH/OLAW. Antibodies are
considered customized if produced using antigen(s) provided by or at the request of the investigator (i.e., not purchased "off-the-shelf").

Sensitization protocols may vary, however Complete Freund’s Adjuvant (CFA) may be used only once during the immunization process. Further immunizations must use Incomplete Freund’s Adjuvant (IFA) or the antigen alone. When sufficient antibody titers are reached mice are euthanized and the spleen removed for cell fusion.

**Priming** of the mouse peritoneal cavity using pristine must not exceed 0.2 ml, as higher doses cause noticeable distress^4-6^.

**Ascites** production is initiated by injection of hybridoma cells into the peritoneal cavity. The development of ascites leading to abdominal distention results in discomfort and distress^7^. The mice must be observed and the observations documented a minimum of twice daily by the investigator. Mice must be weighed prior to injection and a minimum of every other day beginning the day after hybridoma injection. Ascites fluid must be collected before body weight becomes 20% greater than the weight obtained prior to the injection, the abdominal distention is greater than a typical pregnant mouse, the body condition score deteriorates, or if mice are unable to reach food or water.

**Ascites fluid collection** is a one time collection procedure performed on mice that haven been euthanized. Multiple peritoneocentesis is not allowed because^7,8^:

1. Mice with ascites show signs of pain and distress, including decreased activity, and decreased feed consumption.
2. Survival times decrease with additional peritoneocentesis.
3. Ascites tumors disseminate with time.
4. Removal of ascites fluid results in circulatory shock.
5. Body condition score deteriorates over time as tumor burden increases.

**References**

6. Colwell DE, Michalek SM, and McGhee JR. Method for generating a high frequency of hybridomas


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