

Clemson University

Institutional Animal Care and Use Committee (IACUC)

Policy Number: 4.3

Policy Title: The Use of the Mouse Ascites Model for Monoclonal Antibody Production and the Purchase of Custom Antibodies

Section 1: Purpose

The following policy provides a minimum standard for the use of the mouse ascites model for monoclonal antibody production and the purchase of custom antibodies.

Section 2: Scope

This policy applies to all animal use protocols that involve the induction of ascites in mice for the purpose of producing monoclonal antibodies. The policy also applies to personnel purchasing antibodies produced by a supplier specifically for a research project using a custom technique or materials (custom antibody production).

Section 3: Policy

Guidelines

1. Tissue-culture methods for the production of mAb are the default method unless there are clear scientific reasons why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product.
2. When the mouse ascites method for producing mAb is used, every reasonable effort should be made to minimize pain or distress, including frequent observation, limiting the number of taps [i.e. peritoneocentesis], and prompt euthanasia if signs of distress appear.
3. The specific guidelines for consideration by Principal Investigators when developing animal study proposals and for the IACUC when reviewing proposals involving the mouse ascites method are:
 - a. The volume of the priming agent should be reduced to as small a volume as necessary to elicit the growth of ascitic tumors and at the same time reduce the potential for distress caused by the irritant properties of the priming agent. Although 0.5 ml Pristane has been standard for adult mice, 0.1-0.2 ml has been found to be as effective for many hybridomas.
 - b. The time interval between priming and inoculation of hybridoma cells as well as the number of cells in the inoculum are determined empirically. Inocula range from 10^5 - 10^7 cells in volumes of 0.1 - 0.5 ml and are usually administered 10 - 14 days after priming. Generally, very high concentrations are associated with greater mortality and concentrations $< 1 \times 10^5$ cells elicit fewer ascitic tumors and these tend to have a smaller volume yield. Cell suspensions should be prepared under sterile conditions in physiological solutions.

- c. Hybridomas should be MAP (mouse antibody production) or PCR tested before introduction into the animal host to prevent potential transmission of infectious agents from contaminated cell lines into facility mouse colonies and possibly to humans handling the animals.
 - d. Animals should be monitored at least once daily, seven days a week by personnel familiar with clinical signs associated with ascites production and circulatory shock. Mice should be weighed daily beginning 5 days after hybridoma injection and no more than 20% weight gain should be allowed from baseline weight.
 - e. Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort or interfere with normal activity. Manual restraint or anesthesia may be used for tapping. Aseptic technique should be used in withdrawing ascitic fluid. The smallest needle possible that allows for good flow should be used (18-22 G needle).
 - f. Animals should be monitored frequently over several hours following the tap to observe possible signs of shock due to fluid withdrawal. Pale eyes, ears and muzzle and breathing difficulties are indicative of circulatory shock. Shock may be prevented or treated with 2 -3 ml warm saline or lactated ringers administered subcutaneously.
 - g. The number of taps should be limited, based on good body condition of the animal. A maximum of three survival taps (the 4th being terminal) are recommended. Additional taps should have individual IACUC approval. Aseptic technique should be used. Manual restraint or anesthesia may be used. If the exudate is bloody, cloudy or particulates are observed grossly, even after the first tap, the mouse should be euthanized immediately.
 - h. Animals should be euthanized appropriately before the final tap or promptly if there is evidence of debilitation, pain or distress. Signs of distress include hunched posture, rough hair coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems, and solid tumor growth.
4. Prior to approval, the IACUC must determine that:
- a. The proposed use is scientifically justified
 - b. Methods that avoid or minimize discomfort, distress, and pain have been considered (this includes in vitro methods)
 - c. In vitro methods have been found unsuitable.
5. Justifications for in vivo monoclonal antibody production
- a. When a supernatant of a dense hybridoma culture grown for 7-10 days (stationary batch method) yields a monoclonal antibody concentration of less than 5 mg/ml, or if other systems are used and concentrations obtained are less than 500 mg/ml (hollow fiber system) or 300 mg/ml (semi-permeable membrane system).
 - b. When more than 5 mg of monoclonal antibody produced by each of five or more different hybridoma cell lines is needed simultaneously. It is technically difficult to produce this amount of monoclonal antibody since it requires more monitoring and processing capability than the average laboratory can achieve
 - c. When analysis of monoclonal produced in tissue culture reveals that a desired antibody is diminished or lost.
 - d. When a hybridoma cell line grows and is productive only in the animal.

- e. When more than 50 mg of functional monoclonal antibody is needed, and previous poor performance of the cell line indicates that hollow-fiber reactors, small- volume membrane-based fermentors, or other techniques cannot meet this need during optimal growth and production.

Custom Antibodies

OLAW has made the determination that purchase of custom made antibodies produced in animals requires IACUC approval. Accordingly, Clemson University requires an animal use protocol be submitted for activities requiring custom made monoclonal or polyclonal antibodies. Custom made refers to antibodies made from specific antigens that is then used to immunize animals to produce antibodies. This applies to situations where a Clemson researcher contracts with a commercial entity and provides them with the immunizing polypeptide or requests them to generate a specific antigen which they then use for the purpose of generating antibodies commercially. IACUC approval of the purchase of custom antibodies can be expedited by completing and submitting the IACUC form “Animal Use Protocol for the Commercial Production of Custom Antibodies”

<http://media.clemson.edu/research/compliance/arc/custom-antibodies.docx>

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