

I. Introduction

The ACC recognizes that monoclonal antibodies (MAbs) are important reagents used in biomedical research, in diagnosis of diseases, and in treatment of such diseases as infections and cancer. Antibody producing cells are created by the fusion of an immortal cell line and splenocytes harvested from a mouse immunized with an antigen of interest. MAbs are produced using either in vitro (culture of antibody producing cells in various devices) or in vivo (mouse ascites model) methodologies. Whenever possible, MAbs should be produced using in vitro methods; however, it is recognized by the scientific community and the ACC that under some circumstances MAbs must be produced using in vivo methods. If in vivo methods are required to produce MAbs, efforts should be directed at implementing procedures targeted at reducing pain and distress. This includes limiting the methodology to highly trained/skilled individuals familiar with the procedures.

The following guidelines for the production of MAbs were developed to ensure consistency of technique and to minimize potential pain and distress.

II. Institutional Guidelines for Experiments involving Ascites Producing Tumors:

1. The University of Illinois at Chicago Biologic Resources Laboratory is the only campus service unit recognized by the ACC for the production of monoclonal antibodies. Investigators interested in this service should contact Dr. James Artwohl at (312) 996-1217.
2. Priming of the peritoneal cavity is typically accomplished through an intraperitoneal injection of pristine. The pristine priming dose should not exceed 0.2 ml IP. If incomplete Freund's adjuvant is used for priming it is recommended that 0.2 ml be administered IP.
3. After priming, a hybridoma cell suspension is injected into the peritoneal cavity of the animal. This leads to the development of a tumor and subsequent ascites. Factors that affect the survivability of the animal include the type of tumor and number of cells inoculated. Current standard protocols usually call for the injection of 10^6 cells, which lead to survival times of approximately 17-20 days.
4. Animals should be weighed at the time of hybridoma inoculation. Thereafter animals should be monitored for signs of illness and weighed at least daily beginning no later than the 5th day after inoculation.
5. Ascites should be removed by abdominal tap when a body weight increase of 20-25% of the baseline occurs. Thereafter, the body weight increase should not exceed 25% of the baseline and a four-day period from the time of first collection through the third and final collection should be targeted.
6. Removal of peritoneal fluid (20 gauge needle or smaller) should be performed by trained personnel using appropriate sedation and/or analgesia.
7. Mice should not undergo more than three abdominal taps to collect ascites. The third tap is to be done as a terminal procedure.
8. The ACC recognizes that there is variability among hybridoma lines and that the kinetics of certain lines might allow for more than three taps. A PI can therefore request more than three taps if they can

scientifically justify the request based upon hybridoma kinetics and animal well-being. A reduction of animal numbers is not an adequate justification.

9. The ACC recognizes that there is variability among animals and hybridoma lines and any animal exhibiting clinical signs consistent with pain and distress (such as roughed hair coat, hunched posture, decreased activity, tachypnea, dyspnea and pallor of ears and muzzle) or hemorrhagic/cloudy ascites should be euthanized even if it is before the third abdominal tap.

III. Completion of the UIC Protocol for Animal Use:

When producing monoclonal antibodies, the Principal Investigator must recognize the potential for pain and distress as he/she completes the UIC Protocol for Animal Use. He/she must describe the procedures that will be used to minimize pain and/or distress in Form A, item 8g. In addition, Form A, item 13 (including a statement justifying why in vitro alternatives are not appropriate) and Form B, item 9, must be completed to gain approval from the UIC Animal Care Committee.

If there are any questions regarding monoclonal antibody production in animals contact a member of the BRL veterinary staff at 312-996-7040.

Reference:

- *Monoclonal Antibody Production, A Report of the Committee on Methods of Producing Monoclonal Antibodies. National Academy Press, Washington, DC, 1999.*
- *Peterson, N.C., Behavioral, Clinical and Physiologic Analysis of Mice used for Ascites Monoclonal Antibody Production, Comp. Med., 2000, 50 (5) 516-526.*