UBC ANIMAL CARE COMMITTEE
POLICY 010
MONOCLONAL ANTIBODY PRODUCTION POLICY

Date Approved: July 28, 2008

Background

Monoclonal antibodies (mAb) are used extensively in basic biomedical research, in diagnosis of disease and in the treatment of illnesses, such as infection and cancer. The production of a mAb requires immunization of an animal, usually a mouse, obtaining immune cells from the spleen and making them immortal by fusing to a cancer cell to form a hybridoma, which actively secretes the mAb. To obtain sufficient quantities of mAb, the hybriboma cells must proliferate and there are two methods for growing these cells: injecting them into the peritoneum of a mouse or using in vitro cell culture methods. Historically, the former, in vivo method, was commonly used. However, with this method, the injected hybridmoma cells promote production of ascites fluid. While this fluid contains high concentrations of the mAb, excessive ascites fluid and the need for single or multiple sampling of the fluid can cause significant pain and distress to the animal. The in vitro cell culture method avoids these problems and has now become a viable alternative to the in vivo method, except in rare circumstances. Accordingly, the UBC Committee on Animal Care (ACC) strongly advocates the use of the in vitro method for mAb production.

Policy

mAbs should only be produced using the in vitro cell culture method, following immunizing animals with the protein of interest. The in vivo, mouse ascites method for production of mAb will only be approved when there is sufficient justification, which must be detailed in the ACC Protocol Form.

Procedures

1. If a researcher wishes to use the in vivo mouse ascites method for mAb production, the ACC protocol form should document the specific reasons why the in vitro method cannot be employed. Reasons that will be considered by the Committee include:

- The hybridoma cell lines generated do not adapt well to tissue-culture conditions.
- Downstream purification or concentration from in vitro systems leads to protein denaturation and decreased antibody activity.
- The tissue culture method yields mAb that do not reflect the normal modification of proteins with sugars and influence binding capacity and other critical biologic functions of mAb.
- The contamination of valuable hybridoma cell lines with fungi or bacteria requires prompt passage through a mouse to save the cell lines.

2. If the ACC does grant approval for in vivo mAb production, the researcher must adhere to the following procedural guidelines:

- The route of injection should be subcutaneous or intra-peritoneal.
- The maximum volume for hybridoma injection in a mouse should be 0.50 ml (500 μL).
- If priming is required, the lowest possible dose should be employed and the volume not exceed 0.20 ml and the injection can only be given once.
- Use of Freund's adjuvant for priming is not encouraged if other less irritating adjuvants or no adjuvant can be employed. If any priming agent is used, careful and documented monitoring of the animal is required post-injection.
- If blood sampling is required, a sampling method approved by the ACC must be used.
- Animals must be weighed daily. When weight is 20% above baseline, sampling of ascites fluid must occur.
- Non-terminal sampling of ascites fluids can occur only once, but a second sample. can be collected following euthanasia.
- The mouse should be sedated or anesthetized for the non-terminal sampling of ascites fluid and no larger than a 22 gauge needle should be used.
- Animals displaying distress, pain or loss of body condition must be euthanized immediately.
- A rigorous monitoring protocol, involving a score sheet, must be in place with daily documentation of the animals' condition and more frequent monitoring should be initiated if there is any deviation from normal appearance and behavior.

Additional Information can be found in the following publications:

Monoclonal Antibody Production: A Report of the Committee on Methods of Producing Monoclonal Antibodies Institute for Laboratory Animal Research, National Research Council, National Academy Press, Washington DC, 1999.

Guidelines on Monoclonal Antibody Production, National Health & Medical Research Council of Australia, 2001.