MONOCLONAL ANTIBODY PRODUCTION

1. PURPOSE

1.1. The intent of this Animal Care and Use Procedure (ACUP) is to describe monoclonal antibody production and ascites fluid collection in mice. This ACUP is approved by the Cornell Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its application.

2. SCOPE

2.1. This ACUP is intended for use by Cornell investigators and Center for Animal Resources and Education (CARE) at Cornell University staff.

3. INTRODUCTION

3.1. Monoclonal antibodies (MAb) are immunoglobulins which have selected antigen specificity and are usually produced from a single clone of hybridoma cells. In vitro methods are to be used for the production of monoclonal antibodies (MAb) unless there are clear scientific reasons why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product (see SECTION 9.3 for examples of scientific justification for the use of mice in the production of MAb).

3.2. If an in vivo method is justified, the antibody producing cell line is introduced into mice for expansion via ascites (accumulation of fluid in the abdomen). The purpose of this document is to describe the methods by which ascites is induced in and collected from mice, and the limits that must be followed in the interest of animal welfare.

3.3. Contact CARE at care@cornell.edu for more information or for assistance.

4. MATERIALS AND EQUIPMENT

4.1. Abdominal tap materials (e.g. 20-22 gauge needles, syringes, and antiseptic)
4.2. Euthanasia materials (e.g., CO₂ chamber)
4.3. Scale for weighing mice
4.4. Sterile Fluids (0.9% saline or Lactated Ringer’s Solution)
4.5. A priming agent (i.e. Freund’s Incomplete Adjuvant (FIA) or Pristane)
4.6. Hybridoma cell suspension
5. PROCEDURES

NOTE: See section 9.1 for parameters affecting ascites production. See section 9.2 for clinical and pathophysiological effects of ascites production.

5.1. Animal Selection

5.1.1. Use mice of the same strain (syngenic) for immunization to produce the hybridoma clone and subsequently to produce a histocompatible MAb.

NOTE: BALB/c mice are often the strain of choice, as many of the parental myeloma cells used in the fusion processes are derived from BALB/c mice. The use of female retired breeders is advantageous since the relaxed abdominal musculature will better tolerate ascites.

5.2. Priming

5.2.1. Priming agents prevent impairment of hybridoma cell growth and cause serous fluid secretion into the peritoneal cavity.

5.2.2. Prime adult female mice (minimum 6 weeks old, ideally retired breeders) with Freund’s Incomplete Adjuvant (FIA) or other priming agent, such as Pristane.

5.2.3. Administer the priming agent into the peritoneal cavity (IP) once. If administering FIA as the priming agent, do not exceed a volume of 0.3 mL IP. If administering Pristane as the priming agent, do not exceed a volume of 0.2 mL IP (see ACUP 401 Recommended Volumes for Administered Substances for further guidelines).

5.2.4. Wait 7-10 days before injecting the hybridoma cell suspension (obtained via in vitro cell culture propagation).

5.3. Hybridoma Inoculation

5.3.1. Prior to injection, test hybridoma cells for the presence of adventitious viral and Mycoplasma agents with the Mouse Antibody Production (MAP) test or PCR techniques (see ACUP 619 Tumor and Cell Line Testing).

5.3.2. Inject primed mice (IP) with up to 3 x 10⁶ hybridoma cells in a maximum volume of 1.0 mL. (see ACUP 401 Recommended Volumes for Administered Substances for further guidelines).

5.3.3. Monitor mice at least once daily for the first 7 days following hybridoma injection.

5.3.4. Once ascites is noted (abdominal swelling is typically apparent within 7-10 days of hybridoma injection), assess mice at least once every 12 hours.

5.4. Ascites / Tumor Growth

5.4.1. Weigh mice daily beginning 4 days after inoculation to monitor progression of the ascites producing hybridoma. Document the weight of each mouse and retain for laboratory records.

5.4.2. Do not allow mouse body weight gain to exceed 20% of the normal body weight of age- and sex-matched animals of the strain (see ACUP 402 Humane
Intervention Points for further details). Promptly address any other observations of unusual behavior or symptoms of pain or distress (e.g., page CARE veterinary staff at 1-800-349-2456).

5.5. Ascites Fluid Collection

5.5.1. A maximum of three survival taps (the 4th being terminal) are permitted.
5.5.2. If the abdomen of the mouse is visibly distended, administer 1-2 mL of replacement fluids (0.9% saline or Lactated Ringer’s Solution) subcutaneously prior to collection of ascites.

NOTE: Fluid removal carries the risk of hypovolemic shock, hemorrhage, edema and death; thus, fluid replacement is necessary.

5.5.3. Perform the abdominal tap by inserting a hypodermic needle into the caudal aspect (i.e., closer to tail than the chest) of the abdominal cavity with the hub of the needle at an approximate angle of 30 degrees.

NOTE: A large gauge needle permits rapid collection of the viscous ascites fluid; however, needles larger than 20 gauge can cause significant tissue damage

5.5.4. Collect fluid that flows from the needle into a sterile container via gravity. A maximum of 4-5 mL of ascites fluid may be collected at the first (i.e. survival) tap.
5.5.5. Palpate the abdomen of the mouse to determine the presence of an intra-abdominal discreet, palpable tumor.

NOTE: Humane euthanasia may be indicated if a mass is palpated (see ACUP 402 Humane Intervention Points for further details).

5.5.6. For survival taps, monitor the mice closely for the first 60 minutes post-tap. Continue to perform ascites monitoring per sections 5.3.3 and 5.3.4 of this document.

Note: Treat signs of distress with humane euthanasia of the animal (see ACUP 301 Rodent Euthanasia).

6. PERSONNEL SAFETY

6.1. Medical Emergencies: CALL 911.
6.2. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the CARE Occupational Health and Safety webpage for more information.

7. ANIMAL RELATED CONTINGENCIES

7.1. Post contact information for emergency assistance in a conspicuous location within the animal facility.
7.2. Contact CARE veterinary staff for assistance with determination of humane endpoints.
7.3. Emergency veterinary care is available at all times including after working hours and on weekends and holidays by using the CARE (pager 1-800-349-2456)
7.4. Non-emergency veterinary questions & requests for care, email CARE veterinary staff at care@cornell.edu.

8. REFERENCES

8.3. Guidelines for the Use of Adjuvants & Antibody Production (Monoclonal Antibodies), Duke University:
http://vetmed.duhs.duke.edu/GuidelinesforMonoclonalAntibodies.html
8.4. Monoclonal Antibody Production – Mice (SOP #405), McGill University:
8.5. ACUP 301 Rodent Euthanasia:
8.6. ACUP 401 Recommended Volumes for Administered Substances:
8.7. ACUP 402 Humane Intervention Points:
8.8. ACUP 619 Tumor and Cell Line Testing:
8.9. CARE Occupational Health and Safety webpage:
http://www.research.cornell.edu/care/OHS.html
9. APPENDIX

9.1. Parameters Affecting Ascites Production

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
<th>Comment</th>
<th>Reference (see text)</th>
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</thead>
<tbody>
<tr>
<td>Animal Age</td>
<td>Highest MAb(^a) concentrations in mice aged 43-75 days.</td>
<td>Brodeur et al. 1984</td>
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<tr>
<td>Sex Males</td>
<td>Males have a longer period of secretion, higher concentration/mL and greater volume of ascites, probably due to testosterone. Takakura et al. 1967</td>
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<tr>
<td>Strain Nud mice</td>
<td>Nude mice (irradiated mice) and SCID(^a) mice can be used in case of nonsyngeneic hybridoma cells. Yields can be increased by use of BALB/c-derived cross-bred F1 hybrids. Pistillo et al. 1992; Brodeur and Tsang 1986; Stewart et al. 1989</td>
<td></td>
<td></td>
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<tr>
<td>Hybridoma Cells</td>
<td>Some cells show a large variety in growth pattern in animals and tend to grow poorly in some animals. C.F.M.H., personal communication (see text)</td>
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<tr>
<td>Number of Cells</td>
<td>Cell number affects the duration of secretion. Recommended number of cells is between 6 x 10⁵ and 5 x 10⁶ per mouse. Johnson 1995; Brodeur et al. 1984</td>
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<tr>
<td>Primer Product</td>
<td>By using FIA(^b), the interval between priming and hybridoma cell inoculation can be shortened and the animals survive more taps. C.F.M.H., personal communication (see text)</td>
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<tr>
<td>Volume</td>
<td>Optimum volume is 0.5 mL, but a lower volume (0.1-0.2 mL) has also been shown to be effective and to cause less distress. C.F.M.H., personal communication (see text); Gillette 1987; FSU 1998</td>
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\(^b\)Mab: Monoclonal Antibody; SCID, Severe Combined Immunodeficient; FIA, Freund’s Incomplete Adjuvant.

9.2. Clinical, Pathophysiological, and Pathological Effects of Ascites Production

<table>
<thead>
<tr>
<th>Clinical Effects</th>
<th>Pathophysiological Effects</th>
<th>Pathological Effects</th>
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<tbody>
<tr>
<td>Abdominal distension</td>
<td>Anorexia</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>Decreased activity and body mass</td>
<td>Anemia</td>
<td>Infiltrative tumor growth</td>
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<tr>
<td>Shrunken eyes</td>
<td>Dehydration</td>
<td>Adhesions in the abdomen</td>
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<tr>
<td>Difficulty with walking</td>
<td>Tachypnoe</td>
<td>Enlarged abdominal organs</td>
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<tr>
<td>Hunched posture</td>
<td>Circulatory shock</td>
<td>Blood in the abdominal cavity</td>
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<tr>
<td>Respiratory distress</td>
<td>Decreased venous, arterial and renal blood flow</td>
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<tr>
<td>Death</td>
<td>Ascites production</td>
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<td>Immunosuppression</td>
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9.3. **Examples of Scientific Reasons for the In Vivo use of the Ascites Method:**

9.3.1. The hybridoma cell line will not adapt well to in vitro conditions.
9.3.2. In applications where several different mouse MAb are required for injection into mice at high concentrations, the in vitro method can be inefficient.
9.3.3. MAb from mouse ascites might be essential for experiments in which MAb are used in vivo in mice.
9.3.4. Rat hybridoma cell lines do not generate ascites efficiently in rats, and usually adapt poorly to in vitro conditions, but usually generate adequate ascites in immunocompromised mice.
9.3.5. Downstream purification can lead to protein denaturation and decreased antibody activity.
9.3.6. Serum-free or low-serum conditions cannot provide sufficient amounts of MAb for some purposes, such as the evaluation of new vaccines against infectious organisms.
9.3.7. Culture methods sometimes yield populations of IgG MAb that are glycosylated at positions different from those harvested from mouse ascites fluid, thereby influencing antigen-binding capacity and important biologic functions.
9.3.8. When hybridoma cells producing MAb are contaminated with infectious agents, such as yeasts or fungi, the cells often must be passed through mice.
9.3.9. Some cell lines cannot be cultured or maintained in vitro.

10. **HISTORY**

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<td>03 DEC 15</td>
<td>Most Recent Annual Review – Reviewed by: Dr. D. Jeffery</td>
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<tr>
<td>25 NOV 14</td>
<td>Revised – Revision Author: Dr. A. Rizzo; Referee: Dr. B. Singh</td>
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<td>28 FEB 08</td>
<td>New Issued – Original Author: Dr. M. Williams; Referee: Dr. B. Singh</td>
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