Subject: Adjuvant Use and Monoclonal Antibody Production

BACKGROUND

The NIH Guidelines for Use of Adjuvants in Research states “The use of adjuvants in animal research requires careful consideration. While relatively nonspecific inflammation may promote robust immunity, the investigator needs to evaluate the effect of associated local and/or systemic pain and distress of the research animal with the scientific benefit that may be gained from the experiment. The use of potent inflammatory agents, particularly Complete Freund’s Adjuvant (CFA), can result in severe side effects. Although it is expected that alternatives to CFA should be used whenever possible, the use of CFA may be scientifically justified for the induction of autoimmune disease models for which currently no comparable alternatives are known to exist.”

CFA is a mineral oil containing suspension of whole or pulverized heat-killed mycobacterium. It induces an intense local inflammatory and granulomatous reaction which can lead to chronic inflammation, skin ulceration/abscess/tissue necrosis, systemic granulomas, and arthritis.

A commonly used method for in-vivo monoclonal antibody production is the mouse ascites model. This method involves injecting a priming agent followed by hybridoma cells IP in mice. This generates ascites fluid, containing monoclonal antibodies, which is harvested from the mouse. The NIH Guidelines for Ascites Production in mice state “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.”

When utilizing adjuvants or using in-vivo methods to produce antibodies it is important to minimize the potential pain and distress associated with these procedures. The use of an analgesic is required unless robust scientific justification is provided and approved in the protocol.

IACUC Policy and Procedure

Adjuvant Use

1. When possible adjuvants other than CFA should be used. These adjuvants will produce a less intense inflammatory response (e.g. Aluminum compounds, squalene-in-water emulsions, monophosphoryl lipid A, Ribi adjuvants).
2. If CFA is required, it must be scientifically justified in the protocol. The protocol must state route of administration, number of injections, volume to be administered, concentration of CFA to be used, source of CFA, preparation of antigen-adjuvant emulsion (sterility, vehicle, pH), preparation of injection site, and monitoring of the animal.
3. CFA is considered to a chemical hazard and appropriate areas of protocol must be completed.
4. Recommendations when working with CFA:
   a. CFA should only be administered once. Incomplete Freund’s adjuvant should be used for successive immunizations.
   b. Sterile technique must be used when preparing the antigen-adjuvant emulsions.
   c. Aseptic preparation of injection site.
   d. Adhering to recommended routes and volumes according to species (Table 1).
   e. Use of smallest possible volume for injections.
   f. Concentrations of CFA should be <0.1 mg/ml when possible.

Table 1. Recommended Volume (ml) of CFA-antigen Emulsion per Site and Route of Administration (NIH Guidelines)

<table>
<thead>
<tr>
<th>Species</th>
<th>Subcutaneous</th>
<th>Intradermal</th>
<th>Intraperitoneal</th>
<th>Footpad</th>
<th>Intramuscular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>&lt;0.1</td>
<td>Not recommended</td>
<td>&lt;0.2</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt;0.1</td>
<td>&lt;0.05*</td>
<td>&lt;0.5</td>
<td>&lt;0.1*</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05*</td>
<td>Not recommended</td>
<td>Not recommended</td>
<td>&lt;0.25*</td>
</tr>
</tbody>
</table>

*Strong scientific justification required
In-vivo monoclonal antibody production

1. Scientific justification is necessary for this procedure. The protocol must state reason that in-vivo method is required and in-vitro methods cannot be used, description of monitoring procedure, endpoints, number of collections, priming agent used (volume/route), hybridoma cells used (number of cells administered).

2. Administration of a priming agent is required for this model. Commonly used agents include Pristane.
   a. Priming agents may induce discomfort so the lowest dose possible must be used.
   b. Recommendations: Pristane: 0.1-0.2 ml

3. Recommend waiting 10-14 days between priming and hybridoma administration.

4. Administering a higher number of hybridoma cells is associated with greater morbidity.

5. Animals MUST be monitored daily:
   a. Baseline weight must be obtained and recorded.
   b. The animals must be weighed daily after hybridoma cell injections. The animals cannot gain more the 20% of starting weight.
   c. Animals must be observed closely for signs of pain or discomfort (hunched posture, rough haircoat, difficulty ambulating).
   d. Animal must be monitored for respiratory problems and signs of shock (pale eyes, ears, muzzle, and lethargy).
   e. If signs above are noted, animals must be euthanized prior to collection of fluids.

6. Fluid collection
   a. Ascites pressure must be relieved prior to abdominal distension causing discomfort.
   b. The peritoneocentesis can be performed under manual restraint or anesthesia.
   c. Aseptic technique must be followed.
   d. Maximum number of survival taps is three.
   e. If signs of shock are observed, animals must be culled.