Use of Adjuvants in Rodents and Rabbits

Policy: The use of adjuvants in research animals must be reviewed and approved by the IACUC. Alternatives to adjuvants, particularly Complete Freund’s Adjuvant (CFA), must be considered and utilized if and when possible. If adequate alternatives are not feasible, undesirable side effects should be reduced or eliminated through the use of appropriate routes of administration, adequate separation of injection sites, and the use of a small amount of inoculum per site.

Rationale: The use of CFA and other potent adjuvants is associated with the potential for pain or distress in research animals. Utilizing alternative adjuvants and administration techniques that are associated with fewer side effects may improve animal welfare. The NIH’s Office of Animal Care and Use (OACU) maintains guidelines to ensure adjuvants are used only when necessary and that researchers employ current recommendations for refinement when consistent with scientific objectives. Because of the potential for complications from certain routes of administration of immunizations, the following guidelines have been established.

Procedures, Guidelines, and Exceptions:
1. Complete Freund’s Adjuvant (CFA): CFA is a water-in-oil emulsion containing mineral oil, a surfactant, and heat-killed mycobacteria that potentiates cellular and humoral antibody responses to immunogens. CFA creates an intense inflammatory reaction and may result in side effects such as granulomatous reactions, necrotizing dermatitis, and adjuvant-related arthritis. Accordingly, it should only be used if scientifically justified and no comparative alternatives exist. CFA may be used only for the first (priming) dose. Subsequent immunizations should be performed using Incomplete Freund's Adjuvant (IFA) or another adjuvant unless explicitly justified. Re-immunization with CFA is rarely warranted. If approved by the IACUC, an interval of at least three weeks should be given between doses.

2. Alternatives to CFA: Alternatives to CFA must be used whenever possible. In many situations, these alternatives produce a robust immune response with fewer side effects than CFA. IFA has a similar composition to CFA, but lacks the mycobacterial component making it less inflammatory and able to be administered multiple times. Some other alternatives include TiterMax®, the RIBI Adjuvant System®, and aluminum compounds. For more information, see OACU, 2019.

3. Preparation and Injection
   a. The inoculum should be neutral pH, free of toxic solvents, and free of extraneous microbial contamination. Millipore filtration of the antigen before mixing with the adjuvant is recommended when possible.
   b. Injection sites should be aseptically prepared by clipping the hair and performing a surgical scrub to prevent contamination and infection. This is probably the major cause of abscess formation in animals.
   c. Separation between inoculation sites adequate to avoid coalescence is encouraged. The volume injected at each site, locations of sites injected, and number of sites/number of injections should be described in the animal use Proposal.
   d. A minimum 2-week period between subsequent inoculations is recommended.
   e. CFA with mycobacterial concentrations <0.1 mg/ml are recommended to minimize side effects, but concentrations up to 0.5 mg/ml are commercially available and may be used. Mycobacterial concentrations >0.5 mg/ml must be scientifically justified and approved by the IACUC.
4. **Routes and Volume of Administration**
   a. The least invasive route of administration should be used and injection sites should be chosen that do not interfere with locomotion or handling. Subcutaneous (SC) administration is the preferred route for CFA and most other adjuvants.
   b. If routes other than SC must be used for CFA, the Proposal must contain a strong justification. Intradermal (ID) injections frequently result in skin necrosis and sloughing; intramuscular (IM) injections can result in temporary or permanent lameness; intravenous (IV) injections can cause pulmonary lipid embolism; and intraperitoneal (IP) injections can cause peritonitis.
   c. The recommended volumes of CFA-Antigen Emulsion are summarized in the table below (adapted from OACU 2019):

<table>
<thead>
<tr>
<th>Species</th>
<th>SC (ml)</th>
<th>ID (ml)</th>
<th>IP (ml)</th>
<th>Footpad (ml)</th>
<th>IM (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>&lt;0.1</td>
<td>*</td>
<td>&lt;0.2</td>
<td>&lt;0.05**</td>
<td>*</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>*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05</td>
<td>*</td>
<td>&lt;0.25**</td>
<td>*</td>
</tr>
</tbody>
</table>

   *Not recommended
   **Only when justified

d. Footpad injection of CFA in rodents is discouraged because animals may develop arthritis, chronic pain and lameness, and secondary infections in the inflamed areas. If this procedure is to be used, it must be described and its use scientifically justified, including documentation that injections in other sites do not produce adequate antibody titers for the specific antigen being used. If used, only one hind foot may be injected and injections must be spaced at 2 week intervals. Animals that have received foot pad injections must be housed on contact bedding rather than wire-bottomed cages.

5. **Post-injection Observations and Treatments:** Animals must be observed daily for adverse reactions for at least four (4) weeks or until any associated lesions have resolved. The Proposal must specifically detail clinical signs or behaviors indicating a need for intervention, such as the use of analgesics. Examples may include significant erythema, reluctance to bear weight, inappetence, and body weight loss. If IP administration of adjuvant and antigen is scientifically justified and approved, animals must be monitored daily for ascites. Abdominal distention is painful and necessitates relief of ascites pressure and clear humane endpoints in the Proposal.

6. **Personnel Safety:** Accidental inoculation of personnel with adjuvants that contain mycobacterial products (e.g. CFA) can result in sensitization to tuberculin as well as chronic, local inflammation which is poorly responsive to antibiotic therapy. Use caution when handling and administering these products.

**References**