GUIDELINES FOR THE USE OF ADJUVANTS FOR ANTIBODY PRODUCTION

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. When consistent with the scientific objectives, e.g. routine antibody production, adjuvants known to produce less intense inflammatory responses should be considered as alternatives to CFA.… In many situations, these alternatives are capable of eliciting robust cellular and humoral local or systemic immune responses with fewer side effects than those commonly seen with CFA…. All adjuvants used in animal research must be approved by the IACUC, and use of adjuvants that could induce a severe reaction must be scientifically justified. (http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf).

Considerations:

When considering adjuvant use, the Institutional Animal Care and Use Committee (IACUC) and the Principal Investigator (PI) must take into account:

1) the species in which these substances are used
2) the anatomical site and route of injection
3) the dose and volume of the agent per site
4) the pain and distress the animal experiences

Complete Freund’s Adjuvant (CFA)

1. CFA is one of the most commonly use adjuvants and is composed of inactivated and dried mycobacteria emulsified together with a solution of a specific antigen. CFA is highly effective in potentiating antibody responses to injected immunogens, resulting in an intense and prolonged inflammatory reaction.
2. CFA should be used only when small amounts of soluble immunogens are available and the use of other less irritating adjuvants is not feasible.
3. Single and repeated administration of CFA can produce ulceration of the overlying skin following intradermal injections, disseminated granulomas after subcutaneous or intravenous injections, inflammation, local abscess or tissue sloughing, and adjuvant-related arthritis.
4. CFA may be used for the initial immunization, while Incomplete Freund’s Adjuvant, which lacks mycobacteria, is the choice for subsequent immunizations.
5. When CFA is used, it should be used at a maximum of 1:1 antigen to adjuvant, and only for the first dose as repeated dosing can produce severe pain and distress. All booster doses should be with Incomplete Freund’s Adjuvant. Booster doses should not be administered closer than one week apart, and preferably two or more weeks from the previous dose.
6. The IACUC requires that the use of CFA be scientifically justified and supporting documentation supplied.
7. An extensive list of references regarding appropriate procedures and alternative adjuvants can be obtained at http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf

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Recommendations: Adjuvants

1. All procedures used in the preparation of the end product must be as sterile as possible. The hair should be clipped and the skin decontaminated with a surgical scrub. Only sterile equipment should be used for injections and always take appropriate precautions to sanitize the injection sites.

2. Every attempt should be made to purify the antigen by eliminating contamination of the polyacrylamide gel, DMSO, or any other eluting chemicals.

3. When gels or nitrocellulose strips containing antigen must be used, reducing the particle sizes to facilitate their injection as a suspension is superior to surgical implantation of entire strips. The use of polyacrylamide gel as an adjuvant is discouraged as it can cause severe ulcerative lesions that are hot to the touch. Its use must be strongly justified and the animals must be closely monitored.

4. The use of the footpad or intravenous routes for the administration of antigen/adjuvant combinations is prohibited in all species unless scientifically justified.

5. Separate multiple injection sites by a distance sufficient to avoid coalescence of inflammatory lesions and care should be taken to use smaller volumes at each injection.

6. Serum antibody titers should be tested at frequent intervals during the immunization regimen to ensure that the animal is subjected to the minimum number of injections needed to produce the desired state of immunization.

7. Caution is advised when selecting the route of administration, as some routes of injection may be less disruptive to the animal than others (e.g., subcutaneous injection vs. footpad administration).

8. Utilizing the footpad for immunizing small rodents may be necessary in studies where it is required to isolate a draining lymph node as a primary action site the target of infection. Under these conditions, care should be taken to limit the quantity of adjuvant-antigen solution injected, use only one foot per animal, and the animal should be housed on soft bedding. Footpad inoculation must not be used for routine immunization of rodents without specific scientific justification.

9. When administration of adjuvants results in pain or distress, analgesics should be administered, DLAM contacted, or the animal euthanized. Excessive swelling, scratching, chewing, and self-mutilation of the injection site are signs of infection, pain or irritation. Any abnormalities should be reported to the DLAM Veterinary Care Staff (919-843-3407).

Species-Specific Information

1. Rabbits:
   a. Intramuscular: Maximum total dose, 2.0 ml with a maximum dose per site of 0.5ml. Use deep injections into large muscle masses such as those at the posterior aspect of the rear limb or adjacent to the lumbar vertebrae.
   b. Subcutaneous: Doses are the same as intramuscular. Sites can be along the back and lateral to the vertebral column or immediately behind the scapulae.
   c. Intradermal: Maximum total dose of 1.0 ml, with a maximum dose per site of 0.05 ml (therefore, 20 sites). Site spacing should be at least 3.0cm and the area to be injected should be prepared as for aseptic surgery. Sites should be restricted to the skin of the back from the shoulders to the hips.

2. Mice:
   a. Intraperitoneal: Maximum of 0.1 - 0.3 ml of adjuvant or pristane. Mice must be observed daily and ascites fluid removed from the abdomen when it is apparent, usually every two days.
   b. Subcutaneous: 0.01 - 0.2 ml, back of neck or inguinal area
3. Guinea Pigs:
   a. **Intramuscular**: Only a single site (thigh) is recommended. Dose not to exceed 0.2 ml adjuvant.
   b. **Subcutaneous**: Multiple sites along the back. Maximum of 0.2 ml adjuvant/site and 4 sites.
   c. **Intradermal**: Multiple sites along the back. Maximum of 0.03 ml adjuvant/site and 6 sites.

See below for recommended volumes and routes for selected laboratory animals (from http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf)

<table>
<thead>
<tr>
<th>Recommended Volume of CFA-Antigen Emulsion (CFA-AE) per Site and Route of Administration</th>
<th>Species</th>
<th>SubQ (ml)</th>
<th>Intradermal (ml)</th>
<th>Intraperitoneal (ml)</th>
<th>Footpad (ml)</th>
<th>Intramuscular (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>&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;0.1**</td>
<td>&lt;0.1**</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>&lt;0.25**</td>
</tr>
</tbody>
</table>

* Not recommended
** Only when justified

**For a Detailed List of Available Adjuvants:** http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf

**Monoclonal Antibody Production in Mice**

Creating ascites in mice for the production of monoclonal antibodies is no longer routinely acceptable. The IACUC is expected to critically evaluate the proposed uses of the mouse ascites method. Prior to approval of such protocols, the IACUC must determine that (i) the proposed use is scientifically justified, (ii) methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, (iii) the latter have been found unsuitable, and (iv) DLAM Veterinary Services has been consulted. In order to use mice for the production of monoclonal antibodies, the PI must supply documentation and scientific justification that sufficient quantities of the relevant antibody cannot be produced by non-animal alternatives.

**Personnel Safety**

Adjuvants that contain mycobacterial products can be an occupational hazard to laboratory personnel and should be handled with extreme care. Primary routes of occupational exposure to CFA include: accidental injection, conjunctival contact via splashing, and skin contact. Reports of accidental needle punctures in humans have been associated with clinical pain, inflammatory lesions, and abscess formation in tuberculin-positive individuals. Eye and skin exposure to CFA via accidental splashing may lead to serious health complications. Occupational exposures involving accidental splashing of CFA into the unprotected eyes of workers during preparation of emulsions and other mixtures has reportedly produced symptoms including severe ocular irritation, scar tissue formation, and temporary/permanent vision impairment. Tuberculin-negative individuals have tested positive in subsequent tuberculin tests after accidental CFA exposure. Safety glasses, gloves and lab coat should be worn in order to avoid exposure.