IACUC Guideline
Use of Complete Freund’s Adjuvant in Laboratory Animals

The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) has adopted the following guidelines for the use of adjuvants in laboratory animals. These guidelines are based on the National Institutes of Health Intramural "Guidelines for the Use of Adjuvants in Research" [1]. The IACUC recognizes that the principle investigator may be best qualified to select the appropriate adjuvant to be used, and the committee will consider justified applications for use of adjuvants outside these guidelines.

Use of Complete Freund’s Adjuvant (CFA) must be specifically addressed in the protocol application, scientifically justified, and a comprehensive search for alternatives considered. The use of CFA experimentally is always considered USDA category E (Penn C).

These Guidelines are specific to the use of Complete Freund’s Adjuvant; additional general information or information on the use of any other adjuvants may be found in the IACUC Guideline “Monoclonal and Polyclonal Antibody Production.”

Background

CFA is a water-in-oil emulsion containing killed, dried Mycobacterium butyricum which has been used to enhance antigenicity and stimulate an immune response greater than antigen alone. Incomplete Freund's Adjuvant (IFA), water-in-oil emulsion only, is used for similar reasons. The intention of the following guidelines is to minimize potential animal discomfort associated with the use of adjuvants in research.

Reduction, Replacement and Refinement

The USDA has determined that the use of CFA may cause more than momentary or slight pain and may cause a severe inflammatory reaction, depending on the species and route of administration [8,9]. The improper or unnecessary use of CFA may cause severe inflammation, indurations, and/or necrosis in laboratory animals [2]. The most severe inflammatory responses in animals are seen following multiple injections of CFA.

Non-painful alternatives must be considered and documented as part of a written narrative describing a literature search for alternatives to the use of CFA [6]. For more information pertaining to adequate literature search techniques, see the IACUC guideline “Literature Search for Alternatives.” Alternatives to consider include those which reduce the number of animals required (e.g. tissue culture, chicken eggs) or utilize less traumatic adjuvants. Refined adjuvants or antibody production alternatives specifically include:

- Incomplete Freund’s Adjuvant (IFA)
- RIBI®
- TiterMax®
- Specol®
- Montamides
- Syntex Adjuvant Formation (SAF)
- Aluminum compounds

APPROVED
5/12/2015
IACUC Guideline
Use of Complete Freund’s Adjuvant in Laboratory Animals

- Subcutaneously implanted chambers
- SuperCarrier®
- Elvax®
- L-tyrosine
- AdjuPrime®
- Nitrocellulose-absorbed protein
- Gerbu adjuvant
- Immune-stimulating complexes (ISCOMS)

Guidelines

1. Non-inflammatory adjuvants or adjuvants that produce a less intense inflammatory response should be strongly considered as an alternative to CFA [3]. CFA must be used only when absolutely necessary and its use must be justified in each protocol.

2. If CFA is to be used, it must be limited to the initial immunizing dose. Any subsequent immunizations should use an alternative adjuvant such as IFA. If more than one dose of CFA is proposed, it must be strongly justified with objective data.

3. Typical routes of injection are listed in Table 1 below. The IACUC prefers the use of subcutaneous injections in all species. Intradermal, intraperitoneal and intramuscular injections should be avoided, but if chosen as the route of administration, additional monitoring and scientific justification is required. Intravenous (IV) administration is prohibited. Even when given by the approved routes, CFA may cause severe local and systemic pathology.
   a. The reaction is less severe when given as an IM injection in the lumbar muscles compared to the hindlimbs. Therefore, lumbar administration is encouraged when IM administration is chosen.
   b. Intradermal injection, in particular, may result in skin necrosis and sloughing.
   c. Avoid sites of injection which are weight bearing, used in restraint, or prone to self-mutilation.
   d. Recent literature has demonstrated hock immunization may be a comparable and more humane alternative to footpad immunization [7]. However if footpad injection is scientifically justified, then only one footpad may be injected in each experimental animal. Because rabbits do not have a true foot pads, this method of inoculation is prohibited. Please see the table below for guidelines on footpad injection volumes in various species. Post-injection, animals should be housed on soft bedding such as alpha-dry.
   e. The production of rodent peritoneal exudate by intraperitoneal administration can result in undesirable side effects including painful abdominal distention. The resulting distress can be avoided by daily monitoring and relief of ascites pressure, or termination of the experiment (see IACUC Guideline on Monoclonal and Polyclonal Antibody Production). Please see the table below for guidelines on IP injection volumes in various species.

Regardless of the route of CFA administration (injection site, dose, or volume), NIH Guidelines outlined in the table below must be followed [1].
IACUC Guideline
Use of Complete Freund’s Adjuvant in Laboratory Animals

Table 1. Recommended Volume of CFA-Antigen Emulsion per Site and Route of Administration
Adapted from NIH Guidelines [1]

<table>
<thead>
<tr>
<th>Species</th>
<th>Subcutaneous (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>
</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>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05**</td>
<td>*</td>
<td>*</td>
<td>&lt;0.25**</td>
</tr>
</tbody>
</table>

*Not recommended
**Only when justified

4. Injection sites must be separated from each other widely enough to ensure continued blood supply to adjacent areas of skin and subcutis. Boosters should be limited to a maximum of three injections with a minimum of two weeks between each injection.

5. All injection sites must be clipped and surgically scrubbed. The use of sterile needles and syringes is mandatory to minimize microbial contamination. Glass syringes may be preferable as the rubber plungers of certain plastic syringes may react with the oil in the adjuvant. If more than one injection is to be given at a time, sedation is highly recommended.

6. The CFA:antigen emulsified mixture of 1:1 is commonly used [6]. The inoculum must be sterile. The antigen should be Millipore filtered to be free of extraneous microbial or other particulate contamination prior to mixing with the adjuvant. If the emulsion is properly prepared [4], a 23 gauge needle on a 1/2 ml syringe will allow for accurate dosing. If the emulsion is incomplete, dosing will be difficult.

7. To reduce excessive inflammatory response, preparations of CFA with a lower mycobacterial concentration (i.e. 0.05 mg/ml to a maximum of 0.5 mg/ml) should be chosen [5].

8. Given the nature of CFA, some degree of pain is anticipated; therefore use of narcotic agonists, mixed agonist-antagonist, or other species-appropriate agents should be considered and used under the direction of a ULAR veterinarian, taking into account scientific objectives. Steroidal and non-steroidal anti-inflammatory agents should be used with caution given their known impacts on immunological processes. For further guidance on analgesics in mice please refer to the IACUC Guidelines on Mouse Anesthesia and Analgesia Recommendations.

Post-inoculation Monitoring
Post-injection observations should be done daily for four weeks following injection, or until lesions have resolved. If complications occur at the inoculation site (e.g. ulceration), or the animal appears to be in pain or distress (which may be indicated by decreased activity, self-mutilation, rough hair coat, or weight loss) it must be reported as a “sick animal” and ULAR veterinary services contacted, so that a treatment plan may be created. For additional information please see the IACUC Guideline on Humane Endpoints for Laboratory Animals. Possible treatments include topical cleansing of the affected area, application of a triple antibiotic ointment, antibiotics or analgesics as needed.
Personnel Safety
Adjuvants containing mycobacterial products are an occupational hazard to laboratory personnel thus should be handled with extreme care. Accidental needle punctures can result in abscess formation and pain at the exposure site [4]. In order to avoid ocular exposure, in addition to proper PPE, safety glasses should also be worn when handling the agent [1].

References


