Purpose – The purpose of this policy is to provide guidance and resources for investigators that require adjuvant use in live animals.

Background – Adjuvants include any compound that enhances the immune response to an antigen. Adjuvants are commonly used for the in vivo production of polyclonal antibodies either to foreign or self antigens. Many adjuvants are commercially available, and selection is based on intended use and desired effect. Examples include vaccine development/use (low immune response), monoclonal/polyclonal antibody production and collection (moderate immune response), and induction of autoimmune disease (intense immune response). No adjuvant is ideal for all situations and all adjuvants produce varying undesirable side effects, including toxicity.

Commonly used adjuvants:

Complete Freund’s Adjuvant (CFA) – Water-in-oil immersion containing heat-killed Mycobacterium tuberculosis and/or mycobacterial cell wall components; CFA induces a very strong inflammatory response at the injection site that can be painful to the animal. Repeated use can produce sterile abscesses, skin ulceration, and skin/tissue sloughing. CFA is typically only given for the initial immunization, followed by boosters of IFA.

Incomplete Freund’s Adjuvant (IFA) – Similar preparation to CFA, except IFA lacks the Mycobacterium tuberculosis component. Because IFA is less inflammatory, it can be used multiple times in the same animal safely.

Other commercially available adjuvants include RIBI®, TiterMax®, Specol®, montamides, SAF, aluminum compounds, MF59, liposomes, and others.

Policy –

All adjuvants/antigens must be prepared using sterile technique. The preferred route of administration for most adjuvants is subcutaneous (SC).

Antigen/adjuvant injection site(s) should be aseptically prepared, including shaving of site followed by disinfection with surgical scrub.

CFA should be the last resort regarding adjuvant choice; its use requires scientific justification along with demonstration of a search for alternative adjuvants (databases such as ALTWEB or ALTBIB) for IACUC approval.

- animal protocols using CFA are automatically classified at as IACUC Category 3 (equivalent of USDA Category E)
- CFA is only allowed to be administered to each animal once (usually initial immunization)
- CFA should be prepared 1:1 (volume) with aqueous antigen
- if possible, prepare concentrations of CFA <0.1mg/ml (may not be possible for auto-immune disease induction)
- inject volume at multiple sites to minimize inflammation and avoid fusion of lesions

Recommended volumes/sites for CFA-antigen emulsion administration (all volumes in microliters, mls) [1]:

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>ID</th>
<th>IP</th>
<th>FP</th>
<th>IM</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>
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<td>&lt;0.05**</td>
<td>&lt;0.5**</td>
<td>&lt;0.1**</td>
<td>&lt;0.1***</td>
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<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05**</td>
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</tr>
</tbody>
</table>

SC = subcutaneous, ID = intradermal, IP = intraperitoneal, FP = foot pad, IM = intramuscular
* = not recommended ** = requires justification *** = only one limb, requires justification

Post injection care – Post injection monitoring and care is required for all in vivo adjuvant use. The injection site should be monitored for at least three weeks (3 times per week) or until all lesions have healed. Lesions that ulcerate, necrose, or slough must be treated under the direction of the veterinary staff. Animals that show overt signs of pain (hunched appearance, poor coat, discharge around eyes, etc.) should receive analgesics (check with veterinary staff regarding choice) [3].

Any exceptions to this policy must have IACUC approval

References -