Animal Research Protection Program Policy

Adjuvant Use

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Background

The use of adjuvants in animal research requires careful consideration because these agents, particularly Complete Freund's Adjuvant (CFA), can result in severe side effects such as nonspecific inflammation. Nevertheless, the use of CFA is scientifically justified in many systems, such as the induction of autoimmune disease models for which no comparable alternatives exist. The effect of associated local and/or systemic pain and distress of the research animal should be evaluated along with the scientific benefit that may be gained from the experiment.

Scope

This policy applies to all adjuvants used in animal research. This includes but is not limited to:

I. currently licensed adjuvants such as aluminum compounds (e.g., Alum), MF59, liposomes, and monophosphoryl lipid A (MPL);

II. adjuvants in pre-clinical development such as Montanides, polymeric microparticles, flagellin, saponins (e.g., Quil A, QS-21), RC529 (synthetic MPL + Alum), cytokines, and immunostimulatory nucleic acids (e.g., Iscom, CpG oligonucleotides);

III. emerging adjuvants such as virus-like particles, nanoparticles, TLR agonists, muramyl dipeptides, tripeptides (e.g., MDP, MTP), and trehalose dimycolate (TDM); and,

IV. procedures or emulsions such as subcutaneously-implanted chambers, TiterMax, EMULSIGENS, Syntex Adjuvant Formulation (SAF), and Specol.

Policy:

I. The use of any adjuvant must be approved by the IACUC. Scientific justification is required for the use of any adjuvant (e.g., CFA) that could induce a severe reaction or cause local and/or systemic pain and distress to the animal.

II. When consistent with the scientific objectives (e.g. routine antibody collection) adjuvants known to produce less intense inflammatory responses must be considered as alternatives to CFA.

III. CFA should be used responsibly and with care in order to avoid or minimize the adverse effects of excessive inflammation. For most applications, CFA is usually necessary only for the initial immunization, while Incomplete Freund's Adjuvant, which lacks mycobacteria, is the adjuvant of choice for subsequent immunizations. Successive immunizations with CFA must be scientifically justified and approved by the IACUC.

IV. Whenever possible, the least invasive methodology required to accomplish the experimental goal should be utilized. Intra-dermal, intramuscular, and footpad injections should be avoided unless scientifically justified.

V. Footpad inoculation must not be used for routine immunization of rodents without specific scientific justification.

VI. Rabbits must not be immunized in their feet because they lack a true footpad.

VII. The guidelines provided in the **Procedures** section of this policy should be considered when using any adjuvant.

Procedures:

Guidelines for Preparation and Injection

The following guidelines have proven effective in eliminating complications after immunization. These include utilization of sterile technique in the preparation of antigen-adjuvant emulsions, aseptic preparation of the

injection site, appropriate injection technique, appropriate routes and sites of administration, adequate separation of injection sites, and use of smaller volumes at each injection site. Specific recommendations are listed below.

I. Antigen preparations should be sterile and, ideally, isotonic, pH neutral, and free of urea, acetic acid, and other toxic solvents.

II. Antigens isolated using polyacrylamide gels should be further purified whenever possible in order to minimize the amount of secondary inflammation/irritation from gel components. If further purification is not possible, then the amount of polyacrylamide contaminant should be minimized by careful trimming. Ultrafiltation of the antigen with a 0.45micron spin column prior to mixing it with the adjuvant is recommended to remove extraneous microbial contamination.

III. The mycobacteria in CFA should be resuspended by vortexing or shaking the ampule or vial. The CFA is then removed from the ampule or vial using sterile technique. Although approaches may vary, one part or less of CFA to one part aqueous antigen solution (v/v) has been recommended. The CFA/antigen emulsion should be mixed deliberately and with care in order to avoid the introduction of air bubbles.

IV. Formulations of CFA containing 0.5 mg/ml of mycobacterial components are commercially available and have been successfully used by many researchers. However, concentrations of < 0.1 mg/ml are recommended in order to minimize the inflammation and necrosis observed with higher concentrations. Some protocols, such as autoimmune disease induction protocols, may require the use of greater concentrations than those available commercially.

V. The use of preparations containing disrupted mycobacterial cells rather than preparations containing whole, intact bacilli may be preferred, since it is difficult to histologically distinguish the latter from live, acid-fast cells.

VI. For favorable results using CFA while minimizing undesirable side effects, use the recommended injection volumes and sites appropriate for the species, size of the animal, and experimental goal (see Table 1). Some routes of injection may potentially be less disruptive to the animal than other routes (e.g., subcutaneous injection vs. foot-pad administration).

VII. It is necessary to separate multiple injection sites by a distance sufficient to avoid coalescence of inflammatory lesions.

VIII. A period of 2 weeks minimum between subsequent inoculations is recommended.

IX. In addition to the route of administration, the site of injection should be chosen with care in order to avoid areas that may compromise the normal movement or handling of the animal (e.g., intradermal injections in the scruff of the neck of a rabbit).

A. Routes of Administration Presenting Special Issues

1. Footpad Immunization:

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. Procedures to address the well-being of the subject animals should be used (e.g., limiting the quantity of adjuvant-antigen solution injected into the footpad; the use of only one foot per experimental animal; and, housing on soft bedding rather than on screens). Alternative sites with potential draining lymph node utility such as the popliteal lymph node in the hock and cervical sites such as the auricular lymph node and superficial cervical lymph node should be used in order to prevent the animal's locomotion from being affected. The recommended maximum footpad injection volumes are 0.01-0.05 ml in mice and 0.10 ml for rats.

2. Peritoneal Exudate:

Please refer to the OHSU policy: Production of Monoclonal Antibodies using Murine Ascites.

B. Post-injection Observations and Treatments

Post-inoculation monitoring of animals for pain and distress or complications at the injection site(s) is essential, and should be done daily for a minimum of four weeks or until all lesions have healed. Supportive therapy may include topical cleansing, antibiotics, and analgesics. If overt pain or distress is anticipated or observed, the use of narcotic agonists, mixed agonist-antagonists, or other species-appropriate agents should be considered and used under the direction of the veterinarian taking into account the research objectives. Steroidal or non-steroidal anti-inflammatory agents should be used with caution due to their known impacts on immunological processes.

C. Personnel Safety

Adjuvants that contain mycobacterial products can be an occupational hazard to laboratory personnel and should be handled with extreme care. Reports of accidental needle punctures in humans have been

associated with clinical pain, inflammatory lesions, and abscess formation in tuberculin-positive individuals. Tuberculin-negative individuals have tested positive in subsequent tuberculin tests after accidental CFA exposure. Safety glasses should be worn in order to avoid accidental splashing of CFA into the eyes.

D. Other Considerations

Scientists preparing antigens for *in vivo* administration in conjunction with adjuvants should be aware of the potential presence of contaminating substances and other characteristics of the injectate which may have additive inflammatory effects. Care should be taken to consider and eliminate additional inflammatory stimuli whenever possible (e.g., non-neutral vehicle pH; presence of by-products of purification such as polyacrylamide gel fragments). The injectate should be kept sterile.

Table 1. Recommended Volume (ml) of Adjuvant-Antigen Emulsion per Site According to Species

Species	SubQ	Intradermal	Intraperitoneal	Footpad	Intramuscular
Mouse	<0.1	Not permitted	<0.2	<0.05*	<0.05
Rat	<0.1	<0.05*	<0.5	<0.1*	<0.1
Rabbit	<0.25	<0.05*	Not permitted	Not permitted	<0.25**

* Only when justified scientifically

** Only one limb recommended unless justified scientifically

Definitions:

CFA: Complete Freund's Adjuvant

IFA: Incomplete Freund's Adjuvant

MPL: monophosphoryl lipid A

MDP: muramyl dipeptide

MTP: muramyl tripeptide

TDM: trehalose dimycolate

SAF: Syntex Adjuvant Formulation

Authority:

PHS Policy on Humane Care and Use of Laboratory Animals

Guide for the Care and Use of Laboratory Animals, 8th Edition

References:

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