Guidelines for the Production of Polyclonal and Monoclonal Antibodies in Rodents and Rabbits

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IACUC GUIDELINES ON ACCEPTABLE IMMUNOLOGICAL PROCEDURES AND APPROVED METHODS FOR USE OF FREUNDS ADJUVANT

INTRODUCTION

The use of Complete Freund's Adjuvant (CFA) and other immunological techniques in laboratory animals has the potential for causing pain and distress. This guideline provides information on approved immunological methods and advice on how to minimize discomfort to the animals.

COMPLETION OF THE PROTOCOL REVIEW FORM RO-5

- Complete answers are required for questions related to immunological techniques: Items 10 and 11. Reference to this guideline may be used in your RO-5 protocol submission.
- Deviation from the IACUC Guidelines must be justified in detail by the investigator and reviewed and approved by the full IACUC.
- Evidence of appropriate training or experience in using animals in research, specifically in immunological and bleeding techniques must be provided.

RO-4A - Faculty Qualifications in Animal Care and Use
RO-4B - Support Personnel Qualifications in Animal Care and Use

GUIDELINES ON ACCEPTABLE IMMUNOLOGICAL PROCEDURES
When beginning an immunization, choosing the correct adjuvant may be difficult. Historically, Complete Freund's Adjuvant (CFA) has been used as the agent of choice. However, many other adjuvants have been developed recently which are effective and create fewer side effects. The committee encourages the consideration and use of alternatives to CFA.
Complete Freund's Adjuvant (CFA) should usually be used when only small amounts of soluble immunogens are available. CFA is considered to be an emulsion consisting of equal volumes of CFA to antigen (1 part CFA or less to 1 part antigen). The administration of CFA requires the concomitant use of an appropriate anlgesic. If large amounts of particulate, or highly immunogenic immunogens are available, other adjuvants should be considered.
An important aspect in immunization procedures is the utilization of skilled, competent, technical staff experienced in the handling of the species being used and in performing the technique. They must be knowledgeable and capable of recognizing signs of distress in all injected animals, and be responsible for taking action when necessary.

Complete Freund's Adjuvant should be used only for the most problematic immunization situations. It must never be given either intravenously or in repeated doses. CFA must not be used in horses.

PREPARATION OF AREA USED IN INJECTION
The area should be clipped thoroughly and prepared aseptically. A Betadyne or Nolvasan Scrub is suitable for disinfection of the skin, followed by wiping the area with isopropyl alcohol pads. The skin should then have either Betadyne or Nolvasan solution applied to the skin prior to inoculation. This has the dual purpose of preventing infection at injection sites and facilitating monitoring of the site.
INTRADERMAL ROUTE
Sound scientific evidence and justification must be available if the intradermal route of injection of CFA is to be used. Frequent ulceration and infections can occur at the site of intradermal injections with CFA.
In rabbits, volumes of inoculum in excess of 0.05 ml. (50 microliters) per site should not be used. The location of the site(s) should be carefully selected so as to prevent mutilation. A minimal number of sites should be selected, and the distance between each site be maximized. Total volume injected should not exceed 1.0 ml.
The intradermal route is inappropriate in the mouse. Nor is it recommended in other rodents.
SUBCUTANEOUS ROUTE
In guinea pigs, up to a total volume of 0.4 ml (400 microliters) of inoculate may be injected subcutaneously dorsally in the neck, divided into two sites. In rabbits, the site of choice is the inter-scapular region (between the shoulder blades skin on the dorsum (back), avoiding the neck where the rabbit is restrained or handled, administering up to 0.25 ml of inoculum (250 microliters) per site, to a maximum of four sites. Smaller amounts .01 ml (100 microliters) may be distributed per site 1, to a maximum of ten sites. The distance between sites should be maximized. In the mouse, up to 0.1 ml (100 microliters) may be administered in the neck region.
INTRAMUSCULAR ROUTE
In rabbits, intramuscular injections of CFA may be administered in the thigh muscle; up to 0.5 ml (500 microliters), preferably in one site. Intramuscular injection of CFA is not recommended for small laboratory animals such as rats, mice, hamsters, gerbils, etc. For larger animals such as cats and dogs, up to 1 ml of CFA injected into the thigh muscles is acceptable. In livestock such as pigs, cattle, sheep and goats, the intramuscular route is acceptable. In poultry , CFA may be injected in the pectoralis muscle.
INTRAPERITONEAL ROUTE
The intraperitoneal route for injection of CFA is permitted in mice and rats only. CFA should be administered only once, and be limited to minimal volumes of up to 0.1 ml (100 microliters).
INTRAVENOUS ROUTE
Freund's Complete Adjuvant is **NOT** to be injected intravenously.
FOOTPAD INJECTION

CFA should not be injected in the feet of rabbits. Footpad injection of CFA in rodents is not permissible unless there is scientific evidence indicating this route is essential as a specific requirement for the production of immune response. In rats and mice, only one footpad may be used. The concomitant use of appropriate analgesics is required if footpad injections are used. Animals should be maintained on soft bedding and not on wire-bottomed cages. The IACUC recommends **against** the injection of complete Freund's adjuvant into the footpad unless the PI can demonstrate that:

a. the antigen is a poor immunogen by other routes;

If the footpads must be used (after approval by the full IACUC committee) the following conditions must strictly be observed:

a. one hind footpad **only** may be injected;
b. the **volume** of material to be injected into the footpad **must not exceed** 0.05 ml for a mouse and 0.1 ml for a rat or guinea pig.
c. although the first injection may utilize complete Freund's, subsequent (booster) injections must be only of incomplete Freund's adjuvant or saline and must be made higher on the leg in the region of the semitendenosus and semimembranosus muscle groups.
d. the concomitant use of appropriate analgesics is required.

INJECTION TECHNIQUE AND RESTRAINT

- The skillful preparation and delivery of the antigen is critical to the success of the immunization and comfort of the animal.
- The injection site(s) should be shaved and prepared with appropriate antiseptic to ensure asepsis and minimize postinjection complications.
- If necessary animals, especially rabbits, may be sedated prior to injection of Freunds Adjuvant. Please consult the Animal Resources veterinary staff for advice on anesthetic/sedative combinations and methods of injecting or bleeding.
- Be sure to deposit the adjuvant in the correct volume, route and site(s). Improper delivery results in tissue necrosis and failure.
- The formulation strength of CFA is important in determining the degree of reaction and side effects.

OBSERVATION OF INJECTION SITES
The injection sites(s) must be observed by the investigator or his/her designate, a minimum of three times per week, for four weeks after each injection.
If an abscess or ulceration develops at any injection site, it must be reported through established channels, e.g., the animal resources supervisor or veterinarian, and must receive appropriate veterinary treatment. Such lesions should be inspected at least three times per week by the investigator or his/her designate, until all lesions are healed.
As a reference, see:
Canadian Council on Animal Care.
CCAC Guidelines on Acceptable Immunological Procedures.
Monoclonal Antibody Production in Mice

1. Antigen preparations containing CFA must be injected SC or IP. The volume must not exceed 0.1 ml.
2. In priming prior to injecting hybridomas, it appears that 0.1 ml is an adequate dose of pristane. In no case should more than 0.2 ml of pristane be injected. Pristane is a suspected carcinogen and should be used with caution.
3. Mice bearing hybridomas must be observed daily, preferably twice a day, for abdominal distension and poor general condition (rough hair coat, dyspnea, and lack of mobility). Weight gain must not exceed 10 percent (8).
4. Ascitic fluid should not be collected more than twice from the same mouse. General anesthesia is recommended. If general anesthesia is not employed the needle used for tapping should be 22 gauge or smaller.
5. It is recommended that BALB/c retired breeders or F1 hybrids of BALB/c be used for the propagation of hybridomas *in vivo*.
6. *In vitro* production of monoclonal antibodies should be considered.
7. For priming, incomplete Freud's adjuvant (IFA) apparently works as well as pristane and hybridoma cells can be injected as soon as one day after IFA priming, versus 14 days for pristane.

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