ST. LOUIS UNIVERSITY
DEPARTMENT OF COMPARATIVE MEDICINE

IMMUNIZATION OF RABBITS WITH COMPLETE FREUND’S ADJUVANT

General Background

Immunization with complete Freund's adjuvant (CFA) is a widely used procedure to stimulate antibody production. However, it is often associated with undesirable side effects such as large inflammatory lesions at the inoculation site, tissue necrosis, and occasionally local sloughing.

Undesirable lesions can be prevented by carefully limiting the quantity injected at each site and widely scattering the injection sites to ensure adequate barriers of normal skin. Trial volume titrations have shown that using 1:1 suspensions of CFA and aqueous antigens, intradermal injections of 0.05ml, and subcutaneous injections of 0.1ml in rabbits produce palpable lumps which do not progress to excessive inflammation or necrosis.

This method of limiting quantity and scattering sites requires more time because more of the back and flanks must be clipped and prepared for injection, and more training and skill are required to instill small quantities of inoculum. However, it is advantageous because higher antibody production is gained by a broader area of stimulation reacting with more regional lymph nodes and by the creation of many small foci of local cell interactions. The favorable results more than compensate for the greater time and skill required.

The peritoneal cavity and the footpad (the rabbit does not have a true footpad) are considered improper sites for the use of CFA.

NOTE: both Incomplete and Complete Freund's Adjuvant are potentially harmful to humans. Avoid oral, skin, respiratory and eye contact with liquid or aerosolized adjuvant. Gloves, safety glasses or face shield and a respirator should be used during antigen preparation and administration, and during equipment cleaning. See appended material safety data sheets.

Immunogen Mixing Instructions

A. Items needed:
   - Immunogen (antigen)
   - Complete Freund's Adjuvant (CFA) or Incomplete Freund's Adjuvant (IFA)
   - 2 appropriate size sterile, Luer-lock glass syringes (2cc or 5cc)
   - 2 hypodermic needles (20ga x 1")
   - Double-headed single-barreled micro-emulsifying needle

B. Using 2 sterile 2cc or 5cc glass, Luer-lock syringes and 2 20ga x 1" hypodermic needles, draw up into separate syringes, the immunogen (antigen) and the appropriate adjuvant (CFA, IFA) in equal volumes, i.e., \(1.0 \text{ ml immunogen} = 1.0 \text{ ml CFA/IFA}\)

   Note: If total volume of adjuvant plus immunogen is greater than 5 mls, then more than one set of syringe pairs will be necessary.
Any injection containing whole serum as a component will require twice the amount of adjuvant volume to serum volume at the outset.

C. Carefully remove hypodermic needles from syringes, expel excess air from syringes, and connect syringes to a double-headed single-barreled Micro-emulsifying needle.

D. Holding firmly onto the syringes, express the immunogen solution rapidly into the syringe containing the adjuvant. (Immunogen→Oil) Pump the material back and forth from syringe to syringe a few times. Stop and observe condition of the mixture. Any of the following three conditions may occur:

1. If material separates visibly into oil and solution, continue pumping rapidly, stopping frequently to observe status until emulsion is achieved.

2. If material appears to separate into discrete globules, pump vigorously. This state usually precedes achievement of emulsion, noted by change in viscosity and color shade.

3. If material continues separating and retains its initial viscosity and shade, the following may aid in achieving emulsion:

   a. Add 250μl (0.25 ml) additional adjuvant each attempt and repeat steps D1 and D2.

   b. Chill mixture and let stand until material separates and repeat steps C1 and C2.

Immunization Procedure

Antigen Preparation: Initial immunization using no more than 1.0 ml antigen emulsified with Freund's Complete Adjuvant in a 1:1 ratio by volume (i.e., total volume not more than 2.0 ml). Subsequent boosts using no more than 0.5 ml antigen emulsified with Freund's Incomplete Adjuvant in a 1:1 ratio by volume (total volume not more than 1.0 ml). Antigen is prepared using sterile glass syringes and micro-emulsifying needle.

Caution: Safety glasses or face shield, respirator, gloves and a laboratory coat shall be worn to avoid eye, mouth, skin, or respiratory exposure when preparing or injecting the antigen or cleaning equipment. Freund's Adjuvants can cause serious health consequences including blindness, granulomas, and positive TB skin tests if carelessly handled! Read the material safety data sheets before working with these adjuvants.

Rabbit Preparation: hair is clipped widely over back and flanks, and skin is prepared with chlorhexidine or similar agent - not alcohol – the day prior to before injection.

Immunization Schedule (Standard)

Day 0 Hair is removed by clipping with #40 blade. Pre-bleed up to 10 ml on Day 0 or Day 1.
Day 1  Initial immunization with CFA @ 0.1 ml/site, subcutaneously, or @ 0.05 ml/site intradermally.

Day 21-42  Boost with FIA, 0.1 ml/site, subcutaneously, or 0.05 ml/site intradermally.

Day 28-56  Test bleed, 7 - 14 days following boost.

Monthly  Boosts with FIA, as needed - not more frequently than 30 day intervals.

**Bleeding:**

**BEFORE BLEEDING, ALWAYS CONFIRM CORRECT TATTOO ID NUMBERS.**

Give 0.15cc acepromazine SQ approximately 15-30 minutes prior to bleeding.

Wrap bunny in a bunny wrap or Bollman restrainer.

Shave the bunny’s ear and wet ear with an alcohol prep pad.  Allow alcohol to dry.

Insert a 20g 1” needle (bevel up) into the medial artery. The trick is to make sure you have control of your blood collection tube. Milk the artery if necessary (it really works).

You can collect up to 10ml/kg/2 weeks. It can be collected all at once or divided up during the entire month. When collecting blood, use red top collection tubes without the SST in it.

After you have collected the blood:

1. Immediately unwrap the bunny.
2. Label the side and lid of the collection tube with the cage card number and investigator name.
3. Call the investigator.
4. Place the blood in the refrigerator until pickup by investigator.
5. Make sure you log all bleeds and injections you have done in the rabbit log book and in medical record. It helps you to keep track of when the last bleeds and injections were done.

The investigator must call you 24 hours in advance for a rabbit bleed. Fill out a green billing sheet with all the proper information.

When an investigator calls for an **exsanguination**:

1. Anesthetize bunny with subcutaneous Ketamine/Xylazine at 44/7 mg/kg, respectively.
2. When deeply anesthetized, perform cardiac puncture. This method usually produces at least 120ml of blood.
3. Euthanize the animal with an overdose of intravenous euthanasia solution or Pentobarbital (> 100 mg/kg IV), perform thoracotomy to assure death and place in cold room and sign animal cage-card off.
REFERENCES


15. Hsieh DST, Rhine WD and Langer R. Sero-order controlled-release polymer matrices for