

## I. Introduction

The production of antibodies to specific and nonspecific antigens is a tool utilized in nearly all fields of biomedical research. Today antibodies are routinely made to a vast array of proteins, carbohydrates, fats, and nucleic acids. In addition modern biochemical, biosynthetic, and recombinant techniques have created increasingly pure antigens, both natural and synthetic, in an effort to induce very specific immune responses. Many of these newer antigens are small and generally weak immunogens and it is necessary to augment their immunogenicity with adjuvants.

For many years Freund's Complete Adjuvant (FCA) has been used to enhance immunologic responses to antigens. FCA consists of an aqueous solution of antigen emulsified in mineral oil containing heat-killed mycobacterial organisms. The adjuvant activity of FCA is two-fold. First, the oil retains the antigen as a depot at the injection sites, leading to prolonged exposure of the antigen to the immune cells, which results in a sustained production of antibody. Second, the mycobacterial components serve as an immunostimulant for both the humoral and cell-mediated immunity, which also facilitates antibody production. The presence of lipid droplets and mycobacterial components at the injection site can cause a granulomatous reaction. In addition, the antigen used with an adjuvant can also contribute to the degree of the granulomatous reaction and tissue reaction at the injection site (1-3).

Alternative adjuvants such as RIBI®, Montanide ISA50, Montanide ISA70, Titermax® and Specol® are available for polyclonal antibody production. Reports in the literature assessing the side effects of these agents also indicate various degrees of granulomatous/inflammatory reactions at the injection sites (4-8).

Adjuvants that have less inflammatory complications do exist but are used to a much lesser extent. Alum and aluminum salt solutions are adjuvants approved by the FDA for human vaccine use. These compounds stimulate only a humoral response that may be inadequate for weak immunogens, which require cellular mediation for a strong antibody response (2). Freund's Incomplete Adjuvant (FIA), which consists of FCA without the mycobacterial component in the oil is commonly used to booster animals given FCA. Alone, FIA has humoral stimulating properties but, like aluminum, FIA does not significantly stimulate cell-mediated immunity. FIA is also capable of causing abscess and granuloma formation (2), but such reactions are generally less severe than those that accompany the use of FCA.

The NIH has published recommendations for the use of FCA (9, 10). In these recommendations NIH stated that FCA is an "important biologic resource for investigators which should be used responsibly and with care to avoid or minimize the adverse effects of excessive inflammation." These articles included recommendations on dosages and locations with specific mention of using caution with footpad and peritoneal inoculation. In addition, they recommend that the use of noninflammatory or less inflammatory adjuvants be considered when possible. Studies subsequent to the publication of the NIH Recommendations for the use of FCA indicate that FCA, when administered appropriately, may be pursued humanely in the production of polyclonal antibodies (1).

In summary, the ACC recognizes that FCA and other commercial adjuvants used in the production of polyclonal antibodies can cause granulomatous/inflammatory reactions. The ACC also recognizes that FCA and other commercial adjuvants can be pursued humanely to produce polyclonal antibodies when administered in an appropriate manner by trained individuals familiar with the procedures. The following guidelines for the production of polyclonal antibodies were developed to ensure consistency of technique and to minimize pain and distress.

## II. Institutional Guidelines For Adjuvant Use:

1. The University of Illinois at Chicago Biologic Resources Laboratory is the only campus service unit recognized by the ACC for the production of polyclonal antibodies. Investigators interested in this service should contact Dr. James Artwohl at 312 (996-1217).
2. Commercial adjuvants should be administered in a manner consistent with the manufacturer's recommendations.
3. Freund's Complete Adjuvant
  - a. Ideally, the antigen should be electroeluted from gels and suspended in physiologic salt solution without detergent. Emulsions should be prepared in glass syringes so that a drop of the emulsion will not spread over the surface of water.
  - b. All injected inoculums should be free of extraneous contamination especially of microbial origin. Non-absorptive Millipore filtration (0.22  $\mu$ m) of antigen prior to adjuvant preparation is recommended. Syringes and needles should be sterile.
  - c. Historically, FCA has been administered via several different routes. At UIC, it is recommended that FCA only be administered via the intradermal (ID) or subcutaneous (SQ) route. Use of other routes, including footpad, intraperitoneal and intramuscular, must be scientifically justified. The use of FCA via any route in rats must be scientifically justified.
  - d. FCA should only be used for the initial antigenic dose. The use of FCA beyond the initial dose must be scientifically justified and approved by the ACC.
  - e. It is recommended that animals be sedated prior to administration of FCA or FIA.
  - f. The hair over the injection site should be clipped and the skin should be cleaned using a povidone iodine scrub and 70% alcohol.
  - g. Protective eyewear, gloves and lab coats should be worn when working with FCA. Individuals should be vigilant to prevent self-inoculation, which has been associated with granulomatous reactions in individuals that are tuberculin skin positive.
  - h. FCA should be administered over the dorsum in small amounts (0.03 ml/site ID or 0.1 ml/site SQ) over multiple sites (20-30 depending on initial volume) using 25 gauge needles. It is important to maintain adequate separation between injection sites to prevent confluence of the adjuvant.
  - i. Ideally, blood should be collected approximately 10 days following immunization with FCA or FIA to determine antibody titers.
  - j. Booster immunizations should be done using FIA. Ideally, booster immunizations should be administered when antibody titers become stable. A general "rule of thumb" at UIC is to administer booster injections at 3-4-week intervals. Animals should not undergo more than four booster immunizations during the initial phase of antibody production. Should you have questions

on antibody titers and/or the need to administer additional booster immunizations contact a member of the veterinary staff.

- k. Booster immunizations with FIA should be administered in the same manner as FCA. It is important that subsequent immunizations be administered in a different location on the dorsum than the previous immunization.
4. Since some antigens are powerful immunomodulators, which can augment the reactive nature of FCA and commercial adjuvants, all animals administered adjuvants for the production of polyclonal antibodies should be observed on a regular basis. Appropriate species-specific post-procedural records must be completed. Observation should include an assessment of the inoculation sites for tissue necrosis and abscess formation as well as an assessment of the animal's activity, food consumption and body condition to determine if analgesics are warranted. A member of the veterinary staff should be contacted immediately should there be concerns regarding the well-being of an animal or if analgesics are warranted.
5. Care should be taken to avoid the inoculation sites(s) when handling an animal that has been administered an adjuvant.
6. The administration of adjuvants for the production of polyclonal antibodies should only be performed by experienced individuals.

### III. Completion of the UIC Protocol for Animal Use

When producing polyclonal antibodies, the Principal Investigator must recognize the potential for pain and distress as he/she completes the UIC Protocol for Animal Use. Form A, item 13 and Form B, item 7, must be completed to gain approval from the UIC Animal Care Committee. The use of FCA in a manner other than described above must be justified and will require full ACC review for approval.

If there are any questions regarding polyclonal antibody production in animals contact a member of the BRL veterinary staff at 996-7040.

#### **References:**

- Halliday, L C., Artwohl, J.E, et. al. *Physiologic and Behavioral Assessment of Rabbits Immunized with Freund's Complete Adjuvant, Cont. Topics*, 2000, 39(5):8
- Stills, H.F. Bailey, M.Q. *Use of Freund's Complete Adjuvant, Lab Animal*, 1991, 4: 25.
- Broderson, J.R.. *A Retrospective Review of Lesions Associated with the Use of Freund's Adjuvant. Laboratory Animal Science*, 1989, 39 (5): 400.
- Leenars, P.P., Hendricksen, A.F., et. al. *Evaluation of Several Adjuvants as Alternatives to the Use of Freund's Adjuvant in Rabbits and Mice. Lab. Anim.* 1998, 32:387.
- Niemi, S.M., Fox, J.G., Brown, L.R., Langer, R. *Evaluation of Ethylene-Vinyl Acetate Copolymer as a Noninflammatory Alternative to Freund's Complete Adjuvant in Rabbits. Laboratory Animal Science*, 1985, 35 (6): 609.
- Lipman, N. S. et. al. *Comparison of Immune Response Potentiation and In Vivo Inflammatory Effects of Freund's and RIBI Adjuvants in Mice. Laboratory Animal Science*, 1992, 42 (2): 15.
- Johnston, B.A., Eisen, H., Fryd. *An Evaluation of Several Adjuvant Emulsion Regimens for the Production of Polyclonal Antisera in Rabbits. Laboratory Animal Science*, 1991, 41 (1): 15.
- Bennett, B., et.al. *A Comparison of Commercially Available Adjuvants for Use in Research. J. Immuno. Methods*, 1992 (153), pp. 31-40.

- *Review of Polyclonal Antibody Production Procedures in Mammals and Poultry. ILAR Journal, 1995.*
- *NIH Intramural Recommendations for the Research Use of Complete Freund's Adjuvant. ILAR News, 1988, XXX (2): 9.*