THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

SUBJECT: Use of Adjuvants and Antibody Production

DATE: April 17, 2024

Guideline for Use of Adjuvants and Antibody Production

The Guide for the Care and Use of Laboratory Animals and the PHS Policy on the Humane Care of Laboratory Animals requires that in vitro methods for antibody production be considered prior to the use of in vivo methods. Additionally, the Institute of Laboratory Animal Research[1] Executive Summary recommends that in vitro methods be used first and when the ascites method is used efforts are made to minimize pain and distress. Alternative methods, rather than *in vivo* production, must be considered before any *in vivo* methods are approved. The use of *in vivo* methods (i.e. mouse ascites) requires scientific justification in the IACUC protocol.

The use of adjuvants in animal research takes careful consideration. The use of potent inflammatory agents, particularly Complete Freund's Adjuvant (CFA) can result in severe side effects, including granuloma formation, tissue necrosis and sloughing, abscesses, and fever[2,3]. Guidelines:

1. The Principal Investigator (PI) must provide a specific rationale for selection of species, adjuvant, route, sites and handling of antigens when completing the IACUC Protocol.
2. Alternatives to CFA should be used whenever possible. Less problematic alternatives are available and should be considered. RIBI Adjuvant System®, Specol®, TiterMax®, Montanide IAS50, and Montanide ISA70 are commonly used as appropriate alternatives. Noninflammatory adsorptive adjuvants such as alum and aluminum hydroxide gel may also be considered.
3. CFA should only be used if no appropriate alternatives are available, and the use of CFA must be scientifically justified in the IACUC protocol. Note that quantity of antibody is not sufficient justification.
4. When approved, CFA should be used only for the initial immunization, with Freund's Incomplete Adjuvant (IFA) used for subsequent booster injections. If more than one dose of CFA must be used, an interval of at least three weeks should be allowed between doses.

5. Injections should be subcutaneous (SC) or, in rodents, intraperitoneal (IP). Choice of other routes, such as intradermal are discouraged and must be scientifically justified by the investigator. For multiple subcutaneous sites, not more than 0.25 ml per SC site should be used for rabbits, 0.1ml SC for rats, and 0.1 ml SC or 0.2 ml IP for mice. If intradermal injections are scientifically justified by the PI and approved by the IACUC, no more than 0.05 ml may be injected at a site. It is recommended that no more than five sites be injected, and distance between sites should be sufficient to avoid coalescing of inflammatory lesions.

   Subcutaneous or intradermal inoculations should not be done in areas over bony protuberances such as the spine.

   No injections should be done in the foot or footpad unless scientifically justified. Procedures to address the well-being of the animal should be addressed in this case, e.g., limiting the quantity of adjuvant-antigen solution injected into the footpad, the use of only one footpad per animal, only on a hindfoot may be injected, and housing on soft bedding. Hock injections, which direct the immune response to the same draining lymph node (i.e., popliteal lymph node) without the incidental impairment of mobility, are recommended as an alternative to footpad injections.[4]

6. 6. The researchers must observe the animal for evidence of ascites, pain or distress, and the injection site(s) for evidence of lesions such as swelling, abscess or fistula formation, and infection or ulceration at the immunization sites a minimum of three times per week for four weeks after each injection. Supportive care may include topical cleaning, antibiotics or analgesics. The animal should be weighed weekly. Observations and supportive care must be documented, and records kept in the animal room. . Any abnormalities noted during observations must be reported to the veterinary staff. For intraperitoneal inoculation, ascites fluid must be collected before body weight becomes 20% greater than the weight obtained prior to the injection, the abdominal distention is greater than a typical pregnant mouse, the body condition score deteriorates, or if mice are unable to reach food or water. Animal(s) should be monitored frequently over several hours following the tap to observe possible signs of shock due to fluid withdrawal. Pale eyes, ears and muzzle and breathing difficulties are indicative of circulatory shock. Shock may be prevented or treated with 2 -3 ml warm saline or lactated ringers administered subcutaneously. The number of taps should be limited, based on good body condition of the animal. A maximum of two survival taps (the third being terminal) are recommended. Additional taps should have individual IACUC approval.

References

1. Institute of Laboratory Animal Research. Monoclonal Antibody Production. 1999.
2. Jackson LR, Fox JG. 1995. Institutional policies and guidelines on adjuvants and antibody production. ILAR Journal 37(3):141-150.
3. Stills HF. 2005. Adjuvants and antibody production: dispelling the myths associated with Freund's complete and other adjuvants. ILAR Journal 46(3):280-293.
4. Leenaars M, Hendriksen CFM. 2005. Critical Steps in the Production of Polyclonal and Monoclonal Antibodies: Evaluation and Recommendations. ILAR Journal 46(3):280-293.