Guideline for Use of Adjuvants and Polyclonal Antibody Production

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\(^1,2\). Alternatives to CFA should be used whenever possible and the use of CFA must be scientifically justified in the IACUC protocol. Note that quantity of antibody is not sufficient justification.

The Principal Investigator (PI) must also provide a specific rationale for selection of species, adjuvant, route, sites and handling of antigens when completing the IACUC Protocol.

When approved, CFA should be used only for the initial immunization, with Freund’s Incomplete Adjuvant (IFA) used for subsequent booster injections. Other adjuvants should be considered before CFA and IFA. CFA should only be used if no appropriate alternatives are available.

Less problematic alternatives to Freund’s adjuvant are available and should be considered. RIBI Adjuvant System\(^\circledR\), Specol\(^\circledR\), TiterMax\(^\circledR\), 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.

Consideration and justification must be given in the animal use protocol for selection of the laboratory animal species, adjuvant, volume per injection site, location of administration, number of sites, and response required. Particularly with the use of Freund’s adjuvant, it is important to note that the severity of potentially painful inflammatory reactions may be minimized by injection of a small volume of inoculum per site and the use of multiple, sufficiently separated, injection sites when appropriate.

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, 0.5 ml SC for sheep and goats, and 0.1 ml SC or 0.2 ml IP for mice. It is recommended that no more than five sites be injected.
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. Sites should be well separated to prevent consolidation of inflammatory responses. 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. 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 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, and housing on soft bedding. Footpad injections must not be used for routine immunization of rodents without specific scientific justification.

It is the Principal Investigator’s responsibility to ensure the animals are regularly checked. Sites of inoculation must be examined daily or an alternate schedule must be described in the Animal Use Protocol and approved by the IACUC. This is in addition to the daily checking done by animal technicians. Investigators should observe the animals for evidence of pain or distress, and for evidence of lesions such as swelling, abscess or fistula formation, and infection or ulceration at the immunization sites. The veterinary staff should be notified if these clinical problems are found. The animal weight should periodically be compared to initial animal weights. This should be indicated in the protocol and documented.

References