Guidelines for use of Adjuvants and Polyclonal Antibody Production

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The Principal Investigator (PI) must provide a specific rationale for selection of species, adjuvant, route, sites and handling of antigens when completing the Animal Use Protocol. When an adjuvant is necessary to accomplish experimental goals, the investigator should select the adjuvant that causes the least amount of associated pain and discomfort. Therefore, the use of other adjuvants causing less inflammation than complete Freund's adjuvant (CFA) is desirable.

Repeated inoculations of CFA, or footpad, lymph node or intradermal inoculations with CFA are not acceptable unless scientifically justified and approved in advance by the IACUC. The PI must provide data showing that usual methods do not give the result necessary for the experiment(s) proposed and that the requested method would give the desired result. Note that quantity of antibody is not sufficient justification.

Adjuvants

Freund’s adjuvant consists of 85% mineral oil or paraffin oil and 15% mannide monooleate (Arlacel A) as an emulsifier. With the addition of heat-killed mycobacteria (M. butyricum or M. tuberculosis), the mixture is termed CFA. CFA is known to commonly produce undesirable side effects including granuloma formation, tissue necrosis and sloughing, abscesses, and fever. Other deleterious systemic effects, such as polyarthritis, have been reported. CFA is considered a human biohazard, such that accidental self inoculation or splash in the eye has been shown to cause painful sequelae not readily amenable to treatment.

Freund’s Complete Adjuvant (CFA) can cause severe inflammation and ulceration at the site of injection if used improperly. 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.

Alternative Adjuvants

Less problematic alternatives to Freund’s adjuvant 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.
Routes of Administration, Volume, Sites

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 protruberances such as the spine. No injections should be done in the foot or footpad.

Frequency of Boosters

The frequency of boosters must be addressed in the animal use protocol. Two to three weeks is generally considered the minimum time period between the initial and subsequent immunizations. Booster immunizations cannot use CFA.

Monitoring

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.

Record Keeping

The date of each injection and any adjuvant used must be recorded on the cage card, in a chart in the animal room, or on procedure stickers.

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