MASSACHUSETTS GENERAL HOSPITAL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

POLICY ON USE OF ADJUVANTS IN RESEARCH ANIMALS

GENERAL POLICY

This policy describes the acceptable use of adjuvants in research animals housed within MGH facilities. Adjuvants are vehicles employed to enhance the immune response of specific immunogens, which are rarely sufficient to induce a satisfactory antigenic response alone. They work through a number of mechanisms serving as antigen-depot-forming substances, as delivery vehicles or inert carriers, as immunostimulators or modifiers, or any combination of these. In addition, the source of the antigen preparation must be considered. Many immunogens are identified and isolated from polyacrylamide gels, which alone is inflammatory and has adjuvant properties. Ideally, the immunogen should be eluted from the gel before immunization. If this is not possible, the gel should be trimmed so that the least amount of gel is administered. Lastly, preparation of antigens for injection in aqueous solution should be performed aseptically such that contaminants (i.e. unwanted toxins, pyrogens, unintended bacteria and other pathogens) are eliminated and the pH of the injection solution adjusted to within physiological limits.

Adjuvant Selection

Many adjuvants can cause moderate to severe inflammatory responses at the site of administration. The Principal Investigator should first consider whether an adjuvant is actually required to induce the desired immune response. Highly aggregated antigens are likely to induce the appropriate immune response without the aid of an adjuvant. Soluble, relatively pure small molecule antigen preparations are less likely to induce an adequate response alone thereby justifying the need for adjuvant-mediated enhancement. Adjuvant type and administration site should be selected based on three criteria: 1) adequate antigenic response; 2) least amount of inflammatory response at the administration site; 3) best site for minimally affecting the normal posture and movement of the animal.

Non-inflammatory adjuvants such as aluminum compounds and subcutaneous implanted chambers should be considered first since they cause less inflammation. Well-developed alternative adjuvants commonly used in immunology studies include the RIBI adjuvant system (oil-in-water emulsion), Titermax (copolymer water-in-oil emulsion), and Montanide ISA Adjuvant (oil/surfactant-based). Water-in-oil emulsions such as Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) will induce more severe inflammation, should be used only when alternatives do not elicit an acceptable immune response, and require a documented literature search for alternatives in the protocol.

Administration Considerations

In addition to selection of the appropriate adjuvant, the administration site must be considered so that exposure to the least amount of pain/distress is possible. Injection sites should be aseptically prepared (clipped of hair, surgically scrubbed and allowed to dry) prior to antigen-adjuvant administration. Compounds can be administered intravenously (preferably small particulate antigens), subcutaneously (preferred for CFA), or intradermally; intramuscular injection is discouraged. Intraperitoneal immunization is permissible only in mice as a single immunization and scientifically justified if CFA is used. Footpad injections using water-in-oil adjuvants (CFA and/or IFA) are particularly painful/distressful and will only be approved when it is documented that there are no other acceptable methods. Because antigens injected into the footpad are processed by the popliteal lymph node, injections made at the tail base or in the area of the popliteal node are a more humane alternative. Aerosol, oral, and intranasal routes are also utilized when an IgA response is desired.

When administering compounds subcutaneously or intradermally, injecting small volumes into multiple injection sites is more beneficial than injection of larger volumes in fewer sites, from both a humane and scientific perspective. Care should be taken that there is adequate separation between injection sites to avoid the coalescing of inflammatory lesions that could lead to tissue sloughing or abscess formation. Booster immunizations may be given to maintain adequate antibody levels long-term. Frequency of booster immunization should be based upon the time required for the animal to process the immunogen and should be given when the titer has peaked and started to decrease. Booster injections containing antigens such as bacteria, virions or cells may be given intravenously as long as they are not likely to cause anaphylaxis; booster injections involving CFA must be scientifically justified. Soluble antigens with a higher risk of causing anaphylaxis should be administered subcutaneously. Booster injections should be given at sites different from the primary immunizations whenever possible. Animals should not be boosted if adverse reactions were noted during a prior immunization.

Monitoring Requirements

Animals used in antibody production need to be monitored closely after administration of primary and booster adjuvant-antigen injections. Anaphylaxis is a common adverse effect that will determine whether further injections should be performed. Animals should be monitored immediately after injection, one hour later and then 2-3 times a day for the first two days post-injection. Daily observations are necessary to ensure that the approved protocol is followed and to ensure the welfare of the animals. In many cases it is appropriate to administer analgesics to animals in immunization studies. These should be given as stated in the approved protocol. It is generally recommended that analgesics are administered prophylatically to minimize the pain/distress. If animals exhibit pain/distress during the study (e.g., not ambulating normally, not eating, depressed activity, self-trauma), then additional analgesics should be provided or the animal should be euthanized. Consultation with veterinarians from the Center for Comparative Medicine (CCM) is recommended.

Post-procedural monitoring and treatments must be documented on the Individual Animal Medical Record, the Rodent Record Card, laboratory notebook or other method in accordance with the Policy on Animal Observation and Record Keeping.

Blood collection for antibody harvest is a critical aspect of the immunization process. The goal of collection is to obtain a suitable volume of undamaged blood while minimizing adverse physiologic effects on the animal. Please refer to the IACUC Policy on Blood Collection for more information.

If investigators are unfamiliar with any of these techniques, they must contact CCM to schedule training or to request veterinary technical services to perform these procedures.

Related Policies:
Policy on Animal Observation and Record Keeping

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