Florida State University
Antibody Production - Adjuvants

The primary purpose of an adjuvant is to enhance the immune response to a particular foreign protein (antigen) of interest. In the context of antibody production for research purposes, adjuvants stimulate the rapid and sustained production of high titers of antibodies with high avidity. This permits ready recovery of antibody for further research in vitro. Adjuvants have the capability of influencing titer, response duration, isotype, avidity and some properties of cell-mediated immunity. The use of adjuvants is required for many antigens which by themselves are weakly immunogenic.

Adjuvants may act through three basic mechanisms. The first is to enhance long term release of the antigen by functioning as a depot. Long term exposure to the antigen should increase the length of time the immune system is presented with the antigen for processing as well as the duration of the antibody response. The second is the interaction the adjuvant has with immune cells. Adjuvants may act as non-specific mediators of immune cell function by stimulating or modulating immune cells. Adjuvants may also enhance macrophage phagocytosis after binding the antigen as a particulate (a carrier/ vehicle function).

The choice of the appropriate adjuvant is exceedingly important from both the aspect of the end result (high antibody response) as well as the immunized animal's welfare. Many of the adjuvants have the capacity to cause inflammation, tissue necrosis and pain in animals. A major charge to investigators is to minimize animal use and discomfort. While for many years the only effective adjuvant available was complete Freund's adjuvant (CFA), this is no longer the case. Today several other adjuvants are available as alternatives and may be suitable for use in an investigator's experiments.

Selection of an adjuvant is based upon antigen characteristics (size, net charge and the presence or absence of polar groups). Adjuvant choice is also dependent upon selection of the species to be immunized. Some studies are now published comparing antibody response after immunization with antigen complexed to different adjuvants. More work remains to be done and current information cannot be applied across the board to all antigen and adjuvant combinations. Adjuvant selection remains largely empirical. Antigens that are easily purified or available in large quantities may be good choices for starting with the least inflammatory adjuvants for immunization. Should antibody response not be suitable, a gradual increase in the inflammatory level of the adjuvant would then be warranted. Antigens which are difficult to come by (e.g. very small quantities are available) may be better choices for complexing with the more inflammatory adjuvants such as CFA. In addition, small molecular weight compounds and others known to be weakly immunogenic, may need to be complexes with CFA to obtain good antibody titers.

Other factors that influence the inflammatory response include antigen preparation, antigen-adjuvant mixture, injection sites (number and location), volume injected per site and condition of the animal. Antigens should be as sterile as possible and free of chemical contaminants. Antigens should also not be extremely acidic or basic. Excessive quantities of antigen-adjuvant should not be injected per site in order to decrease local inflammatory response. Animals used for antibody production should be in overall good health and free of disease.

Available adjuvants include:

Complete Freund's Adjuvant (CFA): A mineral oil adjuvant; uses a water-in-oil emulsion which is primarily oil. For many years the adjuvant of choice was complete Freund's adjuvant. This adjuvant, while potent immunogenically, also has had a significant historyof frequently producing abscesses, granulomas and tissue sloughs. It contains paraffin oil, killed mycobacteria and mannide monoosleate. The paraffin oil is not metabolized; it is either expressed through the skin (via a granuloma or abscess) or phagocytized by macrophages Multiple exposures to CFA will cause severe hypersensitivity reactions. Accidental exposure of personnel to CFA may result in sensitization to tuberculin.

Incomplete Freund's Adjuvant (IFA): Also a mineral oil adjuvant. Composition similar to CFA but does not contain the killed mycobacteria so does not produce such severe reactions. Used for the booster immunizations following the initial injection with antigen-CFA. May be used for initial injection if the antigen is strongly immunogenic.

Montanide ISA (incomplete seppic adjuvant): A mineral oil adjuvant. Uses mannide oleate as the major surfactant component. The antibody response is generally similar to that with IFA. May have a lessened inflammatory response.

Ribi Adjuvant System (RAS): An oil-in-water emulsion that contains detoxified endotoxin and mycobacterial cell wall components in 2% squalene. Multiple formulations are commercially available, dependent on use. Is an alternative to CFA. Lower viscosity than CFA. Results (titers) often comparable to those with CFA. The squalene oil is metabolizable. Lower incidence of toxic reactions.

TiterMax: Another water-in-oil emulsion, this one combines a synthetic adjuvant and microparticulate silica with the metabolizable oil squalene. The copolymer is the immunomodulator component. Antigen is bound to the copolymer and presented to the immune cells in a highly concentrated form. Less toxicity than CFA. Usually produces the same results as CFA.

Syntex Adjuvant Formulation (SAF): A preformed oil-in-water emulsion. Uses a block copolymer for a surfactant. A muramyl dipeptide derivative is the immunostimulatory component. All in squalene, a metabolizable oil. May bias the humoral response to IgG2a in the mouse. Less toxic than CFA.

Aluminum Salt Adjuvants: Most frequently used as adjuvants for vaccine antigen delivery. Generally weaker adjuvants than emulsion adjuvants. Best used with strongly immunogenic antigens. Generally mild inflammatory reactions.

Nitrocellulose-adsorbed antigen: The nitrocellulose is basically inert, leading to almost no inflammatory response. Slow degradation of nitrocellulose paper allows prolonged release of antigen. Does not produce as dramatic an antibody response as CFA. Good for use if only a small amount of antigen can be recovered from gel band.

Encapsulated or entrapped antigens: Permits prolonged release of antigen over time; may also have immunostimulators in preparation for prolonged release. Preparation is complex.

Immune-stimulating complexes (ISCOMs): Antigen modified saponin/cholesterol micelles. Stable structures are formed which rapidly migrate to draining lymph nodes. Both cell-mediated and humoral immune responses are achieved. Low toxicity; may elicit significant antibody response. Quil A is one example, QS-21 is another.

Gerbu^{R} adjuvant: An aqueous phase adjuvant. Uses immunostimulators in combination with zinc proline. Does not have a depot effect. Minimal inflammatory effect. Requires boosting to maintain high titers.

Conjugation of antigens: Small proteins (<15 KD) and non-protein antigens often need to be conjugated to a carrier protein to increase the immune response. Several options are available as carriers including keyhole limpet hemocyanin (KLH, the most commonly used), bovine serum albumin and ovalbumin. Poly-L-lysine has also been used. Enhanced immunogenicity may also beachieved by covalently linking immunogens to liposomes and injecting the membrane-bound-proteins. The choice of carrier should be based on its own ability to stimulate the immune response and its ability to be adequately coupled to the desired antigen.

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