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Comparison of three adjuvants used to produce polyclonal antibodies to veterinary drugs

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Abstract

Two commercially available adjuvants, Gerbu LQ 3000 and Montanide ISA 50V, were assessed as potential replacements for Freund's adjuvant by evaluating their efficacy in the production of polyclonal antibodies to veterinary drugs in rabbits. The aim was to find an adjuvant that could produce a similar (or enhanced) immune response in the host animal without the undesirable side effects associated with Freund's complete and incomplete adjuvant. The assessment involved the examination of each injection site and the characterisation of the resultant antibodies with regards to antibody titre and sensitivity. It was found that the rabbits immunised with Gerbu adjuvant produced some of the most sensitive antibodies. However, titres were relatively low and adverse effects at injection sites were relatively common. Montanide adjuvant produced no adverse effects and the related antibodies were found to be of adequate sensitivity when compared to those from rabbits immunised with Freund's. It was concluded that Montanide ISA 50V could be considered as a suitable replacement to Freund's for the production of polyclonal antibodies, to low molecular weight compounds in rabbits.

Keywords: Adjuvant; Immunisation; Veterinary drugs; Antibody; Enzyme-linked; Immunosorbent assay

1. Introduction

Antibody production in laboratory animals is a commonly used tool in many fields of biotechnology. Low molecular weight antigens are weakly immunogenic and must be coupled to a large carrier protein in order to elicit an immune response in the host animal. The immunogenic complex is usually administered in combination with a suitable adjuvant. Adjuvants have been used since the 1920s to enhance the efficacy of vaccines administered to humans and animals (Cox and Coulter, 1997) by acting as non-specific stimulators of the immune system. However, while adjuvants used for vaccines need to induce a protective titre over a relatively long time with immunologic memory those intended for laboratory antibody production must induce high titres of high avidity antibody within a relatively short time (Jennings, 1995). When choosing an appropriate adjuvant, those most likely to generate the strongest response for the particular immunogen are considered but more importantly careful thought must be given to those least likely to be detrimental to the health of the host animal. An adjuvant can operate through one or more of three basic mechanisms. Firstly it can extend the length of time that the immunogen is

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exposed to the immune system by providing its slow and even release over a long period of time. This ''depot'' effect allows more time for the immune system to process the antigen as well as conferring an increase in the duration of the antibody response and may produce a secondary immune response after a single injection. Secondly the adjuvant can act as a non-specific mediator of immune cell function, either directly or indirectly. Many adjuvants have a degree of surface activity or possess a surface interface, e.g. the block polymer surfactants in oil-in-water emulsions (Hunter et al., 1981). This relatively hydrophobic surface concentrates immunogen and host proteins and effectively displays them to cells of the immune system (Alexander and Brewer, 1995). Many bacteria possess chemicals that can activate cells of the immune system such as the macrophage which in turn can activate T and B cells. Therefore, naturally occurring and synthetic bacterial products have been employed in many adjuvants. However, macrophages can cause excessive inflammation and pain and so the utilisation of bacterial components must be restricted to avoid these adverse reactions (Claassen et al., 1992). Cytokines are small proteins produced by white blood cells that act as chemical messengers between cells and influence growth and differentiation of T and B cells and APCs. They include the interleukins, lymphokines and cell signal molecules, such as tumor necrosis factor and the interferons, which trigger inflammation and respond to infections. These properties have led to their use as adjuvants; they can be administered themselves or produced locally as a secondary effect of immunisation with another adjuvant, e.g. a bacterial product (Leenaars et al., 1997).

Thirdly an adjuvant can act as a vehicle for transporting the immunogen to the lymph nodes. Association with large particulate structures, e.g. liposomes or indeed carrier proteins for low molecular weight compounds is known to increase the delivery of antigen to APCs. The surface area of the vehicle to which the antigen is bound determines the load and density of the antigen during antigen presentation. Soluble immunogens can be converted to particulate material by the vehicle making it more readily ingested by macrophages.

Freund's complete adjuvant (Freund et al., 1937), prepared from a non-metabolizable paraffin oil containing heat killed *Mycobacterium tuberculosis*, has been commonly used in the laboratory for primary immunisations. Booster immunisations are administered with the incomplete version which does not contain the dead bacterium. In nine cases of accidental injury to man with Freund's complete adjuvant five of the patients suffered adverse reactions (Chapel and August, 1976). The formation of local and systemic lesions in laboratory animals, used for the production of antibodies (Claassen et al., 1992), has prompted investigators to consider using alternative adjuvants, of which there is an extensive list. However, a suitable alternative must be capable of producing a satisfactory immune response in the host animal while causing the minimum undesirable side effects.

Other water-in-oil adjuvants include Titermax (Hunter et al., 1981), which combines a synthetic block copolymer adjuvant (CRL8941) and microparticulate silica in a metabolizable oil (squalene), Montanide (Johnston et al., 1991), which uses mannide oleate as the major surfactant and Specol (Boersma et al., 1992), which is composed of a purified and defined light mineral oil (Markol 52) with the emulsifiers Span 85 and Tween 85. RIBI adjuvants (Ribi et al., 1975) are oil-in-water emulsions where antigens are mixed with small volumes of a metabolizable oil (squalene) which are then emulsified with saline containing the surfactant Tween 80. This system also contains the refined mycobacterial products trehalose dimycolate (TDM) and cell wall skeleton (CWS) and a third immunostimulant, bacterial monophosphoryl lipid A (MPL).

Aluminium hydroxide (or phosphate) is a widely used adjuvant especially in producing vaccines for human and veterinary medicines. Antigen is bound by electrostatic forces to the aluminium salt, providing a short-lived depot effect (Stills, 2005).

Gerbu adjuvants (Grubhofer, 1995) employ cationic nanoparticles in a colloidal suspension to replace the classic water-in-oil emulsion. They bind the antigen and immediately carry it to the lymphocytes for phagocytosis. GMDP (*N*-Acetyl-glucosaminyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine), a cell wall subunit of *Lactobacillus bulgaricus*, is a powerful immunomodulator that induces T cell response and long lasting cellular immunity. Additional immunomodulators, cimetidine and saponin, are included as general enhancers of the immune response. The lack of a depot effect means that more frequent immunising may be required but the manufacturer recommends boosting every 28–42 days.

Various studies have been conducted to compare the immune response and monitor for undesirable side effects produced by alternatives to Freund's. Bennet et al. (1992) found Titermax to be an effective alternative to Freund's when used in rabbits, mice and goats while Leenaars et al. (1994) found that rabbits