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Novel human polysaccharide adjuvants with dual Th1 and Th2 potentiating activity

Nikolai Petrovsky*
Flinders Medical Centre, Flinders University, Bedford Park SA 4052, Australia

Abstract

Pure soluble, recombinant and synthetic antigens, despite their better tolerability, are unfortunately often much less immunogenic than live or killed whole organism vaccines. Thus, the move towards the development of safer subunit vaccines has created a major need for more potent adjuvants. In particular, there is an urgent need for adjuvants capable of boosting cellular (Th1) immunity but without unacceptable toxicity. The adjuvant activity of aluminium compounds (aluminium phosphate or hydroxide) was first described by Glenny and colleagues in 1926. Surprisingly, despite the description of over one hundred adjuvants in the scientific literature, alum remains the only adjuvant approved for human use in the USA. Unfortunately, alum has no effect on cellular immunity and is faced with increasing concerns regarding potential for cumulative aluminium toxicity. Why then has alum not been replaced in human vaccines? Despite the enormous number of candidates, potency has invariably been associated with increased toxicity, and this more than anything else has precluded their use, particularly in prophylactic vaccines where safety issues are paramount. Hence, there is a major unmet need for a safe efficacious adjuvant capable of boosting cellular plus humoral immunity. The extensive data on inulin-based adjuvants indicate that these are excellent candidates to replace alum as the adjuvant of choice for many vaccines. Particular advantages offered by inulin-based adjuvants is that they induce cellular in addition to humoral immunity and offer excellent safety, tolerability, ease of manufacture and formulation. Thus, adjuvants based on inulin have enormous potential for use in vaccines against both pathogens and cancer.

Keywords

Adjuvant; Vaccine; Inulin; Complement; Cellular; Immune; Th1; Th2

1. Introduction

Some of the features involved in adjuvant selection are: the antigen, the species to be vaccinated, the route of administration, the likelihood of side effects and the requirement for a cell-mediated or humoral antibody response [1,2]. Ideally, adjuvants should promote an appropriate immune response, (Th1 or Th2), be stable with long shelf life, biodegradable, cheap to produce and not themselves immunogenic [3]. Freund et al. in 1936, developed an emulsion of water and mineral oil containing killed Mycobacteria, thereby creating Freund's complete adjuvant (FCA), which remains amongst the most potent of known adjuvants and a particularly powerful stimulant of both cellular and humoral immunity [4]. Unfortunately FCA causes severe reactions and is too toxic for human use. A persuasive argument in

*Tel.: +61 8 82044572; fax: +61 8 82045987. nikolai.petrovsky@flinders.edu.au.

favour of inulin-based adjuvants is that they can provide immune responses matching FCA without the toxicity (Fig. 1).

2. Safety of inulin-based adjuvants

The benefits flowing from adjuvant incorporation into any vaccine formulation have to be balanced with the risk of adverse reactions induced by these compounds. Unfortunately, strong adjuvant activity is often correlated with increased toxicity, as exemplified by FCA. A major challenge in adjuvant research is to increase adjuvant activity while reducing toxicity [5]. Adverse reactions to adjuvants include local pain, inflammation, injection site necrosis, lymphadenopathy, granulomas or sterile abscesses. Systemic reactions include nausea, fever, adjuvant arthritis, uveitis, anaphylaxis, organ specific toxicity and immunotoxicity, immunosuppresion or autoimmune diseases [1,6]. There are also increasing community concerns regarding the use of metals, such as aluminium in parenteral vaccines due to possible links to Alzheimers disease and other neurodegenerative disorders. To date inulin-based adjuvants have been tested with a wide range of different antigens in multiple animal species with no significant toxicity.

3. Alternatives to γ-inulin adjuvants

Adjuvants can be classified according to their source, action mechanisms or physico chemical properties [1,7]. Although many adjuvants have been proposed in each of the above classes over the years, these have failed to be successful in humans, chiefly because of toxicity, poor immunogenicity, manufacturing difficulties, instability or cost.

4. Toxicity of alum-based adjuvants

There is a high proportion of moderate to severe granulomas when alum-based vaccines are injected subcutaneously or intradermally [8,9]. Other limitations of alum adjuvants are increased IgE production, allergenicity and neurotoxicity [8,10–12]. Under conditions of reduced renal function, aluminium is accumulated in the body and becomes highly toxic causing fatal neurological syndrome and dialysis-associated dementia. Aluminium intoxication has also been associated with amyotrophic lateral sclerosis and Alzheimer's disease.

5. Alternative human adjuvants

Calcium phosphate has been used for diphtheria-tetanus-pertussis vaccines but overall it is a weak adjuvant thereby limiting its broader use. The saponin Quil A, an aqueous extract from the bark of *Quillaja saponaria* and extracts, mainly QS-21, have been studied as alternatives to alum when strong cell-mediated responses are required [13,14]. In addition to pain on injection, severe local reactions and granulomas, toxicity includes severe haemolysis [5,15–17] making such adjuvants unsuitable for human uses other than for life threatening diseases, such as HIV infection or cancer [18]. Muramyl dipeptide (MDP) [19] and other derivatives from Gram-negative bacteria, such as lipopolysaccharides (LPS) and monophosphoryl lipid A [20] have also been used as human adjuvants although toxicity remains the single biggest barrier to the use of such adjuvants for human prophylactic vaccines. Oil and water emulsions including Montanide, Adjuvant 65, and Lipovant although good at inducing cellular immunity are similarly too toxic for human prophylactic vaccines [21,22]. Hence, adjuvant toxicity is the biggest single factor behind the reason why alum remains the only adjuvant approved for human use by the FDA.

6. Advantages of inulin-based adjuvants

Inulin is a natural storage polysaccharide of Compositae, and is approved for parenteral human use for renal function studies [23]. It contains only fructose with small amounts of glucose and is essentially a linear (unbranched) b-D-(2 → 1) polyfructofuranosyl a-D-flucose. γ-Inulin and related compounds, such as algamulin have been successfully tested in combination with antigens including ovalbumin, tetanus toxoid, syncytial respiratory virus, E7 protein of Human Papilloma Virus, glycoprotein D from Herpes Virus 2, Hepatitis B surface antigen, Influenza, *Haemophilus influenzae* and *Plasmodium falciparum* antigens across a wide range of species including mice, rats, rabbits, dogs, horses, monkeys, and man [24–26]. Inulin-derived adjuvants produce strong Th1 and Th2 immune responses as demonstrated by antibody isotyping (Fig. 1). Of note, no major toxicity of inulin has been demonstrated in any of the species tested, with the only significant finding being the occasional development of small granuloma when very high doses are injected subcutaneously. This excellent tolerability contrasts markedly with the experience of other Th1 adjuvants.

7. Regulatory requirements for adjuvant approval

Significant regulatory and other hurdles exist to approval of new adjuvants. In addition to pre-clinical studies on the adjuvant itself, the combined antigen-adjuvant formulation also needs to be subjected to toxicology prior to commencement of phase I clinical trials [27]. Pre-clinical toxicology evaluation is normally conducted in a small animal species, such as mice, rats, or rabbits and should use the same administration route proposed for human use. The dose and frequency of vaccination for pre-clinical toxicology should be similar or higher to the proposed dose for humans in order to maximize the ability to identify potential safety problems [27]. Nevertheless, many adjuvants appear to be able to pass these animal tests and yet still turn out to be unsatisfactory once administered to humans. It is, therefore, reassuring that in a pilot Phase 1 human study an inulin-based adjuvant was demonstrated to be safe and effective with minimal toxicity [28]. Results on the capacity of inulin-based adjuvants to enhance the immune response consistently show that inulin adjuvants are equal or superior to alum at eliciting antibody responses, and in some instances are even equal in potency to the gold standard, FCA. In addition, they have the benefit over alum that they also stimulate cellular immunity as reflected by Th1 antibody isotype induction. This justifies further development of inulin-based adjuvants for use in prophylactic and therapeutic vaccines.

8. Conclusions

The move away from live or whole killed vaccines to poorly immunogenic purified subunit vaccines requires the development of more potent adjuvants that are nevertheless free of significant toxicity. Many pathogens including viruses, such as HIV require cellular immunity for protection. The currently available human adjuvant, namely alum, is ineffective for this purpose. Whilst several hundred different adjuvants have been proposed over the last few decades, the vast majority have not been successful in being approved for human use, with limitations including lack of efficacy, unacceptable local or systemic toxicity, difficulty of manufacture, poor stability, and prohibitive cost. Inulin-based adjuvants are relatively unique in exhibiting few of these limitations and have the advantage that they are potent inducers of both cellular and humoral immunity, making them suitable for a wide spectrum of prophylactic and therapeutic vaccines.

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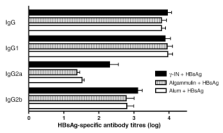

Fig. 1.

γ-Inulin potentiates Th1 response to HBsAg. Groups of C57/B6 mice were immunized with Hepatitis B surface antigen (HBsAg) (1 μg/mouse) in γ-inulin, algammulin or alum. After the second immunization total HBsAg-specific IgG2a were significantly higher for the γ-inulin group whereas total IgG and IgG1 titers were comparable in all groups.