

Combination adjuvant platform for human and animal vaccines



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A novel combination adjuvant platform for human and animal vaccines

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Abstract

Adjuvants are critical component of vaccines. They are being used to enhance and extend the overall immune response, to drive the response towards a specific type of immunity, and to reduce the need for multiple booster immunizations. Here we report the development of a combination adjuvant platform consisting of three immune stimulators, namely host defence peptides, polyphosphazenes and PolyI: C/CpG ODN. The adjuvant platform was co-formulated with a variety of human and animal vaccines and tested in mice, pigs, sheep, koalas, and fish. When co-formulated with a wide range of viral and bacterial antigens including *Bordetella pertussis*, Respiratory Syncytial Virus (RSV), *Chlamydia trachomatis* and influenza antigens, a single immunization induced 100-1, 000 fold stronger humoral immune responses (IgG, IgG1, IgG2a), a much earlier onset of immunity, a clear shift towards a more balanced or Th-1 type of response and extended duration of immunity of up to two years in some cases. The vaccines were highly effective in neonates of less than 7 days of age and provided complete protection against a lethal challenge with *B. pertussis* or RSV. Furthermore, the polyphosphazenes in this combination allow the assembly of microparticles that when lyophilized were stable for several months. Intranasal immunization with these microparticles induced strong mucosal immune response in the upper respiratory tract. Moreover, the adjuvant platform was highly effective in the presence of maternal antibodies. In

summary, we developed a novel vaccine platform for neonates, which provided more balanced, long lasting and fully protective immune responses in neonates after a single vaccination only.

Introduction

Vaccine provides huge public health benefits for reducing the burden of infectious diseases. Live vaccines generate effective immune responses but have been associated with a number of safety concerns, including improper attenuation and reverting back to virulence. Nonliving vaccine antigens i. e. whole, inactivated viruses and specifically recombinant or highly purified subunit vaccines are often weakly immunogenic and need adjuvants to enhance the immunogenicity of vaccine based on antibodies and effector T cell functions to prevent infection. Adjuvants are critical components of vaccines, usually used to stimulate faster, stronger, and long-lasting immune responses to vaccines. It has various roles in vaccine formulations i. e. enhance immune responses to vaccine antigens, provide faster onset of immunity, improving immune responses to immunization in infant or elderly populations, whose immune system are immature or waning, dose sparing, either reducing the quantity of antigen required in the vaccine preparation or reducing the immunization schedule (Coffman, Sher et al. 2010). Adjuvants are absolutely required in subunit vaccines due to the poor immunogenicity of such antigens.

There is no single universal adjuvant which can cover all the vaccine requirements. Using single adjuvant has a number of limitations, including induction of weak, improper, and short lived immune responses. For

example, alum and MF59 (Kenney and Edelman 2003) are the universally
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approved adjuvant for human vaccines. Both normally induce a Th2 biased immune responses but not cellular immune responses which required for immunity against intracellular infections. In many novel adjuvant technologies, using multiple adjuvants in combination often act synergistically by stimulating and activating a various type of immune cells. Combination of adjuvants platform is promising and beneficial for suboptimal vaccines and particularly advantageous for vaccines against specific and more susceptible populations, such as neonates and the older adults. In some aspects, the neonate immune system is similar to that of the elderly, both having diminished anti-microbial activity by immune cells, reduced antigen uptake and presentation by antigen presenting cells and compromised adaptive immune responses (Simon, Hollander et al. 2015). Strong adjuvants may provide an approach to boost immune responses in the both neonates and elderly populations. Most of the studies have combined a delivery system with an immunostimulatory adjuvant, especially combinations with the TLR agonists and MPL. Combination adjuvants have only recently been vigorously explored. Some combinations have been tested in humans and large animals and have yielded promising results. Recently, we developed a novel combination adjuvant platform (TriAdj) which is highly effective with wide range of animal and human vaccine.

Novel Combination adjuvant platform for animal vaccines:

Veterinary vaccines have been used for several of years, and have an important role in protecting animal health, animal welfare, food production, and public health. They are a cost-effective method to prevent animal disease, improve the food production, greatly reducing the need for

antibiotics to treat food and companion animals and reduce or prevent transmission of zoonotic disease to human. Interestingly, the challenges related with vaccinating animals are stability, low cost, ease of administration etc., require new solutions in vaccine development. Freund introduced a combination of mineral oils and bacterial cell components (Freund's complete adjuvant) for the improvement of vaccine immune responses (Freund et al., 1937). However, many nations are not using the Freund's adjuvant in animals due to its reactivity and side effects. Alum and emulsions based formulations have been successfully used since long time in a wide variety of animal vaccines. However, with an advanced understanding of the immune system, many new adjuvants (Saponins, Liposomes, virosomes, particle based and TRL ligand) have recently been developed for veterinary applications. A wide range of adjuvants has been successfully used in commercial vaccines for animals and several new technologies are currently in preclinical development.

Over the past decade, we have seen several new combination adjuvants, typically contain two and three individual adjuvant components, including MF59™ (Novartis Inc.), AS™ (Glaxo Smith Kline Inc.), IC31™ (Valneva Inc.) etc. We recently developed a novel combination adjuvant platform (TriAdj) that is contained of three components, namely toll-like receptor (TLR) agonist either PolyI: C or CpG ODN and an immunostimulatory host defense peptide (HDPs) in polyphosphazene carrier system (Kindrachuk, Jensen et al. 2009; Garg, Latimer et al. 2013). Synthetic PolyI: C and CpG ODNs are well known potent adjuvant with various vaccine antigens and have been shown to enhance immune responses by activating

monocytes/macrophages, dendritic cells, natural killer cells and B cells, and induce the production of proinflammatory cytokines (Krieg 2002; Trumfheller, Longhi et al. 2012). The second component, HDPs are derivatives of natural host defense peptides, which are cationic amphipathic peptides with microbicidal, chemotactic and/or immunomodulatory properties (Yeung, Gellatly et al. 2011). HDPs are involved in a range of immune functions including immune cell recruitment (neutrophils, monocytes, macrophages, T cells and mast cells), innate immune activation, and wound healing (Jenssen, Hamill et al. 2006). The third component, polyphosphazenes are synthetic water-soluble biodegradable polymer with immunostimulatory properties, and forms non-covalent complexes with variety of viral and bacterial antigens and/or other adjuvants to enhance their stability, immunogenicity and allow multimeric presentation (Mutwiri, Benjamin et al. 2007; Andrianov, DeCollibus et al. 2009; Kovacs-Nolan, Latimer et al. 2009; Awate, Wilson et al. 2012).

When vaccine antigens were co-formulated with this combination, we found much faster onset of immunity, highly effective even after a single immunization and significantly long lasting, robust, protective immune responses against variety of animal pathogens including bovine viral diarrhea virus, bovine respiratory syncytial virus and porcine epidemic diarrhea virus. The combination adjuvant is stable, cost effective and highly effective in a variety of animals including pigs, sheep, cattle, koalas, cotton rats and mice (Polewicz, Gracia et al. 2011; Khan, Waugh et al. 2014; Snider, Garg et al. 2014; Garg, Latimer et al. 2015). For example, formulation of E2 protein of bovine viral diarrhea virus with TriAdj resulted in strong humoral

and cell mediated immune responses, leading to significant protection following pathogen challenge of calves (Snider, Garg et al. 2014). Fusion protein of bovine respiratory syncytial virus co-formulated with TriAdj developed significantly higher antibodies and interferon gamma secretion (Kovacs-Nolan, Mapletoft et al. 2009). Similarly, formulation of S1 domain of porcine epidemic diarrhea virus, and outer membrane protein of chlamydial major with TriAdj enhanced humoral and cell mediated immune response in pig and koalas respectively (Khan, Waugh et al. 2014; Makadiya, Brownlie et al. 2016).

Novel Combination adjuvant platform for human vaccines:

Several combination adjuvants consisting of a variety of immunomodulators such as Immune stimulating complexes (ISCOMs), montanides, nanoemulsions, and Adjuvant Systems have been developed in recent years and are currently being tested with human vaccines in preclinical and clinical trials. The novel adjuvant platform, TriAdj was co-formulated with various human vaccines and tested in mice, pigs, sheep, koalas, and fish. This adjuvant platform is highly effective against a variety of infectious diseases.

TriAdj was shown to promote the induction of strong immune responses to various viral and bacterial antigens in multiple animals. For instance, TriAdj in combination with fusion protein of human respiratory syncytial virus (hRSV), mediated the induction of robust, balanced and long-term protective immunity by stimulating long-lived neutralizing antibodies, memory B and CD8+ T cells against hRSV (Garg, Latimer et al. 2013; Garg, Latimer et al. 2014). In addition, mucosal vaccination with TriAdj formulated antigen

induced both systemic and local immunity in neonates, even in the face of <https://assignbuster.com/combination-adjuvant-platform-for-human-and-animal-vaccines/>

maternal antibodies (Garg, Latimer et al. 2015). Similarly, when TriAdj was used with pertussis toxoid of *Bordetella pertussis*, strong and protective immune responses were found in both mice and pigs against lethal infection with *B. pertussis* (Gracia, Polewicz et al. 2011; Polewicz, Gracia et al. 2011). Furthermore, the vaccine formulated with TriAdj induced a prompt onset, longer duration than existing commercial vaccines and effective after a single vaccination even in the presence of maternal antibodies. (Polewicz, Gracia et al. 2013). TriAdj platform was also formulated with influenza virus antigens or chlamydia antigens, which induces strong immune responses in vaccinated animals (Kindrachuk, Jenssen et al. 2009; Shim, Ko et al. 2010). The combination of adjuvants was also shown to be suitable for maternal immunization. Vaccination of pregnant animals with TriAdj formulated human vaccines resulted in efficient transfer of maternal antibodies and protection from subsequent challenge of the offspring (Elahi, Buchanan et al. 2006; Garg, Latimer et al. 2016). These results indicate that maternal immunization with TriAdj formulated antigens might be an alternative, safe and effective approach to provide protection against pathogens in newborn and young infants. Furthermore, the adjuvant platform can be formulated into microspheres (100 nm to 2 μ m) to enhance the mucosal and systemic immune response following intranasal vaccination (Garlapati, Garg et al. 2012). The TriAdj is expected to have multiple applications for the development of vaccines against multiple respiratory pathogens and possibly other infectious agents.

Mechanisms of action of novel Combination adjuvant platform:

A number of mechanisms of action were identified for this novel combination adjuvant. For example, TriAdj as a mucosal adjuvant increased antigen uptake by dendritic cells, improved dendritic cell maturation, and more efficient transported to local draining lymph nodes to present the antigen to T cells (Garg, Latimer et al. 2013). TriAdj with antigens promoted the production of chemokines, cytokines and inflammatory cytokines, followed by recruitment and activation of several immune cell populations including dendritic cells, macrophages and neutrophils to the upper and lower respiratory tract, that leads to strong and long-term protective immune responses of this novel adjuvant formulation (Sarkar, Garg et al. 2016). This was further correlated to the induction of local humoral and cell-mediated immune responses, including production of large numbers of IgA secreting memory B cells as well as effective memory CD8⁺ T cells. TriAdj also promoted increased germinal centre reactions and effective B cell activation and development in the lungs following mucosal immunization (Garg, Theaker et al. 2016).

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