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Review

Vaccine adjuvant formulations: A pharmaceutical perspective

Luis A. Brito, Padma Malyala, Derek T. O'Hagan*

Novartis Vaccines, Cambridge, MA, USA

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ABSTRACT

Formulation science is an unappreciated and often overlooked aspect in the field of vaccinology. In this review we highlight key attributes necessary to generate well characterized adjuvant formulations. The relationship between the adjuvant and the antigen impacts the immune responses generated by these complex biopharmaceutical formulations. We will use 5 well established vaccine adjuvant platforms; alum, emulsions, liposomes, PLG, and particulate systems such as ISCOMS in addition to immune stimulatory molecules such as MPL to illustrate that a vaccine formulation is more than a simple mixture of component A and component B. This review identifies the challenges and opportunities of these adjuvant platforms. As antigen and adjuvant formulations increase in complexity having a well characterized robust formulation will be critical to ensuring robust and reproducible results throughout preclinical and clinical studies.

1. Introduction

Based on the long and extensive history of activities in vaccines, it is clear that adjuvant development is a difficult and challenging endeavor. Most of the adjuvant technologies described in the extensive literature available have failed to progress beyond early pre-clinical studies. However, vaccinologists do not lack potent adjuvants, as there are literally hundreds available for use already, with many more being described annually. Nevertheless, there are very few 'successful' adjuvants, if we define success very narrowly as those adjuvants which have actually been included in a licensed vaccine product. There are important lessons to be learnt from these few success and from the many failures; that must be applied more broadly if greater success is to be achieved in the future (Box 1). Within this review we will focus on adjuvant technologies that we believe have the potential to be 'successful' in the long term, and we will highlight the attributes which allow us to offer this optimistic perspective. However, we will not only restrict ourselves only to the narrow definition of success defined above. While we are aware that clinical progression and potential inclusion in products is of broad interest to most researchers in the field, we are also aware that many groups are focused only on pre-clinical studies and are more concerned with adjuvants of maximal potency. Nevertheless, many of the considerations that we will highlight should be of interest and importance to everyone operating in the area, since all groups should have a strong desire to ensure that their experiments are performed with high quality materials that have been characterized sufficiently so that they can be reproduced by others, and repeat experiments will have a similar outcome. Well-defined and fully characterized formulations for use in pre-clinical studies are an area that we believe has been very much underappreciated in adjuvant science. Hence we will take a different approach in this review which we believe has not been taken previously, we will focus much more on pharmaceutical aspects of adjuvants, including a more accurate definition of what they really are, where the antigen is (e.g. is it in a stable and reproducible distribution), and what is the potential for long term stability for all components. Table 1 outlines basic pharmaceutical attributes of the vaccine adjuvant classes that will be discussed throughout this review. These are very basic and pragmatic considerations that are often overlooked, but necessary if we are to see more success in adjuvant development in the future.

Vaccines have evolved since the initial observations that inoculation of naïve individuals with pustules or scabs from small pox infected individuals yielded protective immunity. Historically vaccines generations of vaccines were often represented by whole pathogens, either attenuated or inactivated, which contained numerous inherent components (adjuvants) which could activate innate immunity upon administration. However, the advent of recombinant DNA technology and advances in conjugation chemistry, etc. have allowed the field to move away from whole pathogen based vaccines toward highly purified homogeneous recombinant subunit antigens and glycoconjugates [1]. Sometimes these antigens alone are not sufficiently immunogenic to induce protective immunity, so adjuvants are often added to ensure robust immune responses. Moreover, there is a trend to develop complex combination vaccines, comprising multiple immunogens from the same pathogen, or combinations of new and/or existing vaccines.

* Corresponding author.
E-mail address: derek.ohagan@novartis.com (D.T. O'Hagan).

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Box 1: Lessons learned from development of adjuvants

1. Risk/benefit analysis for the adjuvanted vaccine must be favorable in relation to pathogen threat, incidence of disease and consequences of infection.
2. Vaccines are used in healthy people, often children, risks must be minimal.
3. Must show a clear need for the adjuvant to be present.
4. Presence of second adjuvant must have clear impact on potency, while not significantly changing risk/benefit analysis.
5. Large safety data base will be needed, likely including an analysis of potential impact of adjuvant on auto immune diseases.
6. Pre-clinical toxicology studies may not be predictive of adverse events in humans.

Adjuvants will become increasingly necessary to help overcome competition between the many different antigens and to ensure that the combination products induce the same level of protective immunity as all the single components.

There is a range of approaches currently available to develop vaccines, which can present antigens to the immune system in a variety of different ways. Some attenuated viruses or bacteria are able to mimic a natural infection without overt pathogenicity, and can potentially stimulate all arms of the immune response and produce a robust protective response e.g., rotavirus, polio, influenza. But not all pathogens can be successfully attenuated to create a safe live vaccine; moreover some cannot even be grown in culture conditions. Alternate approaches to vaccine development include whole inactivated pathogens that are typically inactivated by chemical means. This approach inevitably results in the presence of components of the pathogen which can directly activate the innate immune response e.g., bacterial cell walls, nucleic acids, etc. Other approaches entail producing defined antigens of pathogens, usually surface expressed antigens, using recombinant DNA technology, or by creating synthetic protein/polysaccharide conjugates using polysaccharide components of bacterial cell walls and an established carrier protein (e.g., DT or TT) to provide T cell help. Unfortunately recombinant proteins and even protein/polysaccharide conjugates often induce immune responses that are inferior to live attenuated or inactivated vaccines, since they no longer contain the components of the pathogen that was able to activate innate immunity. Therefore, adjuvants are necessary to improve the immune responses to these highly purified and well-defined antigens. It is our contention that we need to create adjuvant formulations of these antigens, which are equally well characterized and defined as the antigens. Typically this has not occurred and many ill-defined adjuvant components have been added to vaccine antigens to create poorly characterized vaccines for pre-clinical use. Alternative classes of antigens that have gained much prominence recently due to significant commercial and scientific success are virus like particles (VLPs). VLPs have been used as vaccine antigens for immunization against a variety of diseases including influenza, hepatitis B, enterovirus 71 (EV71), HIV and HPV [2]. VLPs are distinctive amongst recombinant antigens in that they are inherently particulate antigens. Antigens presented in any particulate structure, including a VLP format, have been shown to be more immunogenic than standard soluble antigens on many occasions [3]. VLP's have also been established as a means to deliver nonparticulate heterologous antigens on their surface, through chemical conjugation or gene manipulation, since antigenic potency is enhanced due to their multivalent presentation on the VLP surface, which promotes B cell interactions [4]. For the purpose of this review, VLPs will not be considered as an adjuvant approach, since optimization is more dependent on protein engineering and expression systems than pharmaceutical sciences; nevertheless VLP's still need to be included in well-defined formulations which often contain additional adjuvants e.g., alum.

An adjuvant is any component added to a vaccine which enhances the immune response and is defined by what they do and not what they are. Therefore, there are many different kinds of adjuvants with different kinds of structure and chemical composition, which inevitably leads to confusion about what is being discussed. In an attempt to simplify the overall story, many groups have tried to group adjuvants into two groups, 'immune potentiators' or 'delivery systems'. Immune potentiators, as the name implies, elicit their effects by direct activation of the immune cells to increase immune responses [5]. These adjuvants are generally the components of whole pathogens, or synthetic versions of these (cell wall components or nucleic acids, etc.), and differ from 'delivery system' adjuvants such as alum, emulsions and other particulates, which generally work by promoting the uptake of co-administered antigens into the immune cells. There are many different classes of immune potentiators, including agonists of various innate activation systems, including the Toll like receptors (TLRs), NOD Like receptors (NLRs), RIG-I like receptors (RLRs), and C-Type Lectin receptors (CLRs) [3]. These innate immune system components are collectively termed as the 'Pathogen Recognition Receptors' (PRRs) and are present on many antigen-presenting cells (APCs) such as macrophages, B cells and dendritic cells [6]. These receptors are activated by their natural ligands which are pathogen-associated molecules such as unmethylated CpG DNA, lipopolysaccharide (LPS) and double-stranded RNA, which leads to upregulation of co-stimulatory receptors such as CD80 and CD86, and secretion of pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-6 [3]. The TLRs can be further classified into intracellular and extracellular receptors, based on their location in immune cells. TLRs 1, 2, 4–6 are expressed on plasma membrane and recognize bacterial components, whereas TLRs 3, 7, 8 and 9 are expressed in intracellular compartments and are nucleic acid sensors [7]. Examples of established TLR agonists which are exploited as adjuvants include Monophosphoryl Lipid A (MPL), a TLR 4 agonist, CpG oligonucleotides, which are TLR 9 agonists and Resiquimod, a small molecule imidazoquinoline, which is a TLR 7 agonist [5,8,9]. However, despite the distinctions that have been established, immune potentiator adjuvants are increasingly being combined with delivery systems to ensure that poorly immunogenic antigens induce sufficient responses to ensure protective immunity. This review will focus on some basic formulation and characterization aspects of these new generation adjuvants and highlight the challenges that they will face on the path to potential success.

To summarize our key messages in this review, vaccine formulations are complex biopharmaceutical products, which often contain multiple different active components, which need to be stable in a wide range of storage conditions. Vaccines are mostly comprised of a collection of discreet individual proteins, for which changes in structures have the potential to negatively impact the immune response. Therefore, techniques for protein characterization and definition are a key tool in vaccine formulation, as too is the ability to both quantitatively and qualitatively characterize the individual components within a complex mix, e.g., characterization of a mixture of individual antigens already adsorbed to an alum adjuvant. Hence, vaccine formulations need to have some key fundamental attributes that are necessary for the successful development of all pharmaceutical dosage forms. They need to be scalable, robust and reproducible, with the necessary tools in place to characterize all the individual components appropriately. It is our belief that the field of vaccinology would benefit greatly if these key characteristics were considered more often in the early stages of vaccine development, including during basic research studies. Too often we

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