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A Review on Novel Delivery Vehicles for Vaccines Development

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ABSTRACT

The immunogenicity of pure recombinant and synthetic antigens used as vaccines are lesser effective than the vaccines used in previous two decads which were either live, attenuated or killed whole organism. Hence the quality of current vaccines can be improved by either incorporating immunomodulators or adjuvants with novel delivery vehicles like liposomes, immune stimulating complexes (ISCOMs), micro/nanospheres and micro/nanoparticles. Adjuvants are immunological agents that are used in a vaccine to enhance the recipient's immune response to the supplied antigen, thus minimizing the amount of injected foreign material. This review discusses the current status and appllictions of various vaccine adjuvants and delivery vehicles developed till date. A detailed discussion on adjuvants and delivery systems with special emphasis on chitosan in vaccine formulation will be done. Applications of chitosan and its derivatives will be reviewed and their proposed mechanisms in the enhancement of immune responses will be discussed.

Keywords: Adjuvant, antigens, chitosan, immunomodulators, novel delivery, vaccine.

1. INTRODUCTION

Vaccination and immunization is the easiest and effective way to protect individuals from debilitating infectious diseases. The use of vaccines has increased for successful immunization since last two decades, contributing significantly to an increase in life expectancy and improving the quality of life from childhood to the elderly. Vaccines are either used for prophylaxis or therapeutic purposes and act by presenting an antigen to the immune system in order to provoke an immune response to supress an infectious pathogen or a disease process [1]. Vaccines may contain either live-attenuated pathogens, killed or inactivated forms of these pathogens, or purified or recombinant material such as proteins. Active immunization with vaccines has advent as one of the safe and effective method for a large number of diseases and considerable efforts have done to improve the efficacy of vaccines in order to provide optimal immunization [2].

Currently most of the injected vaccines have reported failed to stimulate or generate a mucosal antibody response [3]. Mucosal delivery of vaccines is much important against the diseases caused by pathogens that either invade through, or cause disease at mucosal surfaces [4]. Thus it is needed to search a novel vaccination approach that may have a combined response for both systemic and local mucosal sites.

There are clear distinct advantages observed in mucosal vaccination over injectable vaccination, including easy and self administration, lesser side effects with reduced risk of infections and disease transmission, for example, HIV via contaminated syringes and finally lower costs with better patient compliance. However, the hurdle in mucosal delivery of antigens is its poor immunogenic effect. Therefore, an appropriate vaccine formulation is desired that must be target specific and generate the effective adaptive immunity by stimulating appropriate innate immune system, as well preventing the antigen from physical elimination and enzymatic degradation.

Vaccines do not guarantee complete protection from a disease [5]. Sometimes, this is because the host's immune system simply does not respond adequately or at all. This may be due to a lowered immunity in general (diabetes, steroid use, HIV infection, age) or because the host's immune system does not have a B cell capable of generating antibodies to that antigen. Even if the host develops antibodies, the human immune system is not perfect and in any case the immune system might still not be able to defeat the infection immediately. In this case, the infection will be less severe and heal faster. One can improve the quality of vaccine production by incorporating immunomodulators or adjuvants with modified delivery vehicles viz. liposomes, immune stimulating complexes (ISCOMs), micro/nanospheres apart from alum, being used as gold standard. Adjuvants are typically used to boost immune response. Most often, aluminium adjuvants are used, but adjuvants like squalene are also used in some vaccines, and more vaccines with squalene and phosphate adjuvants are being tested. Larger doses are used in some cases for older people (50–75 years and up), whose immune response to a given vaccine is not as strong [6]. Adjuvants can be used for multiple purposes: to enhance immunogenicity, provide antigen-dose sparing, to accelerate the immune response, reduce the need for booster immunizations, increase the duration of protection, or improve efficacy in poor responder populations including neonates, immunocompromised individuals and the elderly. The other studied adjuvants play major signaling roles within the immune system and have the advantage with exception of high biocompatibility and low toxicity [7].

2. ADJUVANTS

An adjuvant is defined as any compound that enhances the immune response against a vaccine antigen. The word 'adjuvant' comes from the Latin word 'adjuvare', means 'help' or 'to enhance', can be defined as any product or association of components that increases or modulates the humoral or cellular immune response against an antigen. Adjuvants may be a molecule, compound or macromolecular complexe that boost the potency and longevity of a specific immune response to antigens, causing only minimal toxicity or long-lasting immune effects on their own[8]. Adjuvant can also be included in vaccine to guide the

type of immune response generated. This may be especially important when developing vaccine for cancer, human immunodeficiency virus (HIV) or mucosal immune system[9]. Adjuvants have also been shown to protect antigens from degradation, although this generally depends on the nature of adjuvant. For example, chitosan-adjuvate nanoparticles were found to stabilize ovalbumin while on the other side, the model protein antigens are actually destabilized by the traditional aluminium salt adjuvants [10].

Adjuvants have limited or no efficacy unless they are appropriately formulated. Criteria involved in selecting the formulation for a given vaccine include the nature of the antigenic components, the type of immune response desired, the preferred route of delivery, the avoidance of considerable adverse effects, and the stability of the vaccine [11-12]. Ideally, adjuvants should be stable with long shelf-life, bio-degradable, cheap to produce, not induce immune responses against themselves and promote an appropriate immune response (i.e. cellular or humoral immunity depending on requirements for protection)[12]. There are marked differences on the efficacy of adjuvants depending on the administration route (e.g. between mucosal and parenteral routes). Hence, new vectors, antigen delivery systems and adjuvants compounds need to take into account the characteristics of the proposed administration routes [13].

Traditional live vaccines based on attenuated pathogens (inactivated viruses or bacteria) are often sufficiently immunogenic without the presence of adjuvants. Nowadays vaccine development is focus on making much safer vaccines by using subunit vaccines, such as purified protective proteins or carbohydrates, rather than whole microorganisms and hence adjuvants are becoming increasingly important for vaccine developers as many of these novel subunit and split-vaccines are insufficiently immunogenic on their own[14-15]. The adjuvants can be classified based on their five potential modes of action: (i) immunomodulation (modification of cytokine networks), (ii) presentation (maintenance of antigen confirmation), (iii) cytotoxic T-lymphocytes (CTL) induction, (iv) targeting specific cells, and (v) depot generation.

In other way adjuvants can be classified into two separate grouping [8]:

Immunostimulants: these act directly on the immune system to increase the response to antigens that stimulate immune responses. Examples include Toll-like receptor (TLR) ligands, monophosphoryl lipid A[16], cytokines [GM-CSF], IL-2), saponins and bacterial exotoxins (cholera toxin and heat-labile toxin);

Vehicles (delivery systems): these present vaccine antigens to the immune system in an optimal manner, including controlled release and depot delivery systems, to increase the specific immune response to the antigen, and can also serve to

deliver the immunostimulants . Examples include: mineral salts (aluminium), emulsions [17-19]; virosomes [20-21] and liposomes; biodegradable polymeric microparticles; and immune-stimulating complexes (ISCOMs). These carriers share some of the following properties: protection of antigen from degradation following its administration by different routes including mucosal, ability to sustain the antigen release over an extended period of time, intracellular delivery of antigen contributing to cytotoxic T-cell stimulation and targeting at APCs. Hence, with the aim of eliciting broad immune response especially with strong cellular compounds, the trend has been to combine adjuvant or to formulate these to achieve depot formation, recruitment and activation of APCs in the presence of the desired antigen [22].

3. DELIVERY SYSTEMS

To potentiate the effect of vaccines novel delivery vehicles are in use apart from the adjuvants, these include particulate carrier systems such as ISCOMs, liposomes and polymeric micro/nanoparticles that are under investigation for vaccine delivery. Encapsulation or adsorption of antigens onto particles resulted in the induction of significantly enhanced immune responses in comparison to alternative approaches such as solution or gel formulations[23]. Particulate delivery systems offer several advantages as adjuvant/delivery systems for vaccines. Microparticles have a similar size to the pathogens, which the immune system has evolved to combat, hence they are taken up like pathogens by antigen-presenting cells (APCs)[24]. Particulate delivery systems can be used to present multiple copies of antigens on their surface, which may lead to an optimal B-cell activation[25]. If needed different adjuvants can also be incorporated into the particulate systems which may improve the immune response in addition to decreased adverse effects caused by those adjuvants.

Preparation of particulate systems most commonly involve polyanhydrides, polyorthoesters, hyaluronic acid and poly(lactic- co-glycolic) acid (PLGA)[27-29]. However to prepare such particles organic solvents are required which may lead to the degradation of antigen and hence alginates or chitosan can be used to encapsulate a wide variety of antigens without using any organic solvents[30-31]. Particulate systems having size smaller than 10 μm have shown a significantly improved immune response[32]. Particulate systems can be for vaccine delivery can be either biodegradable or non-biodegradable in nature.

4. MAJOR ADJUVANT/ DELIVERY VEHICLES

4.1 Alum based adjuvants

Alum salts principally aluminium phosphate and hydroxides have been the most widely used human adjuvants.

Being weeker adjuvants alums are rarely induce cellular immune responses however it may slow down the rate of antigen release and thus increases the duration of antigen interaction with the immune system [33].

4.2 Complete Freund's adjuvant (CFA)

Freund's adjuvants are considered as one of the most effective adjuvants available for raising antibodies in test animals. Complete Freund's adjuvant contains heat-killed mycobacteria, which is a primary agent responsible for stimulating antibody production, but has also been attributed to a number of undesirable side effects [34]. However the undesirable side effects of CFA like pain, suffering and morbidity in test animals and potentially serious health and safety threats. Freund's adjuvant is effective in stimulating cellular immune response and may lead to the potentiation of the production of IgG and IgA.

4.3 Adjuvants emulsions

This class includes *oil-in-water* (o/w) or *water-in-oil* (w/o) emulsions like IFA (incomplete Freund's adjuvant) and Adjuvant 65 but the toxicity associated with these emulsion include inflammatory reactions, granulomas and ulcers at the injection site and restrict their use.

4.4 Bacterially derived adjuvants

These may be either toxin or nontoxin type:

Toxins include Cholera toxin (CT) a protein complex secreted by the bacterium Vibrio cholerae which shows an enhanced immunogenecity when given intranasally[35]. Pertussigen a killed Bordetella pertussis is used as a parenteral adjuvant and enhances the cellular immune response[36]. Clostridium difficile toxin is composed of Toxin A and Toxin B and have the ability to act as mucosal adjuvants[37]. Shiga toxin (STx) is a protein toxin of Shigella dysenteriae, Type-I, can induce both humoral and cellular immune responses[38]. Staphylococcal enterotoxins are basic proteins produced by certain Staphylococcus and have the ability to act as mucosal adjuvants [39].

Non-toxin proteins include Muramyl dipeptide (MDP) derived from the cell wall of mycobacteria and one of the active components in CFA. MDP is known to be a potent inducer of interleukin-1 (IL-1), which can activate macrophages and T-cells[40]. Lipopeptides derived from bacterial lipoproteins are potent adjuvants for parenteral immunization[41]. Proteosomes a multi-molecular preparations of meningococcal outer membrane protein and used for intranasal immunization which showed a high level of anti-toxin IgA in lung and intestinal secretions [42]. Liposomal adjuvants are synthetic spheres consisting of lipid layers that can

encapsulate antigens and act as both vaccine delivery vehicle and adjuvants that can enhance both humoral and cellular immunity to proteins and polysaccharide antigens [43].

Quil-A is a component of saponin, a detergent derived from the plant *Quillaja saponaria* molina, which is one of the biologically active components of ISCOMs. Saponins induce a strong adjuvant effect to T-dependent as well as T-independent antigens [44]. Immunostimulating complexes (ISCOMs) are 40 nm cage-like particles that form spontaneously when cholesterol is mixed with Quil-A. ISCOMs stimulate a strong response for all immunoglobulin classes. These also stimulate cellular immune response as measured by T-cell responses and delayed-type hypersensitivity. Perhaps a unique feature of ISCOMs is their ability to induce CD8+ specific cytotoxic responses [45]. Several complex carbohydrates of natural origin stimulate cells from the immune and reticulo-endothelial system. γ-inulin is a potent adjuvant inducing humoral and cellular immunity without the toxicity.

4.4 Cell-based adjuvants / delivery systems

Dendritic cells may provoke a potent lymphocytes response and are increasingly being tested for their ability to act as adjuvant in therapeutic vaccines [45].

4.5 Cytokines as adjuvants

A large number of cytokines have been evaluated alone or in combination for their effects on immunity. Different cytokines were studied as adjuvants to induce antigen specifi c serum/ mucosalantibody and cell-mediated immunity. The most notable cytokine adjuvants studied to date include granulocyte/macrophage colony stimulating factor (GM-CSF), IFN, IL-1, IL-2, IL-6, IL-12, IL-15, IL-18 and chemokines [46].

4.6 Polymeric particles

4.6.1 Biodegradable

A variety of biopolymers exists from which nanoparticles for drug delivery can be synthesized, however, the most commonly studied polymers are poly (D,L-Lactide-co-glycolide) (PLG) and poly lactide (PLA). These biodegradable, biocompatible polymers are extensively studied for the use in the formulation of vaccine antigens (i.e. proteins, peptides, DNA, etc.)[47]. In these formulations, antigen can either be entrapped or adsorbed to the surface of the particles. These can act as depot from which the encapsulated antigen is gradually released. The adsorbed antigen may offer improved stability and activity over encapsulated antigen by avoiding formulation and acidic pH conditions caused by the degradation of the polymer [48].

4.6.2 Non-degradable

Among the various non-degradable nanoparticles gold, latex, silica and polystyrene are used due to their prolonged residence in tissues. A study in humans using these particles without electroporation produce a relatively low immune response after vaccination with DNA-gold particles-GM-CSF transfected analogues tumour cells [49]. Another approach for DNA delivery is through particle bombardment or Particle Mediated Epidermal delivery (PMED) or the "Gene Gun" approach. While the delivery efficiency of this technique is quite low, only small amounts of DNA are required to achieve a significant immune response. Clinical trials have shown that this approach can elicit both humoral and cellular immune responses, making it one of the only consistently successful DNA vaccine delivery approaches [50].

4.7 Virosomes

Virosomes are unilamellar empty enveloped particles composed of membrane lipids and viral membrane proteins associated with vaccine antigen which results in enhanced immunogenicity. Virosome technology has been most advanced in influenza, in association with protein or peptides, but it is rapidly being used for other antigens as well. A potential advantage or application of this technology is to take advantage of the physical properties of virosomes in terms of uptake by APCs, as well as the chemical composition, and compatibility with adjuvant molecules derived from lipid-A [51].

4.8 Virus-like particles

Virus like particles (VLPs) use the nature's own mechanisms and structural principles to trigger the immune system for protective effects. VLPs are essentially non-infective virus consisting of self-assembled vial envelope proteins without accompanying the genetic material. Virus like particles maintain a morphology and cell-penetrating ability similar to infective viral particles. The VLPs have also been shown to stimulate both cellular and humoral immunity [52].

4.9 Viral-vectored vaccines

Viral-vectored vaccines consist of a non-replicating virus that contains some defined genetic material from the pathogen to which immunity is desired. Such vaccines are also commonly referred to as live recombinant vaccines since the immune system has evaluated to respond to viruses, this would seem to be an ideal way to deliver an antigen. Advantages of viral-vectored vaccines include their ease of production, a good safety profile (at least in some cases), ability to potentiate strong immune responses, potential for nasal or epicutaneous delivery and mucosal immunization [53].

5. CONCLUSION

Vaccine delivery vehicle and adjuvants have long been of great interest for vaccine development in the clinical and basic immunology. All adjuvants appear to stimulate components of the innate immune system, but the diversity of mechanisms used by even a short list of well-studied adjuvants is impressive. Adjuvants currently used in humans enhance humoral immunity, but many new adjuvants in clinical or pre-clinical development are focused on enhancing specific types of T-cell responses and generating the multi-faceted immune responses that may be needed for challenging diseases such as malaria and HIV. The translation of these experimental adjuvant and delivery systems to human clinical application remains a major challenge in adjuvant and vaccine research. and development due to the substantial differences in physiological and immunological responses between species as well as safety concerns. Thus, although there remain many unresolved issues and hurdles related to the final clinical application of the current experimental adjuvant and delivery systems, there is now sufficient evidence to predict that effective adjuvant and delivery systems with acceptable side effects will be introduced for vaccination in the next decade.

6. ACKNOWLEDGEMENT

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