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Trends in Adjuvant and Vaccine Delivery Systems

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Abstract

Since time immemorial, adjuvants have played a very crucial role in a vaccine formulation. Mere antigen discovery does no good until unless combined with an effective adjuvant. The modern era of vaccinology has transcended way beyond the conventional approach of prophylaxis, where it is driven more towards achieving specific and targeted effects. With the health sector alarmed with the dawn of dread and neoteric diseases/etiology, vaccination is the most effective aid at hand. The gradual advances made in antigen discovery need to be complemented with new generation adjuvants for multidimensional effects. Though conventional adjuvant strategies still canvas the industry, but the foresight of the eminent threat warrants drastic revolution in this sector. Adjuvants basically fall under two broad categories viz., depot/delivery adjuvants and immunopotentiators, encompassing a wide spectrum of the compound with the prospective future application. Each compound embodies a specific characteristic mode of interaction with host immune system. These compounds need further study and explorations with clinical trials directed to their position in vaccine formulation of the newer generation.

Keywords: Adjuvant; Antigen; Vaccine; Aluminium adjuvants; Tensio-active adjuvants; ISCOM; Saponin; TLR-based adjuvants; Virosomes; Virus-like particles

Introduction

Vaccines are considered to be the greatest intervention in medical science where our immune system is programmed in a calculative manner and primed for defence against an invading pathogen. Since the emergence of the concept of vaccines, the health sector has seen an epochal success in the field of immunoprophylaxis [1]. In combination with the modern age therapeutics and hygiene practices, vaccines have contributed significantly in disease control and prophylaxis [1]. Vaccines are the most targeted preventive weaponry, gifted to the health sector by science. But with the emergence of various infectious and non-infectious diseases, we foresee a huge baffling task over the field of vaccinology for global health. "Prevention is better than cure"- a well-known phrase justifying immunisation yet incomplete in the modern age where the word prevention is more defined in nature by How to? When to? and What to? Though each question encompasses a vast expanse of study, this review mostly manoeuvres' over one particular aspect of how to complement vaccines for better stability and lasting immune response. The answer that lie in hand is "adjuvants", which in itself is a huge evolving field dedicated for effective vaccine response and delivery. Although the prime objective of adjuvants is to enhance immune response, but the modern era health sector has extrapolated the adjuvant technology for a range of specific effects. Antigen and delivery system forms two essential components of a vaccine. With a range of antigen engineered and developed over time, demands amalgamation with a suitable adjuvant for targeted results. This review will focus on the various adjuvant and delivery systems that are /can be used in the health sector.

Brief history

The gradual progress of adjuvant through the course of time can be grouped into four phases which began with the adjuvant development for toxoid vaccines, followed by the use of oil and aluminium adjuvants, that was taken over by synthetic and second-generation depot systems and finally the adjuvant for targeted immune response which are the subject of exploration in the present day. Looking back in time, it was Smith in 1907 who demonstrated administration of toxin/antitoxin in immune precipitating ratios could provide enhanced protection [1]. Similarly, the addition of oil along with killed salmonella antigen was the first study to highlight the use of a depot/delivery substance [2]. Gradually, many substances were tested for enhancing the immunogenicity like agar, tapioca, lecithin, starch, oil, saponin, salts of calcium and magnesium, killed *Salmonella typhi*, and even bread crumbs [3,4]. Among the vast list of tested components, the most successful was the aluminium salt adjuvant. The first aluminium salt adjuvant was formulated along with diphtheria toxoid [5]. Freund in 1930s emerged with the use of a water-in-oil emulsion which basically consisted of a mixture of one volume of 10% Arlacel A (Mannide monooleate) and 90% mineral oil with one volume of antigen solution. The use of commonly available mineral oil Drakeol and the surfactant Arlacel A continued due to a prevalent hypothesis that non-metabolizable oil was required for full activity of the adjuvant [6]. Freund's adjuvant became established as the "gold standard" for most vaccines. While on the other hand, formulation of water-in-oil adjuvants using metabolizable oils like peanut oil was also under development [7]. Thus emerged Adjuvant 65 which consists of Arlacel A and aluminium stearate as the stabilizer was reported to be of similar potency to Freund's in both animal and human vaccination with influenza virus [8]. However, the tumorigenic property of Arlacel A kept this formulation from achieving licensure [9]. The approach of water-in-oil emulsions for adjuvant purposes was set back severely but reappeared in the 1990s with the Seppic-produced systems. Subsequently, with modern research plunging into the depth of

adjuvant-mediated pathways in the host, it was realised that a complex interaction with immune cells like Langerhans cells, macrophages and dendritic cells might play a significant role in host immune modulating. So a series of bacterial extracts derived from *Mycobacterium tuberculosis*, *M. avium*, and saprophytic strains of mycobacteria were tried and tested for their effect on immune cell stimulation. Additional activity was also found in DNA and RNA digests [10]. Structural work on a few key strains like *M. bovis*, *Nocardia rubra*, and *Listeria monocytogenes* led to isolation of muramyl dipeptide (MDP) from their cell wall [11]. Subsequently, several works continued in a similar aspect with cell wall derived factors like Wax C, Wax D, phosphatide, and cord factor fractions [12]. The antibody-enhancing adjuvant activity of both poly A:U and polyribo I:C was demonstrated with rabies vaccine. In addition, polylysine/carboxymethyl cellulose–stabilized poly I:C mediated interferon induction in primates was taking a promising turn [13-15]. By the 1970s, the possibilities of using purified Gram-negative endotoxin as an adjuvant was also under consideration for the fact that it could significantly enhance antibody titre and that both the adjuvant and endotoxin properties were separable by the acylation and desterification of lipopolysaccharide mixtures [16]. With the discovery that extracts from Quillaja saponaria has adjuvant property [4], its active component - saponin was incorporated successfully into foot-and-mouth disease vaccine trial [17]. Later on, Dalsgaard developed a purified mixture called Quil A which was more effective and caused fewer local reactions. Quil A is a mixture of more than 25 different saponin molecules. One of them, the saponin QS21, is being investigated for possible beneficial adjuvant effects on the human immune system [18].

The modern pragmatic shift towards the use of recombinant antigens in vaccine formulation had a successful breakthrough with the first recombinant DNA-generated vaccine made against hepatitis B [19]. This encouraged further perpetuating in the direction of producing a spectrum of recombinant and subunit vaccines, which needed to be complemented for immunogenicity, presentation, stability and delivery by new generations of adjuvants. Carrier molecules like liposome and targeting of phagocytic cells by nanospheres were brought in for experimental and clinical trials [20]. MF59, IRIV (immunopotentiating reconstituted influenza virosome) which marked the post-alum adjuvant era are the result of a dynamic change in the field of adjuvant technology [21,22].

Characteristic and mode of action of adjuvants

For an adjuvant to be suitably placed along with a vaccine formulation, certain characteristic traits need to be ascertained, such as it should not risk induction of autoimmunity or allergy, with no teratogenic effects, and should have a no or very low incidence of adverse events. Apart from the detrimental effects, the chemical composition should be well defined, demonstrated to be carcinogen-free and biodegradable, and the type of immunity induced should be specific for the particular vaccine [23].

The mode of action of adjuvants could be broadly grouped into two types, basing on the effects they elicit, with one class of adjuvants exerting a depot effect or delivery of the antigen thus decreasing the degradation kinetics of the antigen and increasing the probable encounter with immune cells. In contrast, the second class of adjuvants enhances interaction with the immune cell by directly targeting them, so are also described as the immunopotentiators [20].

Types of adjuvants

Till date, the vaccine industry has experienced enormous types of adjuvants which basically fall under the group described above, but are more specific and targeted in their action. Different class of adjuvants individually or when cocktailed can complement a particular vaccine with enhanced immunogenicity. There are accumulated examples of some of the adjuvants which have been used or are under trial for vaccine formulation, and would provide researcher and clinicians with a glimpse of the diverse trends in the technology.

Delivery/Depot adjuvants

Mineral salt based adjuvants: Aluminium adjuvants

Aluminium salts such as aluminium hydroxide, aluminium phosphate and potassium aluminium sulphates (alum) are the commonly used aluminium compounds in alum adjuvants. They are the most widely used adjuvants in human vaccines but lack the ability to invoke a cell-mediated immune response. They act by enhancing the antigen retention at the site, thus allowing detection by the immune cells. Aluminium salts activate the nucleotide binding domain-like receptor protein 3 (NLRP3). Other mechanisms of action may involve complement, eosinophil and macrophage [24]. Despite the broader success, they are usually criticised for being associated with various local and allergic reactions. Studies have also shown that higher level of aluminium in the body causes a fatal neurological syndrome and dialysis-associated dementia [18].

Other mineral salts have also been tested for the depot effect like the salts of calcium, iron and zirconium. In particular, calcium phosphate has been used for diphtheria, tetanus, and pertussis (DTaP) vaccines [25]. The basic advantage of calcium salt over the aluminium was that it mimics the natural physiological component and of which was well tolerated. It induced higher IgG levels than IgE [26,27].

Tensio-active adjuvants

The saponin, a derivative of *Quillaja saponaria* has been used as an alternative to alum because of its strong cellular responses. Saponins are triterpenes with better stability and biocompatibility [27]. Squalene and its hydrogenated form squalane both have been ideally suited for making stable and non-toxic emulsions [20]. These compounds induce strong cytotoxic CD8+ lymphocyte responses. Quil A is a purified saponin derivative and has also been successfully used for veterinary applications.

Emulsions

This class includes oil in water or water-in-oil such as FIA, montanide, adjuvant 65, and lipovant [28]. Their mechanism of the action revolves around the release of antigen and the stimulation of antibody-producing plasma cells. However, they are too toxic for routine prophylaxis in humans. Different types of oil have been used under various conditions to explore a more stable, potent and less toxic formulations [29]. Montanide is a family of oil-based adjuvants that have been used in experimental vaccines in mice, rats, cats and dogs, using natural, recombinant and synthetic antigens. In humans, montanide has been used in trial vaccines against HIV, malaria and breast cancer [30]. Presently vaccines employing montanide adjuvants are available for immunoprophylaxis in animals.

Liposome

Liposomes are bilayered spherical vesicles made of phospholipids. They can be either multi-lamellar or uni-lamellar based on the number of lipid bilayers present in their structure. Liposomes are highly flexible delivery systems, able to carry both hydrophobic and hydrophilic substances [31,33]. They are relatively nontoxic and can act as both vehicle and immunopotentiator. Their potency depends on the number of layers, electric charge and composition [32]. They have the ability to enhance both humoral and cellular immune responses. Liposomes have been used widely under experimental conditions, but so far not registered for human use.

Polymeric microsphere

Polymeric micro/nanoparticles have drawn attention in terms of their use as delivery systems for vaccine antigen and therapeutics. Among particulated and polymeric systems, poly (DL-lactide-co-glycolide -PLGA) microspheres have been extensively studied. This compound controls the time of antigen release by altering their degrading kinetics thus making the system more stable [33].

They are broadly classified into two types viz., biodegradable and non -degradable. The biodegradable class encompasses polyesters, polylactides, poly-ε-caprolactone, Polyanhydrides, polyphosphazenes, and polyvinylpyrrolidone which can effectively substitute conventional classes of delivery adjuvants delivering targeted results with lesser toxicity. In contrast, the non-degradable nanoparticles include different materials such as latex, gold, silica, and polystyrene which are being evaluated as antigen carriers for increasing antigen persistence [34].

Immunostimulating complex - ISCOM

Morein et al. in 1984 described the immunostimulating complex (ISCOM) as a particulate antigen delivery system, composed of antigen, cholesterol and phospholipid [35]. ISCOMs have shown to enhance the immune response by simultaneously promoting both humoral and cell-mediated responses, including enhanced cytokine secretion in a variety of experimental animal models. Similarly, ISCOMATRIX is a particulate adjuvant comprising cholesterol, phospholipid and saponin but without antigen. ISCOMs and ISCOMATRIX combine the advantages of a particulate carrier system with the presence of an inbuilt adjuvant (Quil A) and so have been found to be more immunogenic [36].

Immunopotentiators

Microbial adjuvants

Bacteria derived substances constitute a major potential source of adjuvants because of their immuno-stimulatory capacity. Cell-wall peptidoglycan or lipopolysaccharide of Gram-negative bacteria enhances the immune response against co-administered antigens despite themselves not being very immunogenic. Such an activity is mediated by toll-like receptors, activating the danger signals which in turn fire the host immune system [18]. Components from different bacterial species like *Mycobacterium* spp., *Corynebacterium* spp., *Bordetella pertussis* and *Neisseria meningitides* have been used for in vivo studies but failed due to their toxigenic effect [29]. However, researchers achieved tremendous success in isolation of the immunogenic fraction of the bacterial cell wall N-acetylmuramyl-L-alanyl-D-isoglutamine, also called muramyl dipeptide (MDP), thus negating the toxic effects of using the whole organism. The unique nature of this adjuvant is the ability to stimulate humoral and cell-mediated immune responses under different conditions/modes of administration [37]. Subsequently, many other compounds were also derived from bacteria like threonyl-MDP, trehalose dimycolate (TDM) which simulates both humoral and cellular immune responses. DNA containing CpG motifs and lipopolysaccharide (LPS) from Gram-negative bacteria enhances the cellular immune response in addition to B-cell mitogen. It was found that the major toxigenic and immunogenic factor was lipid A component of LPS which could be hydrolysed to obtain monophosphoryl lipid A, with all the adjuvant activity but without the toxicity [18].

Cytokines

Cytokines are the recent era of exploration for use as adjuvants. Cytokines are included in the modern classification of adjuvants. Of the innumerable cytokines, few have gathered attention like IFN-γ for its ability to enhance cellular immune response [38] and Granulocyte-macrophage colony stimulating factor (GM-CSF) for its ability to activate antigen presenting cells [39]. Cytokine adjuvants are envisioned primarily in combination with DNA vaccines where antigen and cytokine expressing genes can be harboured in the same vector. The effect of IL-12 and IL-15 as a mucosal adjuvant for improved IgA and IgG2a and immunomodulatory role has been highlighted in several studies [24].

Carbohydrate adjuvants

There are many polysaccharides capable of stimulating the immune system, which are usually sourced from plants and fungus. Gamma inulin is a potent humoral and cellular immune response activating adjuvant and is also an activator of the alternate complement pathway thereby activating macrophages [40]. Unlike FCA, gamma inulin does not elicit toxic reactions, rather it forms a suitable combination with another class of adjuvants to be engineer adjuvants targeting cellular and humoral responses e.g., algammulin (gamma inulin/alum hybrid adjuvant). Other carbohydrates which have adjuvant action include glucans, dextrans, lentinans, glucomannans and galactomannans. Levans and xylans, also have immunoenhancing activity [41]. Acemannan, a natural polysaccharide extracted as a mucilaginous gel of the Aloe barbadensi, stimulates generation of cytotoxic T lymphocytes (CTLs) [42]. The most investigated polysaccharide for mucosal vaccine delivery is poly-D-glucosamine (chitosan). This polymer is prepared by the partial deacetylation of chitin. The advantages of chitosan adjuvant lie due to its low production cost, biocompatibility and biodegradability. Its ability to enhance macromolecular penetration across the intestinal and nasal barrier is an admirable trait for its use in mucosal vaccines [34].

TLR-based adjuvants

TLRs play a critical role in the innate immune system and are expressed in a variety of immune cells, including macrophages, dendritic cells (DCs), mucosal epithelial cells, neutrophils and dermal endothelial cells [43]. Adjuvants based on stimulating TLR signalling pathway would prove effective in immune modulation. Several TLR agonists have been established to have adjuvant activities. For example, a TLR4 agonist (3-O-desacyl-40-monophosphoryl lipid A/MPL) adsorbed to alum known as AS04 is currently approved for use against Human papilloma and Hepatitis B virus [44,45].

Laser vaccine adjuvants

The use of non-destructive lasers to alter tissue immune responses is a novel approach to enhance systemic vaccine responses which was initially explored in Russia and further developed in the US. This technology significantly improves responses to both prophylactic and therapeutic vaccines administered to the laser-exposed tissue, particularly the skin. It is speculated that it mostly acts by modulating dendritic cell trafficking by the release of specific signaling molecules from epithelial cells. So this technology can be effectively implemented in case of intradermal vaccines [46].

Virosomes

Virosomes are unilamellar structures composed of membrane lipids and viral membrane proteins. Due to the physical association with viral antigen, it results in enhanced immunity. The potential advantage of this system is easy uptake by antigen presenting cells. A flu vaccine licensed under the name Inflexal V2 in Europe is immunopotentiating reconstituted influenza virosomes (IRIV). Similarly, a number of vaccines are being marketed such as Epaxal™, a hepatitis A vaccine registered in 1994 in several European, Asian and South American countries [22,47].

Virus like particles

The virus-like particles (VLPs) stimulates the immune response by delivering a material that mimics certain structural properties of a virus. The VLPs are essentially non-infective virus consisting of self-assembled viral envelope proteins without the genetic material. The VLPs retain morphology and cell penetrating ability similar to infective viral particles. The VLPs have also been shown to stimulate both cellular and humoral immunity [48].

Conclusion

The adjuvant technology has transcended way beyond the conventional approach of vaccinology. With the vaccine antigen discovery in full swing, the adjuvant technology has matched shoulders with it, so that an effective and targeted formulation can be achieved. Before choosing an adjuvant from the list of adjuvants available, one must be thorough regarding its use and the target that needs to be achieved. Despite several efforts and advances in the field very few have made it to the to the application level. Nevertheless, the role of adjuvants in both therapeutic and prophylactic vaccines is imperative. In the world where therapeutics alone fails to combat evolving drug-resistant microorganisms and the threat of non-infectious diseases like cancer, immunoprophylaxis through a strategic course in vaccine formulation blended with tactical exploration and exploitation of the immune system would relieve both human and animal health sectors of the intimidating situation.

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