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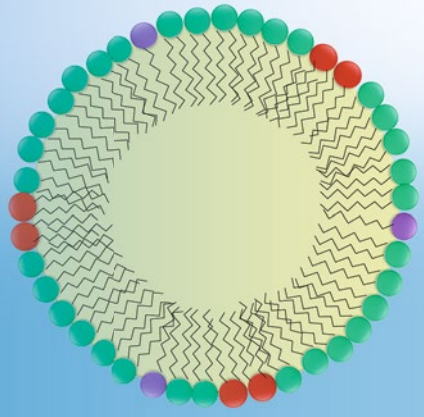

Christopher B. Fox *Editor*

Vaccine Adjuvants

Methods and Protocols

Humana Press

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Vaccine Adjuvants

Methods and Protocols

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Preface

"So far, the results have been very, very exciting…But now we got involved into practical application" --Edgar Ribi [1]

Vaccine adjuvants have an interesting and empirical history, which led immunologist Charles Janeway to refer to them as the "immunologist's dirty little secret." Nevertheless, pioneering work led by Edgar Ribi elucidated structure-function relationships of adjuvant components while emphasizing practical application and manufacturing aspects, leading to the development of the TLR4 ligand MPL® that is now contained in approved human vaccines. In recent years, progress in vaccine adjuvant technology has accelerated to an exciting pace, including FDA approval of adjuvant-containing vaccines such as Cervarix® (2009) and Fluad® (2015), and positive phase III clinical results of adjuvanted vaccines for malaria, shingles, and hepatitis B. In addition to these significant clinical advances, earlier stage progress in adjuvant development including use of synthetic raw materials, novel formulation and characterization approaches, elucidation of mechanisms of action, and technology transfer to developing country institutions is likewise highly encouraging. Moreover, the critical role of adjuvants with regard to global pandemic preparedness is being realized. Given these considerations, there is a clear need for up-to-date information on the practical methods and protocols important for successful adjuvant synthesis, formulation, and evaluation from the experts in the field.

The complex factors involved in the design, synthesis, formulation, physicochemical and bioactivity characterization, and clinical development of vaccine adjuvants are often underestimated, in part because adjuvant access and formulation know-how have historically not been widely available. This collection seeks to elucidate the practical methods necessary for successful adjuvant development, with a particular focus on the synthesis, formulation, manufacturing, and characterization aspects involved. It is anticipated that readers will be empowered to develop effective and stable vaccine adjuvants with product potential through application or adaptation of these techniques. While in some cases there is necessarily some overlap, my intent has been to avoid duplication of material covered in previous books from the Springer Protocols series, including the excellent volumes edited by Derek T. O'Hagan (*Vaccine Adjuvants: Preparation Methods and Research Protocols*, Methods in Molecular Medicine, 2000) and by Gwyn Davies (*Vaccine Adjuvants: Methods and Protocols*, Methods in Molecular Biology, 2010). The reader is referred to these previous books for further information on vaccine adjuvants.

The present volume begins with two review chapters, one focused on an overview of adjuvants in general and the other a specific case study on the development of the CpG adjuvant 1018. Chapters 2–8 concern the in silico design, chemical synthesis, biosynthesis, and/or purification from natural raw materials of specific adjuvant molecules. Chapters 9–15 involve adjuvant formulation approaches, including liposomes, oil-in-water emulsions, aluminum salts, block copolymer gels, biodegradable polymeric particles, and lyophilized cakes. The analytical characterization of adjuvant formulations and adjuvant-containing vaccines is treated in Chapters 16–21, involving particle sizing, vibrational spectroscopy,

antigen-specific fluorescent and gel-based techniques, methods to separate antigens from adjuvants prior to analysis, and stressed stability approaches. Finally, chapters 22–26 involve the biological characterization of vaccine adjuvant activity, including in vitro and in vivo approaches, including modern bioinformatic tools, to measure innate and adaptive immune responses. Given the expansiveness of current adjuvant research and development, it was not possible to include every topic of interest. Nevertheless, a wide range of molecular and particulate adjuvants has been represented in the chapters included here.

It is my sincere pleasure to introduce the reader to this volume on vaccine adjuvants. I hope he or she will find it to be as informative and useful as I have, and that the methods described here by expert hands-on authors will facilitate vaccine adjuvant product development efforts. I have long been impressed with the practical approach and helpful notes featured in the Springer *Methods in Molecular Biology* series. By focusing this volume on the pragmatic aspects of vaccine adjuvants, my goal is to help them become more accessible, manufacturable, and better characterized. Ongoing efforts along these lines should help in removing the "dirty little secret" sobriquet from adjuvants, and in the tradition of Edgar Ribi, turn exciting results into practical applications.

Seattle, WA, USA *Christopher B. Fox*

Reference

1. NIH Oral History Interview (1985) Hamilton, Montana, USA

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Chapter 1

Overview of Vaccine Adjuvants: Introduction, History, and Current Status

Ruchi R. Shah, Kimberly J. Hassett, and Luis A. Brito

Abstract

Adjuvants are included in sub-unit or recombinant vaccines to enhance the potency of poorly immunogenic antigens. Adjuvant discovery is as complex as it is a multidiscplinary intersection of formulation science, immunology, toxicology, and biology. Adjuvants such as alum, which have been in use for the past 90 years, have illustrated that adjuvant research is a methodical process. As science advances, new analytical tools are developed which allows us to delve deeper into the various mechanisms that generates a potent immune response. Additionally, these new techniques help the field learn about our existing vaccines and what makes them safe, and effective, allowing us to leverage that in the next generation of vaccines. Our goal in this chapter is to define the concept, need, and mechanism of adjuvants in the vaccine field while describing its history, present use, and future prospects. More details on individual adjuvants and their formulation, development, mechanism, and use will be covered in depth in the next chapters.

Key words Adjuvant, Alum, Nanoemulsion, Vaccine, Immunopotentiator

1 Introduction

Vaccination has protected the human race from numerous devastating diseases, improved the quality of life, and extended the average lifespan. According to statistics released by National Institute of Health in 2010, vaccines have prevented approximately 2.5 million deaths and countless cases of illness each year [1]. The modern day concept of vaccination was introduced by Edward Jenner in the eighteenth century when he made the connection between the lack of small pox infections and milk maids. Using this observation Jenner took cow pox (which does not cause severe disease in humans) and inoculated individuals. Those individuals were then found to be protected against small pox infection [2]. However, well before Jenner's observation many Asian and African countries had practiced a similar concept of variolation (using infected material to immunize a healthy individual against the same infection) for centuries [2, 3]. This history of vaccination is not only

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interesting but also suggestive of the empirical approach that has always been a trademark of vaccine development from inception until today, despite our increasing understanding of the field of vaccinology [4].

Vaccination is a process of mimicking infection in the body leading to the activation of the immune system for the generation of a potent immune response [5, 6]. Once injected, pathogen-associated molecular patterns (PAMPs) present in the vaccine interact with the pattern recognition receptors (PRRs) on the innate immune cells present at the site of injection (SOI) and initiate an immune cascade. This involves upregulation in the production of chemokines and cytokines that subsequently lead to an increase in the number of antigen presenting cells (APCs) at the SOI. The APCs are involved in antigen uptake and subsequent presentation to T-cells that ultimately are responsible for priming CD4+ and CD8+ responses. These T-helper cells also activate the B cells, leading to the production and secretion of antibodies. Activation of both B and T cells is required for a robust immune response. A portion of the total T and B cells transform into memory cells which can mount an adaptive immune response quickly during future infections [6].

As medical science advanced the crude methodology of Jenner was refined leading to an improvement in the safety and efficacy of vaccines. Building directly from Jenner's work, live attenuated and whole killed pathogens are considered the first generation of vaccines. Live attenuated vaccines contain weakened versions of the pathogen, virus or bacteria. These attenuated pathogens can replicate inside the host leading to long lasting immunity. These vaccines are highly effective; yet there is a concern of reversion to its virulent form. For example the vaccine for Venezuelan Equine Encephalitis has to undergo only two-point mutations to return to virulence, limiting its utility to vaccinating high-risk individuals such as lab workers [7]. Whole killed vaccines on the other hand are incapable of replication as they undergo a viral inactivation step such as crosslinking, or viral splitting. Despite the lack of reversion for these types of vaccines, safety is still a concern. In the 1960s a formalin-inactivated vaccine against RSV in a clinical trial killed an infant subject [8]. This tragedy hampered the RSV field, and until recently no vaccine candidates have entered into late-stage clinical trials [9]. Another type of commercially available vaccine is the inactivated toxoid, e.g., tetanus. These traditional vaccines are still in use as they are highly potent; but in certain disease targets there are safety concerns and issues with the manufacturing process and in some non-cultivable microorganisms this traditional approach does not work [10, 11]. The limitations outlined above led to the introduction of subunit and recombinant protein vaccines. Subunit and recombinant proteins are highly purified antigens which require only a part of the pathogen to generate a protective immune

response. These antigens improved vaccine development as they proved to be safe with no ability to revert to a virulent form and were easier to manufacture and characterize. Also, these antigens exhibit low potency due to fewer PAMPs in comparison to the conventional attenuated or whole inactivated vaccines. Adjuvants were thus introduced in vaccines to enhance the immunogenicity of these weaker antigens and help in improving the overall potency of poorly immunogenic subunit vaccines [11].

Adjuvants are defined as materials added to vaccines in order to improve the immunological response. Adjuvants have many potential benefits such as reducing the frequency of vaccination, reducing the dose of antigen per vaccine (dose sparing), improving the quality of the immune response, and promoting cross-clade immunity and in certain cases they may improve the stability of the final vaccine formulation [12]. Adjuvants have been used to improve immunogenicity of vaccines in immune-comprised patients (e.g., HIV positive), infants, and elderly patients. Adjuvants such as MF59 and AS04 have even improved the efficacy profile of the vaccine in comparison to non-adjuvanted vaccines or placebo [13, 14]. In this chapter we focus on adjuvants which are added specifically to enhance the immune responses of a poorly immunogenic antigen.

Conventional classification schemes based on origin, disease target, route of administration, type of formulation, mechanism of action, intended use (delivery vs. immune potentiation), etc. may not be directly applied to vaccine adjuvants. One way to classify adjuvants is according to different generations—based on how they interact with the immune system and their composition [15]. Particulate adjuvants like alum, emulsions, liposomes, and microparticles can be considered as the first generation of vaccine adjuvants. This first generation can also be considered as antigen delivery systems which promote the uptake of the co-administered antigen from the SOI [15]. The second generation of vaccine adjuvants may be best described as combinational adjuvants as they are comprised of immune potentiators combined with the first generation of vaccine adjuvants, e.g., AS04 which is included in Cervarix® and consists of alum and a TLR4 agonist [15]. AS04 is a part of the adjuvant systems by GlaxoSmithKline which applies a similar concept of combining delivery systems and immune potentiators into one single system; we will discuss these individually in the following sections.

Currently there are many types of adjuvants available for vaccine use being evaluated throughout various stages of vaccine development. Ultimately the selection of an adjuvant for a vaccine should take many factors into consideration. Safety of an adjuvant is the first criteria and it is dependent on the risk to benefit ratio of the intended vaccine. An adjuvant should be safe, well tolerated, easy to scale up and manufacture, pharmaceutically acceptable (in regard to pH, osmolality, endotoxin levels, etc.) with a reasonable shelf life, compatible with the antigen, and economically feasible [16].

Establishing all these parameters while maintaining the safety of the adjuvanted vaccine is a difficult time-consuming process; therefore currently only a handful of vaccine adjuvants are included in commercial vaccines.

2 Current Adjuvants

Although many well-respected academic and industry groups have excellent adjuvant research programs, very few of their discoveries have successfully translated to components in licensed vaccines. In the USA aluminum salts, AS04 (monophosphoryl lipid A [MPL] with aluminum hydroxide), AS03 (oil-in-water emulsion consisting of squalene, alpha-tocopherol, and Tween 80), and MF59 (oil-in-water emulsion consisting of squalene, polysorbate 80, and sorbitan trioleate) are adjuvants included in licensed vaccines [17, 18]. In addition to adjuvants licensed in the USA, Europe has licensed vaccines containing virosomes [17]. Each vaccine adjuvant has had its own challenges and successes. Experiences from previously studied adjuvants and the pharmaceutical feasibility of adjuvants have impacted and directed the development of the future adjuvants.

2.1 Aluminum-Based Adjuvants

Aluminum salt solutions were originally added to growth medium to help purify tetanus and diphtheria vaccine antigens through precipitation, but it was soon discovered that aluminum precipitated antigens were more immunogenic than the soluble antigens [19]. Aluminum-based adjuvants have been used since the 1920s, making them the adjuvant used for the longest period of time and the most frequently used adjuvant in licensed vaccine products with approximately one-third of licensed vaccines containing alum [20]. As a result alum has an extensive track record of safety in vaccines.

Although potassium aluminum sulfate was originally referred to as alum, aluminum hydroxide and aluminum phosphate are more commonly referred to as alum in the vaccine community. Aluminum hydroxide has a crystalline needle like morphology whereas aluminum phosphate appears as amorphous loose aggregates [21]. Alum has been used as an antigen delivery system where the antigen interacts primarily though electrostatic interactions and ligand exchange. The electrostatic interactions of antigen and alum are a function of pH and type of alum. The point of zero charge (PZC) will determine the charge of alum; for aluminum hydroxide and aluminum phosphate the PZC are approximately 11 and 5, respectively [22]. Based on the formulation pH and the isoelectric point (PI) of the antigen, the appropriate alum adjuvant can be chosen to maximize adjuvant-antigen electrostatic interactions by having oppositely charged antigen and adjuvant [23]. Ligand exchange occurs when hydroxide groups on the alum exchange with phosphate groups present on the antigen. Although

association of antigen to alum allows the antigen to remain at the site of injection for longer periods of time, association of antigen to alum may not be critical for immune potentiation [24].

Alum promotes a strong Th2-biased response, also referred to as a humoral immune response. Although the exact mechanism of action for alum is still unknown, proposed mechanisms include depot effect, an inflammatory response which recruits antigen-presenting cells, NALP3 inflammasome activation, release of DNA from cell death causing danger-associated molecule pattern (DAMP) recognition, and enhanced phagocytosis by antigen-presenting cells [15, 25–27]. Despite the success of alum use in many vaccines, it has limitations particularly for use against intracellular pathogens and pathogens that require a strong cellular immune response. In addition, alum is sometimes found to be not potent enough as an adjuvant for some antigens, e.g., influenza vaccines where alum was found to be a poor adjuvant [28, 29].

One approach to overcome the limitations of alum is to use it to co-deliver it with additional adjuvants. Adjuvant system AS04 combines aluminum hydroxide or aluminum phosphate with the immunostimulatory molecule monophosphoryl lipid A (MPL) [30]. Mechanistic studies suggest that alum and MPL do not work synergistically, but alum facilitates the delivery of MPL at the site of injection and increases the duration of cytokines [31]. Monophosphoryl lipid A (MPL) is a modified version of lipopolysaccharide (LPS) that is significantly less toxic but still remains a TLR4 agonist [32]. By including MPL with aluminum hydroxide, both a Th1 and Th2 response can be created [30]. AS04 is currently used in licensed human papillomavirus (Cervarix®) and hepatitis B (Fendrix®) vaccines [30].

Alum-based vaccine formulations have limitations regarding stability. When alum is frozen, alum particles significantly aggregate leading to a decrease in vaccine efficacy when administered [33–36]. To avoid potential freezing, vaccines need to be transported and stored in a very narrow temperature range throughout the cold chain. Although no commercial formulations containing alum are stored frozen or lyophilized, proof-of-concept studies have shown the feasibility of lyophilizing alum formulations [20, 36–38].

2.2 Emulsions

Another approach that has an extensive history of use as vaccine adjuvants are emulsions. The earliest used emulsion designed as a vaccine adjuvant was a mineral oil-based water-in-oil emulsion called Freund's adjuvant. The water-in-oil (w/o) emulsion comes in two forms, complete Freund's adjuvant (CFA) which contains mineral oil, emulsifier, and killed bacteria *M. tuberculosis* and incomplete Freund's adjuvant (IFA) which has the same composition as CFA without the bacteria [39]. Although Freund's adjuvant has a long history of use, it will likely never be included as originally described in human vaccines due to safety concerns; it has been

approved for use in certain large animal veterinary vaccines [40]. Toxicity issues were caused by the non-biodegradable oil, high levels of oil in the emulsion (water-in-oil), reproducibility of emulsion, and poor oil and/or emulsifier quality [41–45]. Despite this, CIMAvax EGF, a therapeutic non-small lung cancer vaccine developed and marketed in Cuba contains another mineral oil containing water-in-oil emulsion adjuvant Montanide ISA 51 by Seppic [46].

To create an adjuvant without the tolerability issues associated with FCA or IFA, oil-in-water emulsions prepared with biodegradable/biocompatible oils such as squalene (e.g., MF59) were developed in the 1980s [47]. MF59 is primarily used in influenza vaccines since it can improve immune responses and improve cross-reactivity to a wide array of influenza strains [48]. MF59 has been shown to be safe and well tolerated with millions of doses administered in over 35 countries [49]. MF59 is composed of squalene, Span 85, and Tween 80 in 10 mM sodium citrate buffer at pH 6.5 with an average droplet size of approximately 165 nm [47, 50]. Recently the mechanism of action of MF59 has been extensively studied and although it is still ongoing various theories have been established [51, 52]. MF59 does not create an antigen depot at the site of injection and the antigen and MF59 are cleared independently from the site of injection. An immune competent environment is created at the SOI leading to an influx of APCs and other immune cells. MF59 also upregulates production of cytokines and chemokines which further attracts the immune cells to the SOI. This migration of APCs leads to an increase in uptake of the antigen, especially by neutrophils and monocytes, and translocation to draining lymph nodes where MF59 also helps in priming the immune responses [51, 52].

AS03, a GlaxoSmithKline proprietary adjuvant, has also been used for influenza vaccines where it enhances immune responses similar to MF59 [53]. The difference in composition (squalene, alpha-tocopherol, and Tween80 in phosphate buffered saline) of AS03 leads to a different mechanism than MF59 [54]. Alpha-tocopherol (vitamin E) has been shown to have antioxidant and immunostimulatory properties which have been found to be critical to the adjuvant effect of AS03 [55]. AS03 and antigen must be delivered to the same site for an enhanced immune response to be achieved but emulsion and antigen do not have to be associated to generate the enhanced immune response [55]. Monocytes and granulocyte recruitment at the SOI are responsible for mechanism of action of AS03 [55].

To further enhance the immunogenicity of AS03, adjuvant system AS02 consists of the AS03 emulsion and incorporates the immune potentiators QS-21 and MPL to induce both strong antibody and cellular immune responses [30, 56]. QS-21 is a saponin from the soap bark tree, *Quillaja saponaria*, and has been found to enhance the immune response by producing high antibody titers,

improving responses for T cell-independent antigens, and promoting CD8+ T cell responses [57].

Another emulsion that contains a TLR4 agonist is stable emulsion with glucopyranoside lipid adjuvant (GLA-SE). GLA-SE contains squalene, glycerol, phosphatidylcholine, glucopyranoside lipid adjuvant (GLA), and pluronic F68 in ammonium phosphate buffer [58]. A synthetic analogue of MPL, GLA has been shown to be more potent per molecule and less toxic than MPL [59]. The particles formed in stable emulsion are 100 nm in diameter [58]. Formulations containing GLA create a Th1 type of immune response. GLA can also be formulated as an aqueous formulation, in a liposome or adsorbed to alum, where each delivery system yields a slightly different immune response [58]. Additional emulsions have been evaluated as adjuvants including AF03 and WEC50; for a more detailed discussion on emulsion adjuvants the reader is referred to previously published reviews [10, 60].

2.3 Lipid-Based Particles

Liposomes are spherical particles containing a bilayer of phospholipids with an aqueous center [61]. Liposomes can be used to deliver both antigen and immunostimulatory molecules [61]. Components can be encapsulated within, associated with the membrane, or adsorbed on to liposomes [62]. Since liposomes alone do not create a strong immune response, they are often combined with immunostimulatory molecules [63–65]. Cationic liposomes have been found to improve immune responses more than neutral or anionic liposomes since cationic liposomes increase the uptake of entrapped antigen to cells [66]. CAF01, a cationic liposomal adjuvant developed by Statens Serum Institute containing DDA (dimethyldioctadecylammonium) and TDB (trehalose dibehenate), is now being clinically tested as a component of a tuberculosis vaccine [67].

Immunostimulating complexes (ISCOMs) were developed in the 1980s. ISCOMs originally had antigen incorporated with Quil A adjuvant with phospholipids and cholesterol [68]. To facilitate antigen association with the 40 nm ISCOMATRIX particles, it was found that the antigen must be amphiphilic [69]. Due to its poor tolerability Quil A is now replaced with more refined saponin preparations [70]. ISCOMATRIX has a dual role: immunomodulation and antigen delivery [69]. While it modulates the immune response by activation of immune cells and upregulation of cytokines and chemokines, it is hypothesized that it interacts with membranes on the cell surface and endosomes to deliver the antigen into the cytosol [69, 71]. As ISCOMATRIX can efficiently induce CD8+ responses it has been used as the gold standard for CTL immune responses [15].

The most clinically advanced liposomal adjuvant is AS01, a liposome composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, MPL, and QS21. Immediately before injection, AS01 is combined with the antigen. It has been reported that

for AS01 to be effective, the adjuvant and antigen must be delivered to the same injection site at the same time which leads to AS01 recruiting antigen-presenting cells to the injection site [72].

2.4 Polymeric Particles

During the 1980s poly(lactide-co-glycolide) (PLG) microparticles began to be evaluated for use as adjuvants [73]. In addition to being biodegradable and bio-compatible, these particles offer the possibility of a single-shot vaccine, thus overcoming the need for booster shots [74]. Since PLG is biodegradable, antigen entrapped within the polymer was able to be released once the particle was introduced into an aqueous environment [75]. Unfortunately, harsh conditions are required to entrap the antigen in PLG which results in a loss of antigen stability [76]. To overcome the loss of antigen stability during incorporation of antigen into the PLG particle, adsorption of antigen on the surface of PLG particles has also been attempted [77]. Since PLG particles induce immune responses only marginally better than alum, further development of this adjuvant has been halted since alum has a long history of use and safety. To increase the immune response generated with PLG particles, immune stimulating molecules have also been entrapped in the microparticle [76, 78]. Polymeric nanoparticles have also been evaluated as adjuvants, but there has been no biological advantage to the use of nanoparticles as opposed to microparticles [79].

3 Future Prospects for Adjuvants

Adjuvant research is an active field due in part to an increased need to improve immune responses of poorly immunogenic antigens, an increased understanding of the molecular mechanism of the innate immune system, improved biophysical analytical techniques for analysis of nanoscale assemblies, and a number of clinical successes in the past 15–20 years. Approval of an adjuvanted influenza vaccine containing MF59 in 1997 in Europe illustrated a path forward for emulsion adjuvants. Emulsions such as AS03, AF03, and SE soon followed a similar path and the field largely focused on the use of squalene as the oil of choice within emulsion adjuvants. Alternate oils have been evaluated further supporting the use of squalene as an adjuvant [80]. Interesting to note is the sizes were not evaluated in that report; we recently identified the size of the oil droplet to be critical to eliciting the appropriate immune response with changes as little as 70 nm impacting immune responses [81]. Recently a series of papers have focused on detailing the mechanism of action of MF59 [52, 82]. This increased mechanistic understanding is useful to benchmark novel adjuvants against a safe well-tolerated class of adjuvants.

A number of adjuvanted clinical candidates have recently gained significant attention. The adjuvant systems developed by

GSK have been advancing through the clinic. Recently scientists at GSK reported >96% efficacy with an $AS01_B$-adjuvanted herpes zoster vaccine in a phase III clinical trial [83]. An AS01-adjuvanted malaria vaccine was recently given a positive recommendation from the EMA [84]. Additionally the AS04-adjuvanted hepatitis B and human papilloma virus vaccines have successfully been in use for a number of years [85]. These examples clearly illustrate how an adjuvant can improve immune responses for vaccines where the mechanism of neutralization is understood in part.

Dynavax's hepatitis B vaccine Hepsilav-B has shown promising results in three phase III trials. Hepsilav-B includes immunostimulatory sequence 1018 which contains unmethylated CpG motifs allowing it to act as a TLR 9 agonist [86]. Several advantages over currently marketed products have been seen in Hepsilav-B including a reduced number of doses required from three doses over 6 months (Engerix-B) to two doses in 1 month to achieve seroprotection, and increased seroconversion in hypo-responsive populations such as obese, smokers, males, and diabetics while maintaining a similar safety profile to approved vaccines [86]. After receiving a rejection on the FDA regulatory filing in 2013 due to insufficient safety data and concerns about adjuvant caused autoimmunity, Dynavax hopes to resubmit the application in 2016 with an increased number of safely immunized patients and positive results from the latest phase III trials [87].

The late-stage failure of the AS15-adjuvanted cancer vaccine is a reminder that a powerful adjuvant alone cannot generate the desired immune response [88, 89]. Deep understanding of biology is needed to generate the appropriate immune response. Although the field of immune-oncology has clearly made significant advances through the use of PD-1 antibodies and CAR-T therapy, the field in general has not yet reached a consensus on how to generate the most potent immune response against cancer cells within the patient. Therapeutic vaccines will rely heavily on adjuvants in order to coax the immune system to break tolerance (in the case of cancer vaccines), generate tolerance (in the case of allergy vaccines), or generate antibodies against poorly immunogenic antigens (e.g., nicotine vaccine).

Early-stage concepts include a recent report from Wu et al. describing a novel small-molecule adjuvant that binds to alum for enhanced responses [90]. Combining an existing well-established adjuvant with a novel immunostimulator leverages the existing safety record of alum while introducing a novel potent adjuvant for improving cellular responses and breadth of response. Recent phase II data for a peptide-based vaccine adjuvanted with the Matrix M2 saponin-based adjuvant was found to be highly effective in reduction of viral shedding for herpes simplex virus [91, 92].

During the 2009 influenza pandemic, a small but significant subset of vaccinated individuals who received AS03-adjuvanted flu vaccine in Europe developed narcolepsy [93, 94]. It was not until

recently that Soheil et al. identified homology between an antigen found in the vaccine and a protein found in the human body to lead to narcolepsy [95]. As the adjuvant improved the overall immune response of the vaccine, it likely helped elicit antibodies against this protein in a subset of patients. This example is a reminder that adjuvants need to be combined with well-defined and well-characterized vaccine antigens. Although vaccine adjuvants can improve responses and lead to improved health, particularly for unmet medical needs, an in-depth understanding of the biology and well-characterized antigens is critical for the field to succeed as a whole.

The increased use of recombinant proteins will inevitably lead to a greater use of adjuvants. Not many vaccines will require the "kitchen sink" approach where multiple immune stimulators are combined to create a varied and long-lasting immune response, although recent late-stage trials are illustrating the clear need to combine different classes of adjuvants for improving responses. As our understanding of the immune system improves through the use of antibody repertoire analysis and deep sequencing combined with other recent bio-analytical advances the immune system will be harnessed not only to be used for preventing infectious disease, but for treating autoimmunity and cancer, and there is a high likelihood that vaccine adjuvants will be a central player in those next-generation treatments.

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