New Generation Vaccine Adjuvants

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Vaccines represent an outstanding success story in modern medicine and are responsible for a huge reduction in morbidity and mortality worldwide, but it is clear that improvements are necessary to enable the development of successful vaccines against some difficult pathogens, including human immunodeficiency virus (HIV), hepatitis C virus (HCV) and respiratory syncytial virus (RSV). Vaccine improvements may include the addition of new adjuvants, which are able to induce higher immune responses, or immune responses with greater breadth, to cover the broad antigenic diversity of some pathogens. New generation adjuvants are starting to become available which may enable the development of new generation vaccines.

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Introduction – Vaccine Safety and Efficacy

The introduction of vaccines into medical practice at the beginning of the twentieth century has had an extraordinary impact on human health, and represents an unparalleled success story. Vaccines are widely considered to be the most safe and effective medical intervention available. In conjunction with the introduction of antibiotics and modern hygiene practices, vaccines have contributed enormously to a steady decline in the mortality and morbidity caused by infectious diseases (**Table 1**). Each year, the currently available vaccines prevent up to 3 million deaths and 750 000 children are protected from serious disability. Nevertheless, since vaccines are mainly used in young children with no pre-existing medical conditions, the level of scrutiny in relation to safety is very high. It is in this very conservative context that the advantages of the introduction of new and novel vaccine adjuvants needs to be considered, and establishing the safety of any new approach will remain a very high priority.

The Need for New and Improved Vaccines

Despite the success of current vaccines, there is a clear need for the development of vaccines against a number of infectious diseases for which vaccines are not yet available, or are inadequate, including human immunodeficiency virus (HIV), hepatitis C virus (HCV), RSV, Neisseria meningitides serotype B, Group A and B streptococcus, tuberculosis (TB) and malaria. Unfortunately, these pathogens have proven exceptionally difficult to control with traditional vaccines, and novel approaches will be required. New vaccines may also be needed to protect against a number of emerging or reemerging infectious diseases, including severe acute respiratory syndrome (SARS), Ebola, Hanta and Dengue viruses. In addition, improved vaccines are necessary to protect against the continued threat of the emergence of pandemic strains of influenza

Table 1 The impact of vaccines on disease burden in the US

Disease	Max. no. cases (year)	Cases in 2001	Reduction in disease (%)
Smallpox	48 164 (1901)	0	100
Diphtheria	206 939 (1921)	2	99.99
Pertussis	265 269 (1934)	4788	98.20
Tetanus	1560 (1923)	26	98.34
Polio	21 269 (1952)	0	100
Measles	894 134 (1941)	96	99.99
Rubella	57 686 (1969)	19	99.97
Mumps	152 209 (1968)	216	99.86
Haemophilus influenzae type b	20 000 (1992)	51	99.75

and the continued growth of drug-resistant organisms. Unfortunately, vaccines may also be required to protect against the threat of bioterrorism. In the broader context, there is an increasing awareness that infectious agents are often the cause of chronic diseases, which might be prevented or treated with new-generation vaccines. Moreover, in addition to their traditional role in preventing infection or disease, vaccines also have the potential to be used as therapeutic agents to treat cancers or chronic infectious diseases.

What are Adjuvants; Why Do We Need Them?

Vaccine adjuvants were first described about 80 years ago and have been used to improve immune responses against non-living vaccines since then. Although the role of an adjuvant is to improve the immunogenicity of antigens, they are often included in vaccines to achieve a range of more specific effects (**Table 2**). Historically, adjuvants have been crucial to the development of vaccines and they are likely to prove even more important in the future. The majority of vaccines currently in development are comprised of highly purified recombinant proteins, or peptides, representing subunits of pathogens. Unfortunately, these vaccines lack most of the features of the original pathogen and are often poorly immunogenic. Therefore, the need for vaccine adjuvants is greater now than ever before. The preferred strategy for the development of new-generation vaccines is to add highly purified synthetic adjuvants, which will activate only the elements of the immune response required for protection, and will not trigger a more generalized activation of the immune response. Vaccines that comprise attenuated live organisms or whole inactivated organisms do not generally require adjuvants.

How Do Adjuvants Work?

Adjuvants are included in vaccines to induce enhanced immune responses to vaccine antigens. Hence, adjuvants are defined by the effects that they achieve, rather than what they actually are, and a very diverse range of compounds and materials can achieve an adjuvant effect. To try to better define how adjuvants actually work, it is necessary to reduce the complexity of the immune response down to some very simple basic concepts. One way to do this is to consider which 'signals' are necessary to induce a successful immune response. With this approach, it begins to become possible to define how adjuvants make important contributions to vaccines, and also it becomes possible to place different kinds of adjuvants into broad groups, to understand better how they achieve their effects.

Table 2 The role of adjuvants in vaccines

Increase antibody responses – bactericidal, virus neutralizing, inactivating etc.
Induce cell-mediated immunity, e.g. T_H1 cytokines (interferon-γ)
Decrease the dose of antigen in the vaccine
Decrease the number of doses of vaccine necessary
Overcome competition between antigens in combination vaccines
Enhance immune responses in the young or elderly, who often respond poorly to vaccines

The signals necessary for a successful immune response to a vaccine antigen can be broken down as follows:

Signal 1 – antigen
Signal 2 – costimulation of immune cells, including antigen-presenting cells (APCs)
Signal 3 – immune modulation
Signal 0 – activation of the innate immune response

Adjuvants contribute directly to all of these signals, but different adjuvants do this in different ways. Some adjuvants can be better defined as 'delivery systems', since they are particulate carriers to which antigens can be associated, to stabilize the antigens, and to allow them to be present for extended periods of time. Hence delivery system-based adjuvants often prolong signal 1. Prolongation of signal 1 has also been called a 'depot effect'. Because delivery systems are particulates with similar dimensions to pathogens, they are taken up by phagocytosis into APCs, the key cells involved in immune response induction. Hence, delivery systems can also contribute to signal 2, and can indirectly activate APC. Immune potentiators are a different broad class of adjuvants, which exert direct stimulatory effects on immune cells (signals 2 and 3), and also initiate the immune response through activation of innate immunity (signal 0). Although immune potentiators are a very broad class of materials, typical immune potentiators are purified components of bacterial cells or viruses, or synthetic molecules that mimic these structurally. Consequently, they are recognized as 'danger signals' by receptors present on immune cells, particularly APC, which are present to 'sense' when an organism is infected. Once these receptors are engaged, the cells respond accordingly through activation of the innate immune response, which provides a first line of defence against pathogens. The most well known of these receptors are the Toll-like receptor family (TLR) (Medzhitov, 2001), which recognize diverse components derived from bacteria and viruses. However, it is clear that there are many other receptor families involved in immune activation and we are only at very early stages of understanding how these receptors and their downstream signals and mediators interact to ensure effective immune activation. Hence, vaccine adjuvants can be divided into two broad groups, based on their principal mechanisms of

Table 3 A simplified classification system for vaccine adjuvants

Antigen delivery systems	Immune potentiators
Alum	MPL and synthetic derivatives
Calcium phosphate	MDP and derivatives
Tyrosine	CpG oligonucleotides
Liposomes	Alternative bacterial or viral components – flagellin etc.
Virosomes	Lipopeptides
Emulsions	Saponins
Microparticles/nanoparticles	dsRNA
Iscoms	Small molecule immune potentiators, e.g. Resiquimod
Virus-like particles	

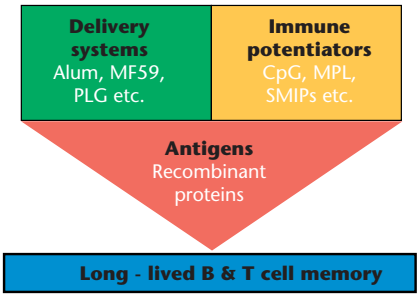

Figure 1 The optimal composition of new generation vaccines. Increasingly, new-generation vaccines will comprise recombinant protein antigens, synthetic immune potentiators, designed to stimulate only the appropriate immune response required for protective immunity and a delivery system, which will physically link the antigens to the immune potentiators and will focus their effects only to the relevant immune cells.

action, they can be classed as antigen delivery systems, or immune potentiators (**Table 3**).

If this simplistic adjuvant classification is linked to a geographical concept of immune response activation, in which antigens which do not reach local lymph nodes do not induce immune responses (Zinkernagel *et al.*, 1997), it allows a clearer definition of the mechanism of action of many adjuvants. The role of a delivery system is to enhance the amount of antigen reaching the cells that are responsible for the induction of the immune response, while immune potentiators are mainly responsible for directly activating these cells. Nevertheless, these simple definitions are often challenged when immune potentiators are included into delivery systems not only to focus their effects on to the immune cells, to maximize their potency, but also to minimize their effects on nonimmune cells. Hence, delivery systems improve the therapeutic ratio of immune potentiators, reduce the dose needed, and improve their specificity and safety. Therefore, optimal new generation vaccine adjuvants will comprise both immune potentiators and delivery systems, which will be designed to maximize potency and safety, through codelivery of antigen and key activation signals (immune potentiators) to the relevant APC populations (**Figure 1**).

What is the Current Status of Vaccine Adjuvants?

The main hurdle to the development of new and improved vaccine adjuvants has always been safety. Hence, although many adjuvants have been extensively evaluated in both preclinical and clinical studies, only insoluble aluminium salts (generically called 'Alum') have been included in licensed vaccines in North America. Alum-based vaccines were originally licensed more than 70 years ago. However, it still remains unclear exactly how aluminium salts work as adjuvants. Antigens are generally adsorbed to Alum adjuvants, which stabilizes antigens and protects them against degradation, so certainly signal 1 is enhanced. In addition, Alum induces an inflammatory response in tissues, which probably contributes to signals 2 and 0. Recent studies have highlighted the importance of interleukin 4 in the antibody response of animals immunized with Alum-adsorbed antigens (Jordan *et al.*, 2004). Moving beyond the use of Alum, an influenza vaccine containing an alternative adjuvant (Fluad™), called MF59 (Podda *et al.*, 2005), was successfully introduced on to the European market in 1997. More recently, some alternative adjuvant approaches have been, or are close to being approved in licensed vaccines.

The Emulsion Adjuvant MF59

MF59 is an oil in water emulsion of squalene oil, which is a naturally occurring substance found in plants and in a range of animal species, including humans. Squalene is an intermediate in the human steroid hormone biosynthetic pathway and is a direct synthetic precursor to cholesterol. Hence, squalene is biodegradable and biocompatible. Eighty percent of shark liver oil is squalene, and sharks provide the original natural source of squalene for the preparation of MF59 emulsion. MF59 also contains two nonionic surfactants, polysorbate 80 and sorbitan trioleate 85, which are included to optimally stabilize the small emulsion droplets. Pre-clinical experience with MF59 is extensive and has been reviewed on a number of occasions, most recently by Podda *et al.* (Podda *et al.*, 2005). MF59 adjuvant has also been shown to be safe and efficacious in humans (Podda, 2001). MF59 can be used with a wide range of antigens and is particularly effective for inducing high levels of antibody responses. The largest clinical experience with MF59 has been obtained with Fluad™, which is a licensed flu vaccine in more than 20 countries, and more than 23 million doses have been distributed. The adjuvant effect of MF59

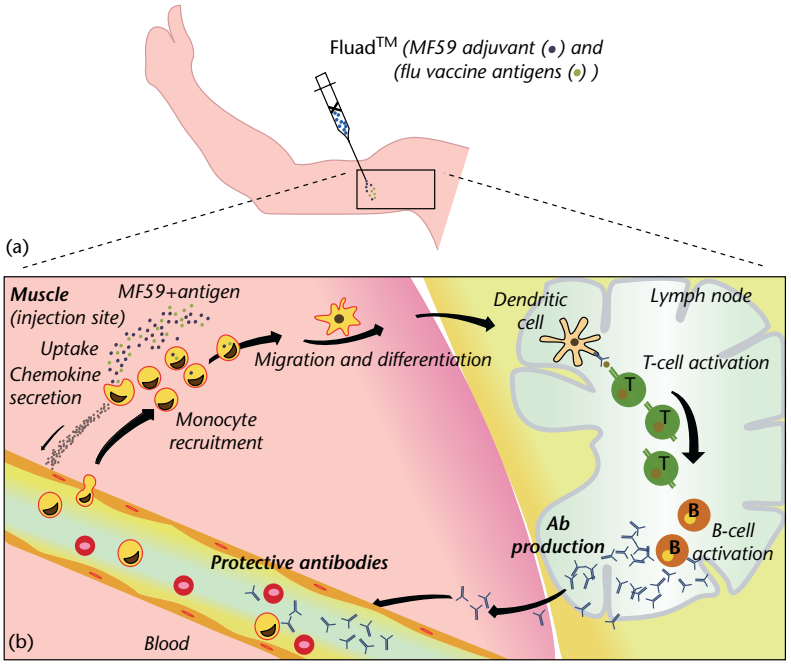

Figure 2 Summary of the mechanism of action of MF59 adjuvant. Following injection of Fluad™ through a syringe and needle into the muscle, MF59 and flu vaccine antigens are taken up by phagocytic cells. These include macrophages which differentiate from blood monocytes and can act as antigen presenting cells (APCs) for T cells. Monocytes and other cell types are activated by the uptake of MF59 adjuvant and respond by secreting chemical messages called chemokines, which are responsible for recruiting further monocytes and additional immune cells from the circulation into the site of injection. Activated macrophages, which contain flu antigens and MF59 adjuvant migrate towards the draining lymph nodes. MF59 uptake also enhances monocyte differentiation into dendritic cells, a type of APC that is very potent at presenting antigens. These APCs also migrate from the tissues to the lymph nodes, where they present flu vaccine antigens to naive T cells, resulting in activation of these cells. While macrophages are thought to act only as APCs for T cells that have previously been stimulated, dendritic cells express high levels of costimulatory molecules which confer upon them the special ability to activate T cells that have not previously encountered the antigen. The flu-specific T cells contribute to further activation of the flu-specific B cells, which are responsible for secreting flu-specific antibodies, which move into the blood circulation and offer protection against influenza infection and disease. MF59 directly enhances the number of flu antigen-specific T cells activated and also results in the secretion of higher levels of flu-specific antibodies into the blood. Reproduced by permission of Derek O' Hagan.

results in a significant increase in antibody titers against flu, compared to conventional nonadjuvanted flu vaccines (Podda, 2001). The increased immunogenicity of Fluad™ was shown to be particularly important in subsets of the elderly population who are at high risk for developing the most severe consequences of flu infection, due to chronic respiratory, cardiovascular and metabolic diseases (Podda, 2001). Additionally, MF59 induces enhanced immune responses against heterovariant flu strains, which is particularly important when the vaccine does not match the circulating strains of the virus perfectly (Del Giudice *et al.*, 2006; Podda, 2001). Extensive clinical evaluations have also established that MF59 adjuvant is very well tolerated (Podda, 2001). MF59 has also been evaluated as a potential adjuvant for inclusion in pandemic influenza vaccines and has been shown to induce a highly significant increase in antibody titres. In addition, MF59 also allowed a significant reduction in the antigen concentration, which might be very important to increase vaccine production capacity in a real pandemic (Nicholson *et al.*, 2001). Broader cross-neutralization against heterovariant pandemic strains was also observed with MF59 (Stephenson *et al.*, 2005), which is important, and might favour the use of MF59 adjuvanted pandemic vaccines for stockpiling.

Although MF59 has been used mainly in adults, it has also been shown to be a safe and efficacious adjuvant in both neonates and toddlers. Moreover, MF59 is generally a more potent adjuvant than Alum, for most vaccines evaluated. In relation to the mechanism of action of MF59, although it is generally considered to be a delivery-based adjuvant, promoting antigen uptake and presentation, MF59 has also been shown to have direct effects on immune cells. MF59 triggers the release of factors from immune cells which promote the recruitment and maturation of additional immune cells (**Figure 2** shows a summary of the mechanism of action of MF59 adjuvant).

Virosomal Vaccines

An alternative approach to vaccine delivery, which is also included in a licensed flu vaccine (Inflexal V™) in Europe is

called immunopotentiating reconstituted influenza virosomes (IRIV), or more simply 'virosomes'. Virosomes represent a modification of an established drug delivery approach in which phospholipids are used to prepare vesicles, called liposomes, which can be used as delivery systems for a variety of entrapped drugs. Liposomes have been used as successful delivery systems for anticancer drugs in marketed products. Virosomes are prepared by detergent disruption of influenza virus to free the viral membrane glycoproteins, followed by addition of phospholipids to allow liposomal vesicle formation, and removal of the detergents. Hence, the membrane antigens from influenza virus, mainly haemagglutinin are presented in a particulate structure of similar size to the native virus. The IRIV concept was first introduced on to the market in 1994, as a delivery system for a hepatitis A vaccine, but was subsequently also used for influenza vaccines. In contrast to the MF59 adjuvanted vaccine, which is focused on the elderly population, who need an improved flu vaccine due to age-related impairment of their immune responses, virosomes are used in all age groups and appear to represent an alternative approach to inactivated whole virus flu vaccines. Inactivated virus flu vaccines were originally introduced in the 1960s, but have been largely replaced by subunit vaccines, which are more highly purified and are better tolerated. Although virosomal flu vaccines are better tolerated than whole inactivated flu vaccines, there is limited evidence to suggest that they are actually more immunogenic than conventional flu vaccines. When virosomal flu vaccines and the MF59 adjuvanted product were directly compared, it was concluded that MF59 induced more potent immune responses (Baldo *et al.*, 2001). Moreover, the safety profiles of virosomal and MF59 adjuvanted flu vaccines appear to be comparable, with both showing only mild and transient local reactions at the injection site. Overall, while it is clear that MF59 offers a significant adjuvant effect for flu vaccines, particularly for pandemic strains, it is less clear that virosomes achieve an 'adjuvant' effect. Rather they appear to offer an alternative means to deliver flu antigens in a particulate structure that is well tolerated and can be administered to subjects of wide age range, including the elderly.

What are the Current Options for Improved Vaccine Adjuvants?

Although Alum and MF59 adjuvants are included in licensed vaccines, they both have some limitations. In preclinical models, neither adjuvants induce potent T-cell immune responses of a T_H1 type, which is normally defined as the ability of antigen-primed T cells to produce γ-interferon in response to restimulation *in vitro*. T_H1 cells are thought to be particularly important to provide protective immunity against some intracellular pathogens. Hence the inability of currently available adjuvants to induce potent T_H1 responses is thought to be a significant factor limiting our ability to develop effective vaccines against some pathogens, including HIV, HCV and malaria. Nevertheless, a range of immune potentiators are becoming available that are able to enhance T_H1 responses (O'Hagan and Valiante, 2003). The first of these, which has been recently included in a licensed vaccine is called monophosphoryl lipid A (MPL), and is a natural product, which is produced by chemically detoxifying bacterial lipopolysaccharide (LPS). LPS, which is also known as endotoxin, is very potent at activating the immune system, but is too toxic for human use. In fact strict specifications exist to ensure that only exceptionally low levels of endotoxins are present in biological products intended for human administration. Nevertheless, an extensive programme in the 1970s identified a reliable and reproducible process for the detoxification of LPS, to allow it to be used as a vaccine adjuvant, without significant adverse effects. MPL was first licensed in Europe in early 2005, to be used in populations who responded poorly to the existing hepatitis B vaccine, due to renal insufficiency. The approved product, Fendrix™, contains the traditional adjuvant Alum, to which a recombinant antigen is adsorbed, but also contains MPL. This same adjuvant formulation, with MPL adsorbed to Alum, is also undergoing late-stage clinical evaluation in other vaccines and will likely gain additional product approvals within the next few years. In addition, the same adjuvant, MPL, is also included in different adjuvant formulations that are undergoing late-stage clinical evaluation. Although MPL has been shown to be a safe and effective adjuvant in a clinical setting, alternative new generation adjuvants appear to be more potent for the induction of T_H1 responses. In pre-clinical studies, synthetic oligonucleotides which mimic signature sequences (CpG) present in bacterial DNA, appear to be very potent T_H1 adjuvants (Krieg *et al.*, 1995). These CpG oligonucleotides are currently undergoing early stages of clinical evaluation as new-generation vaccine adjuvants. In addition, synthetic small-molecular-weight drugs have been identified, which also induce potent T_H1 responses (Wille-Reece *et al.*, 2006).

As an alternative to the use of new generation adjuvants to activate T_H1 immune responses, MF59 adjuvant can be used as a booster vaccine, once a T_H1 response has been established by immunization with live viral vaccines, or with new-generation deoxyribonucleic acid (DNA) vaccines. This approach is currently undergoing clinical evaluation as a potential HIV vaccine candidate.

What is the Best Long-term Approach for Adjuvant Development?

There are many natural products, often extracted from bacteria and viruses, which directly activate immune cells,

Table 4 Advantages of small molecule immune potentiators as adjuvants

Simple and cheap to synthesize
Easy to manipulate drug structure to modify performance/activity
Easy to manipulate physicochemical properties
Significant formulation experience with similar compounds
Potential broad use beyond vaccines for immune modulation

and evaluations of how best to exploit these immune potentiators as vaccine adjuvants is ongoing. However, there is also an increasing interest in the use of synthetic analogues of these agents. Synthetic analogues often have lower manufacturing costs and can be obtained in highly purified forms, which is often in sharp contrast to the heterogeneous natural products. One of the most interesting and promising classes of compounds which have potential to be exploited as new-generation adjuvants are traditional small-molecular-weight drugs (Wille-Reece *et al*., 2006). The discovery that traditional drug-like compounds can function as vaccine adjuvants has required the use of a new terminology, and these compounds have been called Small Molecule Immune Potentiators (SMIPs). The use of SMIPs as adjuvants allows the exploitation of traditional pharmaceutical synthetic approaches, with all the associated advantages, including the ability to manipulate compound structures to control performance. There are numerous advantages which can be realized through the use of SMIPs as adjuvants and these are highlighted in **Table 4**. Given these advantages and the likelihood that more diverse families of SMIPs will be discovered, it appears likely that a number of SMIPs will become available, to allow better manipulation and control of the immune response. However, it is also clear that delivery systems will be required for SMIP-based adjuvants, to ensure that the SMIPs are delivered preferentially to key immune cells and that the immune activation signals are not available to a more broad array of cells, due to diffusion of the drugs away from the injection site. Hence, adjuvant formulations will increasingly comprise one or more potent immune potentiators, which will be designed to induce the specific kind of immune response required, formulated into delivery systems, which will be designed to maximize potency and minimize potential for adverse events, to ensure maximal safety.

Conclusions and Future Perspective

In the last decade, there have been a number of significant advances in technologies designed to identify, express and deliver vaccine antigens. As a consequence, many of the vaccine candidates currently under evaluation look very different to traditional vaccines. In particular, there has been a shift away from the use of whole pathogens or inactivated subunits, towards the use of recombinant purified proteins. Although this has improved vaccine safety, it has also resulted in the need to develop novel adjuvants to improve the immunogenicity of vaccine antigens. The optimal vaccine candidates of the future, particularly from a safety perspective, will contain recombinant protein antigens, purified synthetic adjuvants and a delivery system designed to ensure that both the antigen and the adjuvant are targeted efficiently to APC. Formulation of the vaccine antigens into a delivery system will fulfil two main purposes: (i) to focus the effects of the immune potentiator on to the key cells of the immune system to enhance potency and (ii) to limit systemic distribution of the immune potentiator, to minimize its potential to induce adverse effects. It is clear that novel adjuvants (immune potentiators) and delivery systems will be required to enable the successful development of vaccines against diseases that have not yet yielded to traditional approaches.

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