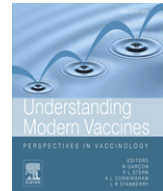Contents lists available at ScienceDirect

Understanding Modern Vaccines:
Perspectives in Vaccinology

Vaccine adjuvants

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Key concepts

- Adjuvantation of vaccines is a well-established concept and practice
- Adjuvants enhance and modulate immune responses to antigens. This is particularly important when the antigens are purified and lack intrinsic innate and/or adaptive immune triggers
- Adjuvants differ in the types and magnitude of immune responses they elicit, hence they must be selected in view of the immune response required to induce immunity to a given pathogen or antigen
- Combinations of adjuvants can take advantage of the properties of each individual component of an adjuvant composition
- Adjuvants are a key tool in developing efficacious vaccines to meet many vaccine challenges

Adjuvant concept

The *adjuvant* concept is more than 80 years old with the first adjuvant present in human vaccines, an aluminium salt (aluminium potassium sulphate, also known as alum), appearing in the 1920s. About 70 years later a licensed vaccine with an alternative adjuvant to aluminium salt was developed (Figure 4.1). The addition of

Figure 4.1 **Use of adjuvants in vaccines**. As with all areas of vaccine development, the availability and variety of adjuvanted vaccines has increased with a greater understanding of immunology. The antigen approach employed for the individual disease is given in parentheses. Aluminium salts were the only adjuvant used in licensed vaccine formulations for human vaccines until the 1990s. Several new adjuvants have been developed and used since.
HPV, human papillomavirus.
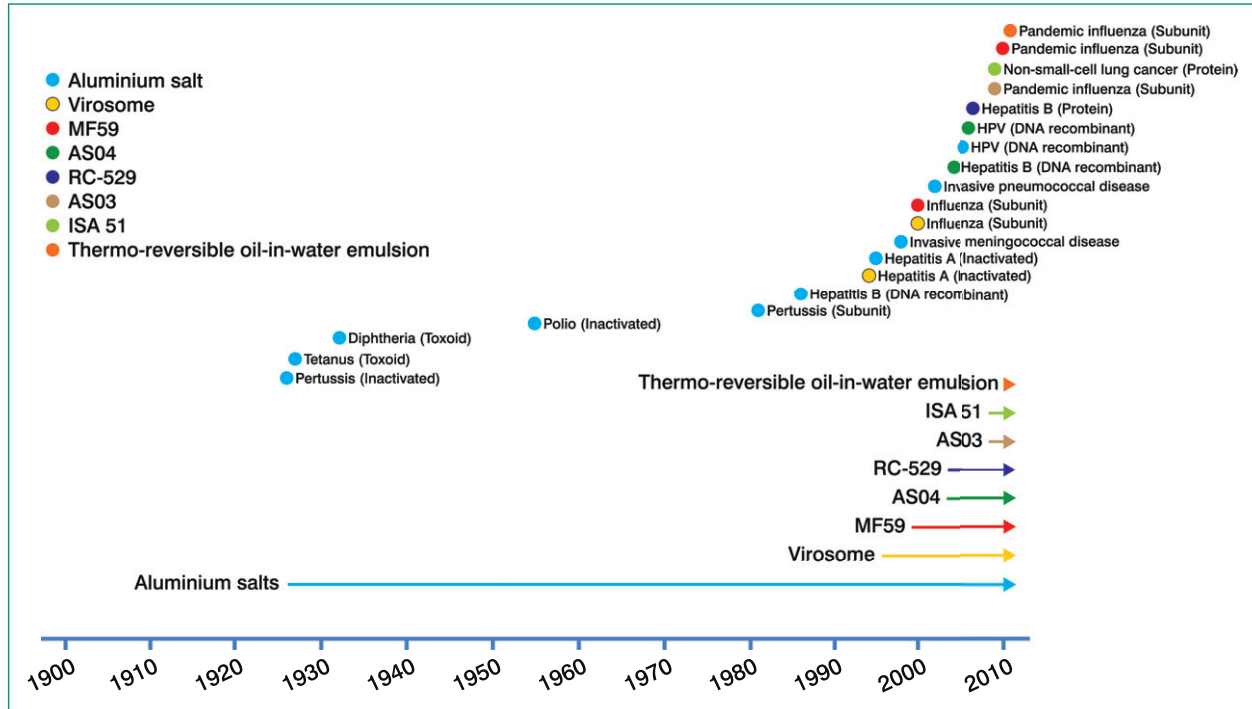
components other than the pathogen or *antigen* to vaccine preparations represents one of the original attempts to improve vaccine efficacy. Adjuvants are substances that can enhance and modulate the *immunogenicity* of the vaccine antigen. In a vaccine, the specificity of the immune response is provided by the antigen and the role of the adjuvant is to amplify this immune response. Live vaccines usually do not require adjuvants as they mimic natural infection and are therefore 'naturally adjuvanted'. Most inactivated (whole or subunit) vaccines do require adjuvants since the

inactivation processes remove, in part or totally, the pathogenic features of the microorganisms that are responsible for triggering the immune response. Inactivated vaccines may retain some of the characteristics that stimulate the *innate immune system* (ie *pathogen-associated molecular patterns* [PAMPs], see *Chapter 2 — Vaccine immunology*), but the amount and context of these PAMPs may be insufficient to provoke long-lasting immunity. Aluminium salts have been sufficient to induce an adequate immune response for most of the licensed inactivated and subunit vaccines.

However, many of the modern vaccines consist of highly purified antigens for which the natural innate immune triggers are not present. These refined formulations often show reduced immunogenicity and therefore require adjuvantation. Classic aluminium salts are not always capable of eliciting the desired immune response and more complex adjuvantation may be required. One of the promising approaches to improve efficacy of newly developed *prophylactic* and *therapeutic vaccines* is the use of innovative adjuvants including the technique of combining different types of adjuvants into single formulations.

Aluminium salts

In the 1920s, Gaston Ramon and Alexander Glenny recognised that several substances added to *toxoids* had adjuvant properties (see *Chapter 1 — Vaccine evolution*). During experimentation with tetanus and diphtheria toxoids in horses, Ramon observed that the addition of bread crumbs, tapioca (both starches) or saponin increased the yields of serum antibodies. In 1926, Glenny formulated the first adjuvanted vaccine by precipitation of diphtheria antigen onto particles of aluminium potassium sulphate. It was believed that aluminium compounds enhanced the response to antigens by extending the time during which antigen is available in the tissue (the so-called depot effect). It is known today that aluminium, like many of the new adjuvants described below, acts by direct activation of the innate immune cells.

Adjuvant selection
There is no universal adjuvant to cover all vaccine needs. The appropriate selection of adjuvants to match the antigens is key to the formulation of novel and efficacious vaccines. For example, different aluminium salts (phosphate or hydroxide) are used depending on the ion charge required for binding to the antigen.

First use of adjuvants
Adjuvants were initially developed for use in animals to increase the yield of serum antibodies for antitoxins.

Other adjuvants

Water-in-oil emulsions as adjuvants were first introduced by Jules Freund in the 1930s. Like aluminium, this adjuvant was designed to release antigen over an extended time period at the injection site, acting as an antigen carrier. The emulsion induced potent immune responses, but the high *reactogenicity* observed in humans was unacceptable. It was later established that the reactogenicity observed was due to impurities present in the mineral oil, and new formulations lacking impurities were subsequently developed.

The need for innovative adjuvants

As mentioned above, aluminium salts work well for traditional bacterial toxoids and many of the currently available vaccines for which *antibodies* are the main correlate of protection. The induction of complex, integrated immune responses for diseases such as human immunodeficiency virus (HIV), has reignited the search for new classes of adjuvants, including improved water-in-oil emulsions with a less reactogenic profile than Freund's original adjuvant.

Table 4.1 shows several adjuvanted vaccines currently available in Europe and the USA, some of which contain single novel adjuvants or a combination of adjuvants.

Understanding the role of adjuvants

Pathogens contain intrinsic triggers of immune defence, PAMPs, which are recognised by cells of the innate immune system and are necessary to elicit a robust immune response (see *Chapter 2 – Vaccine immunology*). Some inactivated and subunit vaccines lose part or most of the pathogen's intrinsic immunostimulatory ability due to the inactivation or purification processes. These vaccines therefore require adjuvants in order to enhance an antigen-specific adaptive immune response.

Expected benefits of adjuvants

1. **Stronger immune priming:**
   - **Faster immune response**
   - **Elevated immune response**
   - **Broader, cross-protective immunity against genotypes or drift variants different from vaccine antigen(s)**
   - **Longer lasting immune response, fewer boosters needed**

2. **Enhanced and effective immune responses in specific low-responding populations, such as the elderly or immunocompromised patients**

3. **Antigen sparing (use of smaller amounts of the antigen) – particularly relevant for pandemic events**

4. **Acceptable safety profile**

TABLE 4.1. EXAMPLES OF ADJUVANTS IN LICENSED VACCINES

Adjuvant	Vaccine	Product
Aluminium salts	DTaP	*Boostrix™*, GSK Biologicals
		Daptacel™, Sanofi Pasteur
		Infanrix™, GSK Biologicals
		Repevax™, Sanofi Pasteur
	DTaP, polio and Hib	*Pediacel™*, Sanofi Pasteur
		Pediarix™, GSK Biologicals
		Pentacel™, Sanofi Pasteur
	DTwP, Hib and hepatitis B	*Quinvaxem™*, Novartis
	DTaP, polio, Hib and hepatitis B	*Infanrix hexa™*, GSK Biologicals
	Hepatitis A	*Avaxin™*, Sanofi Pasteur
		Havrix™, GSK Biologicals
		Vaqta™, Merck & Co
	Hepatitis B	*Engerix-B™*, GSK Biologicals
		Recombivax HB™, Merck & Co
	HPV-6/11/16/18	*Gardasil™*, Merck & Co
	Influenza (H5N1)	*Daronrix™*, GSK Biologicals
		Fluval™, Omnivest
		Panflu™, Sinovac
		Panvax™, CSL Biotherapies
	Pneumococcus (conjugated)	*Prevnar™/Prevenar™*, Wyeth
		Synflorix™, GSK Biologicals
Synthetic MPL (RC-529)	Hepatitis B	*Supervax™*, Dynavax Technologies
Virosomes	Hepatitis A	*Epaxal™*, Crucell
	Influenza (seasonal)	*Inflexal™*, Crucell
		Invivac™, Solvay
Oil-in-water emulsion (*MF59™*)	Influenza (H1N1)	*Focetria™*, Novartis
	Influenza (H5N1)	*Aflunov™*, Novartis
	Influenza (seasonal)	*Fluad™*, Novartis
Montanide™ ISA51	Cancer	*CimaVax* EGF™, Bioven
AS04	Hepatitis B	*FENDrix™*, GSK Biologicals
	HPV-16/18	*Cervarix™*, GSK Biologicals

TABLE 4.1. EXAMPLES OF ADJUVANTS IN LICENSED VACCINES — CONTINUED		
Adjuvant	**Vaccine**	**Product**
AS03	Influenza (H5N1, H1N1)	*Prepandrix*™, GSK Biologicals
		Pandemrix™, GSK Biologicals
		Arepanrix™, GSK Biologicals
Thermo-reversible oil-in-water emulsion	Influenza (H1N1)	*Humenza*™, Sanofi Pasteur

DTaP, paediatric diphtheria, tetanus and acellular pertussis; Hib, Haemophilus influenza type b; HPV, human papillomavirus, AS04, Adjuvant System 04; AS03, Adjuvant System 03; DTwP, Hib and hepatitis B, paediatric diphtheria, tetanus and whole cell pertussis; MPL, monophosphoryl lipid A. The information given in this table is not meant to be exhaustive but to give an overview of the subject matter.

RECOGNITION OF PATHOGENS

Sentinel immune cells are equipped with innate receptors, the so-called *pattern recognition receptors* (PRRs). These recognise PAMPs and allow them to distinguish between different broad types of organism such as bacteria, viruses and *parasites* (see *Chapter 2 — Vaccine immunology*).

The stimulation of these receptors and other signals coming from inflammatory processes and/or tissue damage alerts the immune system and guides it towards the most appropriate type of response. PRRs include the *Toll-like receptors* (TLRs) which are, to date, the best characterised of the PRRs.

> **Possible impact of adjuvants on immune mechanisms**
> 1. **Recognition of PAMPs**
> 2. **Presentation of antigens to T-cell receptor**
> 3. **Recognition of co-stimulatory signals**
> 4. **Intracellular signalling processes in APCs**

A key process in the development of a successful immune response is the initial encounter with the innate immune system as this guides the downstream adaptive response. The specific innate signals received by *antigen-presenting cells* (APCs) strongly influence the magnitude and quality of the ensuing T- and B-lymphocyte responses, the nature of T-cell response, and the induction of memory cells (see *Chapter 2 — Vaccine immunology*).

The innate and adaptive parts of the immune system need to communicate with each other in order to induce the relevant immune response. *Dendritic cells* (DCs), *macrophages* and *monocytes* participate in the presentation of antigens to the cellular mediators of *immune memory*, the *T cells*, which, in turn, promote the activation and maturation of specific *antibody*-producing *B cells*.

They are the link between the innate and adaptive immune response. Based on its nature, an adjuvant can enhance the adaptive immune response to vaccine antigens by amplifying or modulating any of the signals involved in the process of innate immune response activation.

ADJUVANTS MIMIC NATURAL IMMUNE-DEFENCE TRIGGERS

The discovery of PRRs, PAMPs and TLRs, and the recognition of the link between innate and adaptive immunity, has facilitated the development of a series of innovative adjuvants. The main immune mechanisms that can be impacted by adjuvants are summarised in the box on the right. Their general mode of action based on current evidence is shown in Figure 4.2. In general, adjuvants act in a similar way to the immune-defence triggers present in pathogens by interacting with APCs and promoting appropriate immune responses. Based on the different PRRs identified and their associated ligands and downstream effects (see *Appendices, Supplementary Table 1*), one area of research on new adjuvants is the identification of substances able to mimic the effect of one or more natural ligands, eg TLR agonists.

Adjuvants in licensed vaccines
ALUMINIUM SALTS

Different aluminium salts are contained in numerous licensed vaccines (Table 4.2). Aluminium salt adjuvants have complex, heterogeneous physical structures and the antigen is adsorbed to the adjuvant through hydrophobic and electrostatic interactions between antigen and the aluminium salt. Aluminium hydroxide is positively charged at a physiological pH of 7.4 and binds acidic proteins. Aluminium phosphate, on the other hand, is negatively charged and therefore binds basic proteins.

Depending on the hydrophobic interactions with the antigen, the appropriate aluminium salt is selected to maintain antigen immunogenicity and to obtain maximum adjuvant effect (Table 4.2).

The role of adjuvants in vaccines
Adjuvants mimic natural defensive trigger molecules in order to stimulate a strong and comprehensive immune response to the antigen. These triggers may be immuno-enhancers, including exogenous or synthetic microbial derivatives, or endogenous immuno-active compounds such as cytokines, chemokines and co-stimulatory molecules, or other natural compounds such as saponins, squalene or vitamin E.

Main immune mechanisms that can be impacted by adjuvants
Adjuvants help to make an antigen more visible or reactive to the immune system and several different mechanisms of action have been proposed depending on the adjuvant.
'Persistence of antigen' was considered previously to be the result of a simple depot effect. Today, this phenomenon is believed to include features such as improved antigen delivery and enhanced uptake by APCs.
Direct activation of innate immune cells, including APCs, induces the localised release of cytokines and chemokines, through specific receptors (such as TLRs), and influences the subsequent adaptive immune response.
Better immune targeting may be achieved by influencing the type of adaptive immune response induced through an enhanced recruitment and stimulation of APCs at the site of injection and in the regional lymph nodes.

Figure 4.2 Adjuvants: general mode of action based on current evidence. Adjuvants mimic elements from pathogens that are recognised by the innate immune system (1). This results in a local cytokine response and recruitment of various innate cells including monocytes and immature DCs (2). Immature innate cells integrate these proinflammatory signals and begin to mature in APCs. Simultaneously, they migrate to the local draining lymph nodes where they induce T- and B-cell responses (3). This leads to the production of adaptive immune effectors such as CD4$^+$ T cells and antibodies (4). Adjuvants can therefore influence the magnitude and quality of the adaptive immune response via effects on the innate response.

Compared with the same antigen in a non-adjuvanted formulation, the expected benefits of adjuvants are:

• An increased recruitment of innate cells at the site of injection
• An increased number of activated APCs migrating to the draining lymph node
• An increased uptake of the antigen by APCs with a subsequent enhancement and modulation of the adaptive immune response

The immune profile of the adaptive immune response is therefore influenced by adjuvants and may result in an improved cytokine pattern in quantity and quality, a greater diversity of CD4$^+$ T cells, and a wider antibody profile.

DC, dendritic cell; APC, antigen-presenting cell; CD, cluster of differentiation.
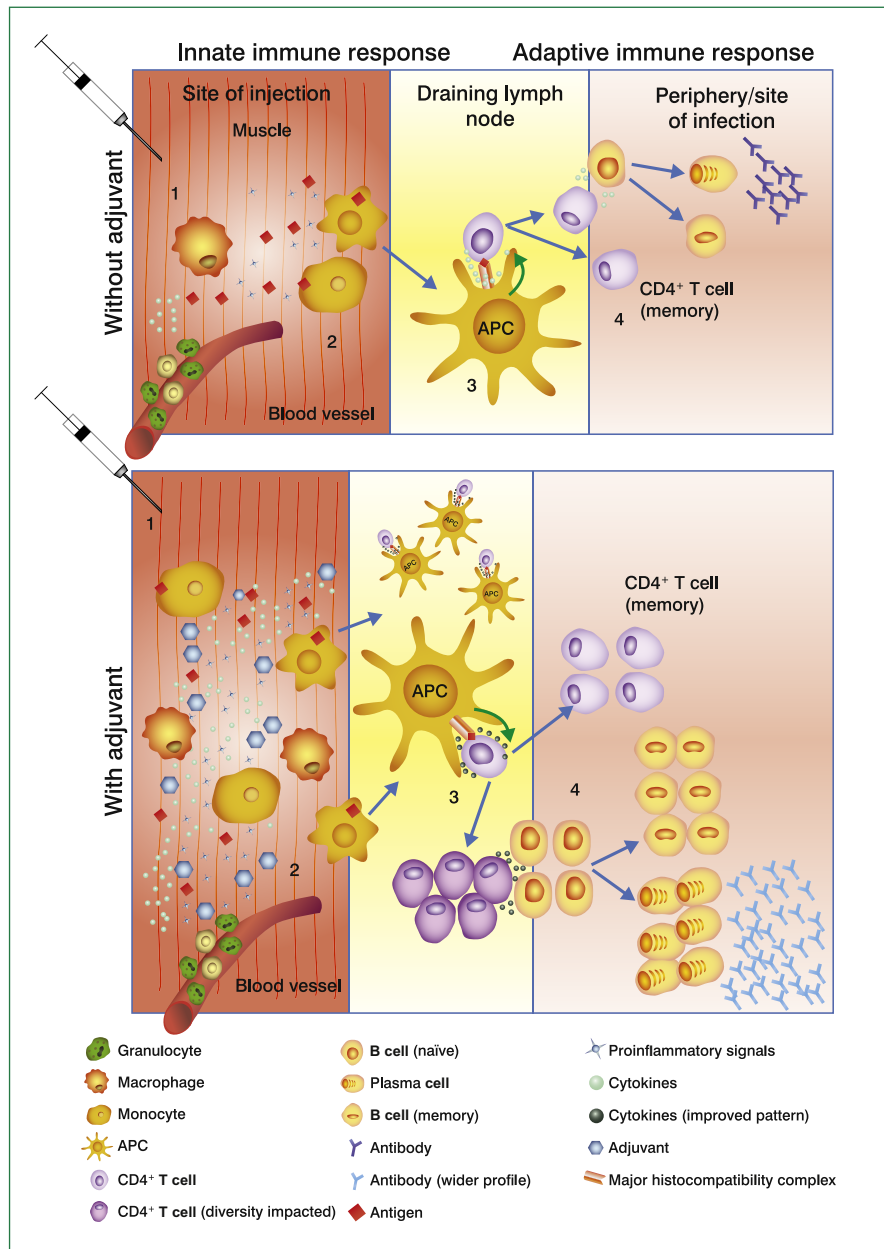
Glenny postulated that aluminium salts were effective adjuvants because they promote antigen persistence and prolong release of the antigen. It has also been suggested that the antigens adsorbed on the aluminium salts are presented in a particulate *multivalent* form, making them more efficiently internalised by APCs. Recent studies have shown that this is not always the case. Most antigens are rapidly desorbed from aluminium salts following exposure to interstitial fluid, therefore adsorption is not always required to achieve adjuvanticity. However, adsorption or entrapment in aggregates might favour a high local antigen concentration and improved uptake by APCs. In addition, insoluble aluminium salts have been shown to directly activate innate immune cells. It has been suggested that the effect of aluminium salts on cells may lead to the production of uric acid *in vivo* from the breakdown of purine nucleotides in apoptotic cells, which act as *damage-associated molecular patterns* (*DAMPs*). DAMPs are generally substances released by stressed or dying cells and are recognised by cells of the innate immune system.

Aluminium salts have recently been shown to activate *in vitro* components of the 'inflammasome' complex, but whether the activation of this pathway is required for the adjuvant effect of aluminium salts *in vivo* is uncertain. Nevertheless, new data also clearly show that aluminium salts have additional effects — beyond promoting persistence of antigen — that account for their adjuvant properties.

Limitation of aluminium salts

As discussed previously, aluminium salts have been used successfully in vaccines against pathogens where antibodies provided the primary mechanism of protection. Aluminium salts exert little effect on Th1-type or *cytotoxic T-cell* responses, which are required for responses against intracellular pathogens. Hence, with vaccines for such pathogens, aluminium salt adjuvants have been found to be inadequate. For example, all attempts to develop a malaria vaccine formulated with aluminium salt as adjuvant have failed so far.

TABLE 4.2. EXAMPLES OF VACCINES ADJUVANTED WITH ALUMINIUM SALTS

Aluminium salt	Vaccine
$Al(OH)_3$ — aluminium hydroxide and/or $AlPO_4$ — aluminium phosphate	Pertussis Diphtheria Tetanus HBV HAV
$(Al)_2PO_4SO_4OH$ — aluminium hydroxyphosphate sulphate	HPV
HBV, hepatitis B virus; HAV, hepatitis A virus; HPV, human papillomavirus.	

Figure 4.3 **Structure of a liposome**. Liposomes consist of phospholipids, molecules with hydrophilic heads and hydrophobic tails. After dispersion in water, groups of phospholipids form small spheres which enclose an aqueous core.
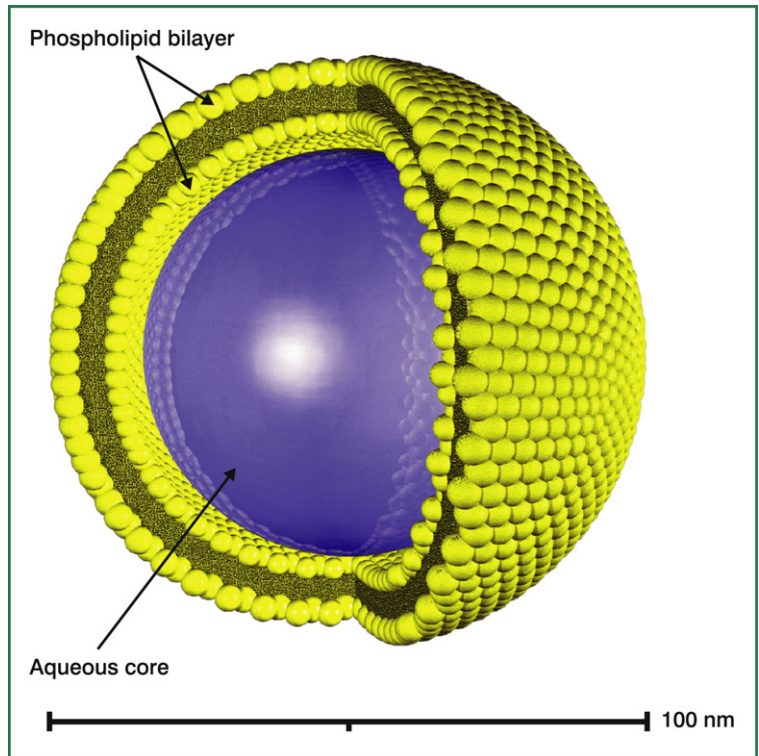
LIPOSOMES/VIROSOMES

Liposomes are artificial vesicles consisting of lipid layers that can encapsulate/intercalate antigens in their membrane and act as antigen-delivery vehicles (Figure 4.3). Some licensed products contain *virosomes* — spherical lipid vesicles that include the functional viral glycoproteins haemagglutinin (HA) and neuraminidase (NA) from influenza. These glycoproteins are thought to facilitate antigen uptake by APCs (Figure 4.4).

The suggested mechanism of action of influenza-based virosomes involves direct interaction of virosome particles with APCs or, in some cases, with B cells which, in turn, activate T cells. The influenza HA antigen targets the virosomes to APCs which engulf it by *endocytosis* and present the antigens to T cells after proteolytic
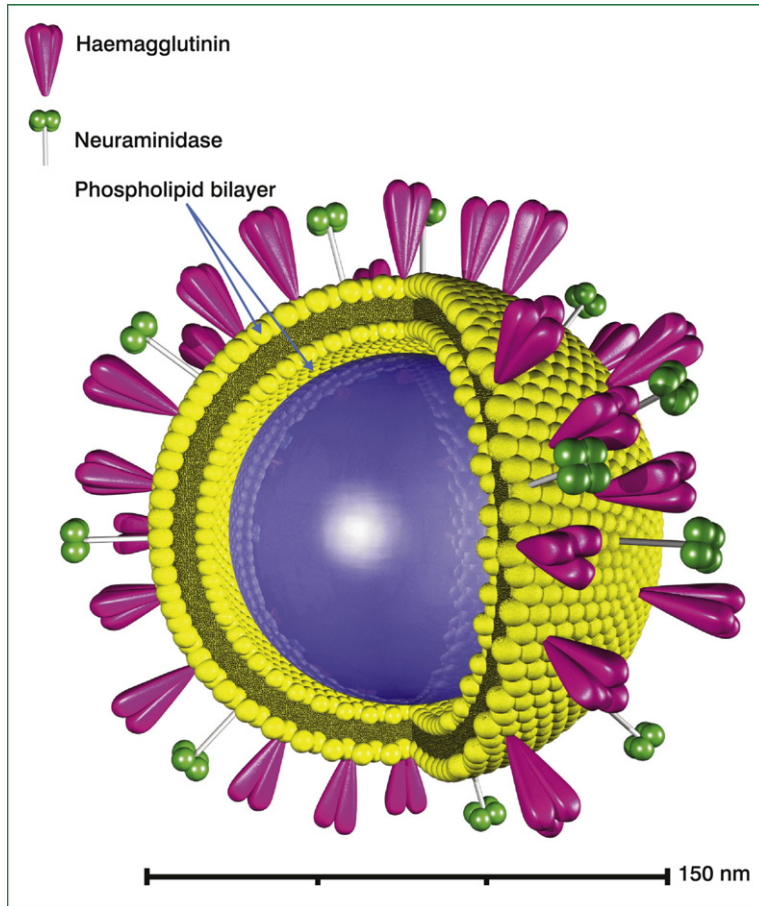
Figure 4.4 **Structure of a virosome**. Virosome technology uses liposome-based structures that have viral proteins embedded into the membrane, for example the haemagglutinin and neuraminidase proteins of an influenza virus. These elements help to target the virosome to the immune system cells, delivering the vaccine antigen.

degradation. Pre-existing immunity against influenza may represent another important determinant for the immunostimulating effect of virosomes. Currently there are two virosome-adjuvanted vaccines, licensed in some European and non-European countries (Table 4.1).

EMULSIONS

Emulsions are based on the combination of two immiscible components, typically an oil and water, with one substance dispersed into the other (Figure 4.5).

Figure 4.5 Emulsions in vaccines. Emulsions consist of two or more immiscible liquids such as water and oil. The minor component (the dispersed phase) is dispersed in the predominant component (the continuous phase). Emulsions are stabilised with amphiphilic molecules, referred to as emulsifiers or surfactants. Depending on the volume fractions and the emulsifier, either an oil-in-water (A) or a water-in-oil (B) emulsion is generated.
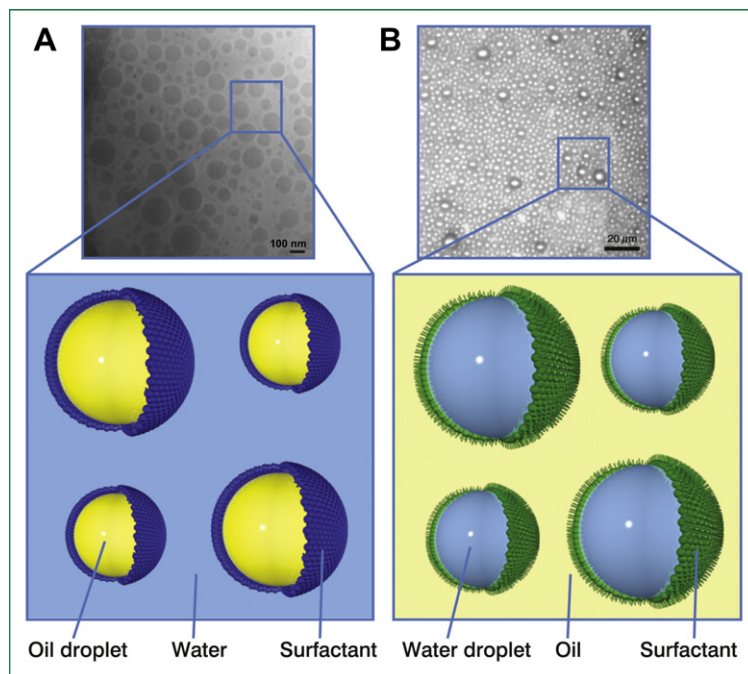
Courtesy of GSK Biologicals (A). Courtesy of Professor Daniel E Resasco, University of Oklahoma, USA (B).

Because of this inherent incompatibility, emulsions need to be stabilised by the addition of *surfactants* or an emulsifier, eg substances like Tween 80 or Span 85. Two different types of emulsions have been developed: water-in-oil and oil-in-water. Both types of emulsion induce high antibody responses, but oil-in-water emulsions tend to have better reactogenicity profiles. Oil-in-water emulsions have been used successfully in licensed vaccines (Table 4.1).

Most oil-in-water emulsions are based on squalene, an organic compound which occurs naturally in all plants and animals, including humans. Squalene is the precursor for the biosynthesis of several steroid hormones, vitamin D and cholesterol. In humans, the highest amount of squalene is found in the sebaceous glands, from which the average secretion is 475 mg squalene per day. Squalene

is used as a skin moisturiser and antioxidant in cosmetics. For pharmaceutical products, squalene is derived from shark liver as this is the purest source available. Importantly, the adjuvant effect of squalene is only observed when the molecule is part of an oil-in-water emulsion.

MF59™

MF59™ is an oil-in-water emulsion made of squalene droplets in a continuous aqueous phase, with a diameter of 167 ± 20 nm (Figure 4.6). *MF59*™ induces recruitment and activation of APCs leading to inflammatory responses. The emulsion acts more
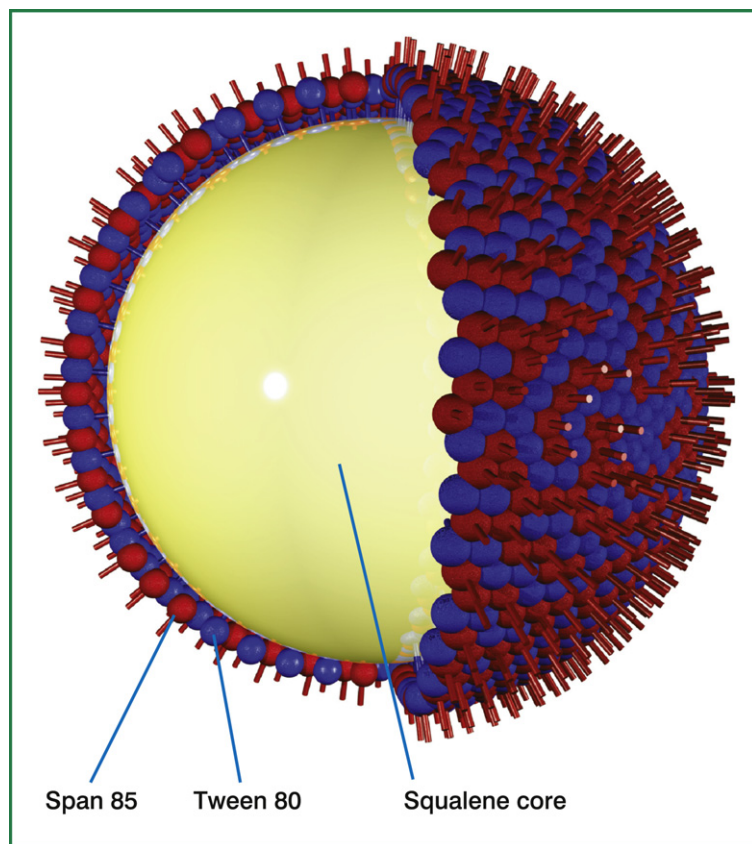

Span 85   Tween 80   Squalene core

Figure 4.6 Structure of *MF59*™. *MF59*™ is an oil-in-water emulsion containing squalene as the dispersed phase and two non-ionic surfactants, Tween 80 and Span 85. Squalene is a triterpene hydrocarbon found naturally in various plants and in the organs and tissues of many animals, including humans.

specifically on macrophages present at the site of injection. A local increase of *chemokine* release (see *Chapter 2 − Vaccine immunology*) influences the recruitment of immune cells from the blood to the site of *vaccination*, creating an amplification loop. *MF59™* also increases antigen uptake by monocytes and enhances differentiation towards a mature phenotype, thereby promoting migration of antigen-loaded cells to the draining lymph node. Compared with aluminium salts, a stronger immune response, eg higher antibody and T-cell response, is elicited (Seubert et al., 2008).

MF59™ is present in licensed seasonal and *pandemic* influenza vaccines (Table 4.1). It enhances immune responses in the elderly population and can facilitate immune responses against specific drift variants of the seasonal influenza virus not included in the vaccine. *MF59™* demonstrated how an adjuvant can improve the immune response to a classical vaccine in a challenging population, such as the elderly, which is affected by immune senescence (Podda, 2001). Clinical studies with an *MF59™*-adjuvanted pandemic influenza vaccine showed antigen-sparing abilities, and for the H5N1 vaccine, the induction of some cross-reactivity versus different viral clades (Banzhoff et al., 2009). The induction of cross-reactive immunity against drifted strains may be very important during a pandemic, as it is very likely that the emerging virus will continue to mutate as the pandemic proceeds.

Thermo-reversible emulsion adjuvant

A thermo-reversible oil-in-water emulsion containing squalene, emulsified with surfactants, is present in the formulation of an H1N1 pandemic influenza vaccine which was licensed in Europe in 2010 (Table 4.1). The mechanism of action has not yet been reported.

ADJUVANT COMBINATIONS IN LICENSED VACCINES

Well-known adjuvants, such as aluminium salts, oil-in-water emulsions or liposomes, are combined with other compounds which act as immuno-enhancers to better modulate and guide

specific components of the immune system aiming to achieve the desired immune response. The more complex formulations, comprising three or more adjuvant components, are designed in particular to induce more potent cellular immune responses (see *Chapter 2 — Vaccine immunology*).
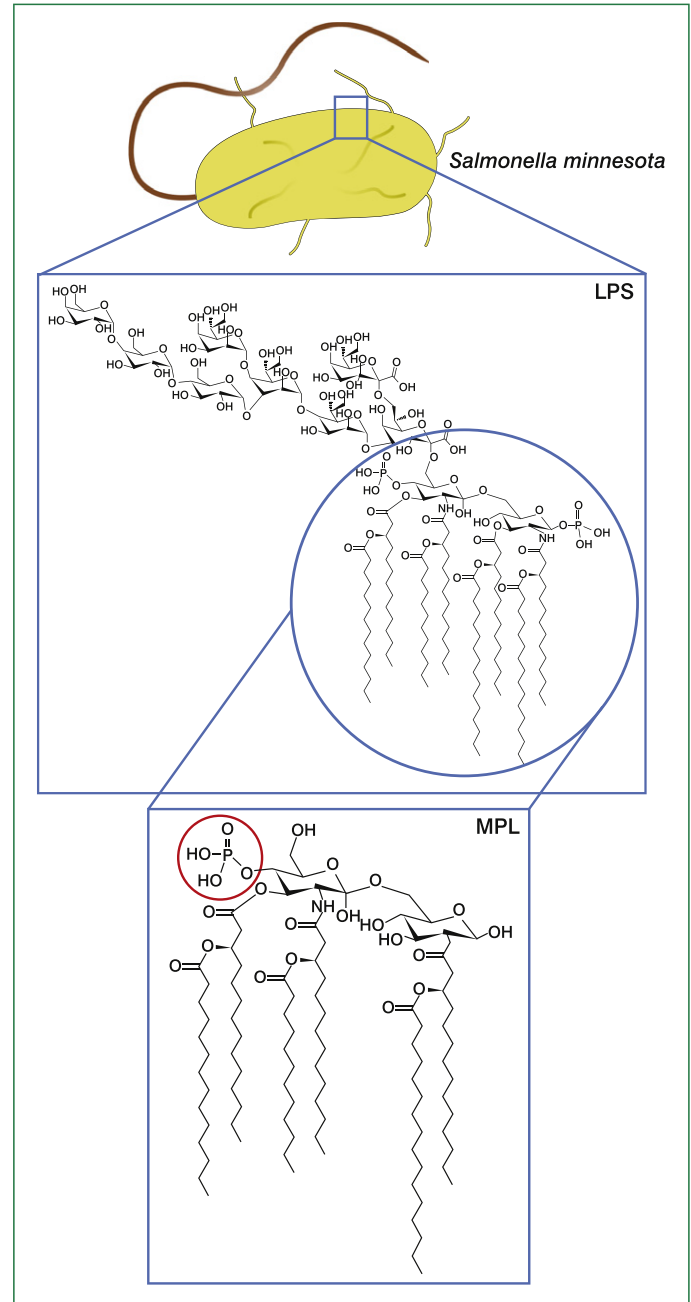
AS04

The first example of a combination of adjuvants is the *Adjuvant System* (*AS*) *04* (*AS04*), which is based on a *lipopolysaccharide* (LPS) derivative, *monophosphoryl lipid A* (MPL) and aluminium salts (Figure 4.7). LPS, derived from Gram-negative bacteria, is a potent immunostimulant and a specific TLR4 agonist. MPL is obtained by mild hydrolysis and further purification of LPS derived from *Salmonella minnesota.* The product has similar immunostimulatory properties to LPS, but lacks the reactogenicity of native LPS. In AS04, MPL is adsorbed onto aluminium hydroxide or aluminium phosphate, depending on the vaccine with which it is used.

In AS04, MPL plays a crucial role in the activation of the innate immune system. Direct stimulation of TLR4 leads to the maturation of APCs, inducing the expression of *cytokines* that in turn enhance the adaptive immune response by stimulating the maturation of Th cells, in particular Th1. Therefore, recognition of MPL by TLR4 leads to enhanced humoral and cellular immune responses. AS04 has to be administered at the same injection site as the antigen — together or within 24 h — to exert its effect. During this period, AS04 transiently induces local innate cell activation and cytokine production, as demonstrated by the local increase in activated antigen-loaded DCs and monocytes, which migrate to the lymph node draining the injection site. Aluminium salt appears to modulate and prolong the cytokine responses to MPL at the injection site. Taken together, these results support a model where the addition of MPL to aluminium salt enhances the vaccine response by prompting increased activation of APCs and downstream enhanced stimulation of Th1 T-cell responses (Didierlaurent et al., 2009).

Figure 4.7 Chemical structure of LPS and MPL. LPS is found in the membranes of Gram-negative bacteria and is an important recognition pattern for the innate immune response. TLR4 agonist MPL is a derivative of LPS that mimics natural defence triggers produced by a pathogen, but with greatly reduced reactogenicity. During the purification process parts of the sugar backbone, one phosphate functionality and two side chains are cleaved from LPS. A synthetic MPL analogue (RC-529) has also been developed and is used in an HBV vaccine (Table 4.1). The RC-529 molecule features a monosaccharide derivative instead of the hexa-acylated disaccharide chain in MPL.

LPS, lipopolysaccharide; MPL, monophosphoryl lipid A; TLR, Toll-like receptor; HBV, hepatitis B virus.

AS04 is currently used in two licensed vaccines (Table 4.1). The first licensed vaccine adjuvanted with AS04 was a hepatitis B virus (HBV) vaccine for pre-haemodialysis and haemodialysis patients, who are relatively poor responders to aluminium-adjuvanted HBV vaccine. In this target population, the vaccine formulation adjuvanted with AS04 significantly enhances the immune response to hepatitis B antigen and induces more rapid, higher and longer lasting *seroprotection* and enhanced cell-mediated immunity (CMI) compared with the aluminium-adjuvanted vaccine (Kong et al., 2005). Similarly, the AS04-adjuvanted human papillomavirus (HPV) vaccine has shown the ability to induce higher antibody levels when compared with the same antigen formulated with aluminium salts (see case study 1, *Chapter 5 — Vaccine development*). Furthermore, the AS04-adjuvanted HPV vaccine provides cross-protection against certain other high-risk HPV types not contained in the vaccine (Paavonen et al., 2009).

AS03

AS03 (Figure 4.8) is a combination of adjuvants, based on α-tocopherol (vitamin E) and squalene in an oil-in-water emulsion with a droplet diameter of 150—155 nm. It is used in pandemic influenza vaccines (Table 4.1). Vitamin E is a lipid-soluble antioxidant with immune-enhancing properties which is present in the human body in muscles, adipose tissues, the adrenal and pituitary glands, and pancreas. The most important function of vitamin E is to maintain the integrity of cellular membranes by protecting their physical stability, and by inhibiting tissue damage caused by oxidation. Vitamin E is exclusively synthesised in plants and found in high amounts in vegetable oils and nuts. Vitamin E is widely used in cosmetics and in foods as a dietary supplement. The vitamin E used in vaccines is of synthetic origin.

Both monocytes and macrophages respond to AS03 with a local production of a range of cytokines and chemokines. Macrophages are the most likely initiators of the cytokine response, whereas recruited monocytes elicit a second wave of chemokine secretion and further innate cell recruitment (Morel et al., 2011).

Figure 4.8 Structure of AS03. AS03 contains α-tocopherol (vitamin E) and squalene in an oil-in-water emulsion, stabilised by the surfactant Tween 80. α-tocopherol is the preferentially absorbed form of vitamin E in humans and modulates innate cytokine production, enhancing cell recruitment and antigen uptake. Vitamin E has immunostimulatory properties.
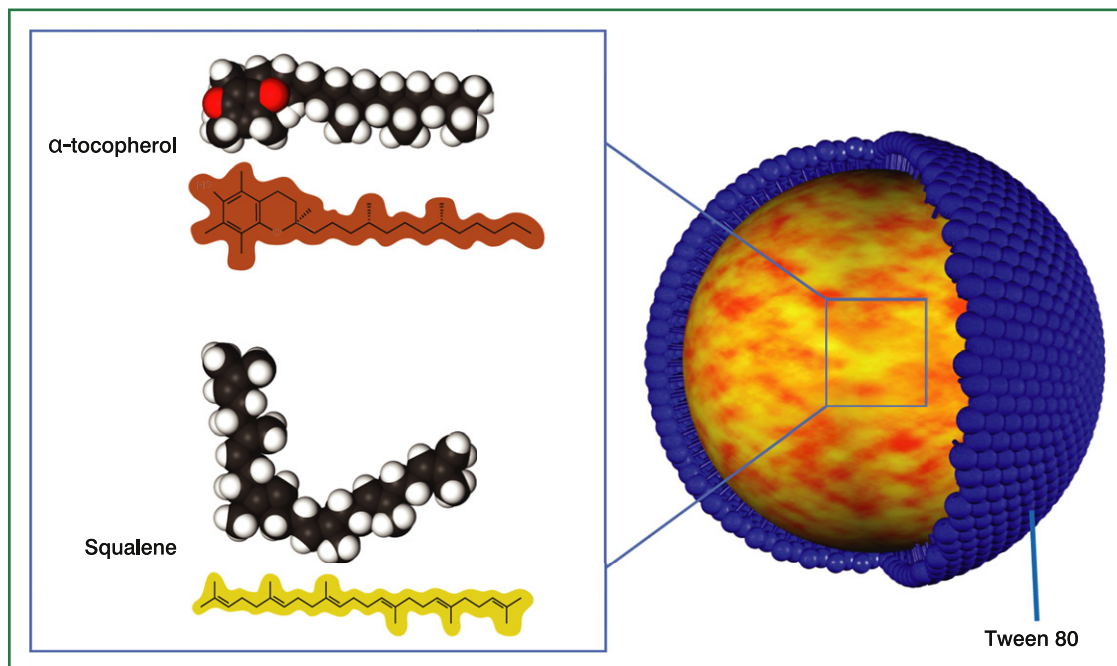
An AS03-adjuvanted pandemic influenza vaccine (Table 4.1) has been shown to allow for antigen sparing, ie less antigen is needed per vaccine dose (Leroux-Roels et al., 2007; Roman et al., 2010). Also a high level of cross-reactive immunity to heterologous strains of H5N1 has been observed (Leroux-Roels et al., 2008).

Other adjuvants in late-stage (Phase III) development

There are currently several newly developed adjuvants being evaluated in Phase III clinical studies. Adjuvants in earlier development phases are described in *Chapter 6 − Vaccines of the future*.

MONTANIDE™

Novel water-in-oil emulsions have recently been developed for use in both therapeutic and *prophylactic vaccines*. *Montanide™* ISA51 is a water-in-oil emulsion containing mineral oil and mannide-mono-oleate as an emulsifier. These emulsions are used as adjuvants with epidermal growth factor (EGF) as antigen in ongoing Phase III studies against cancer. *Montanide™* adjuvants induce a strong immune response with an improved safety profile compared with Freund's water-in-oil emulsion, but mild-to-severe local reactions are still observed in about half of the subjects in clinical trials. For this reason the *Montanide™* adjuvants are applied mainly in immunotherapy. A non-small-cell lung cancer (NSCLC) vaccine containing *Montanide™* ISA51 as an adjuvant was recently registered in Cuba and Chile.

## MICROBIAL DNA IMMUNOSTIMULATORY SEQUENCES

Microbial DNA contains intrinsic immunostimulatory sequences (ISS) which act as ligands of intracellular TLRs, such as TLR9. When recognised by TLRs, ISS can lead to amplification of the adaptive immune response, in particular cell-mediated immunity. Several ISS with distinct biological activities have been characterised and preliminary clinical data show that the use of these sequences in vaccines can enhance humoral and cellular immune responses to the vaccine antigens.

One example of an ISS is CpG 7909 (Figure 4.9), an agonist of TLR9 and an inducer of proinflammatory cytokines. CpG refers to a group of synthetic oligodeoxynucleotides derived from bacterial DNA containing unmethylated CpG motifs. CpG 7909 stimulates TLR9, induces Th1 immunity and cytotoxic T-lymphocyte responses in animals, and is currently in Phase III clinical trials as part of an adjuvanted HBV vaccine (Cooper et al., 2004).

AS01

AS01 combines the effects of three components: liposome, MPL (TLR4 agonist) and QS21. QS21 is a triterpene glycoside derived from a saponin extracted from the bark of the South American tree

Figure 4.9 **Structure of an immunostimulatory sequence**. One example of an immunostimulatory sequence is CpG 7909. CpG-rich DNA is an agonist of TLR9 and an inducer of proinflammatory cytokines. CpG-rich regions are characterised by multiple occurrences of adjacent cytosine (C) and guanine (G) residues in a linear sequence (the p indicates that C and G are connected by a phosphodiester bond). CpG regions are observed at a lower frequency than would be predicted by random nucleotide distribution in the DNA of vertebrates, which is in contrast with the DNA of microorganisms. *CpG, adjacent cytosine (C) and guanine (G) residues in a linear sequence connected by a phosphodiester bond; TLR, Toll-like receptor.*
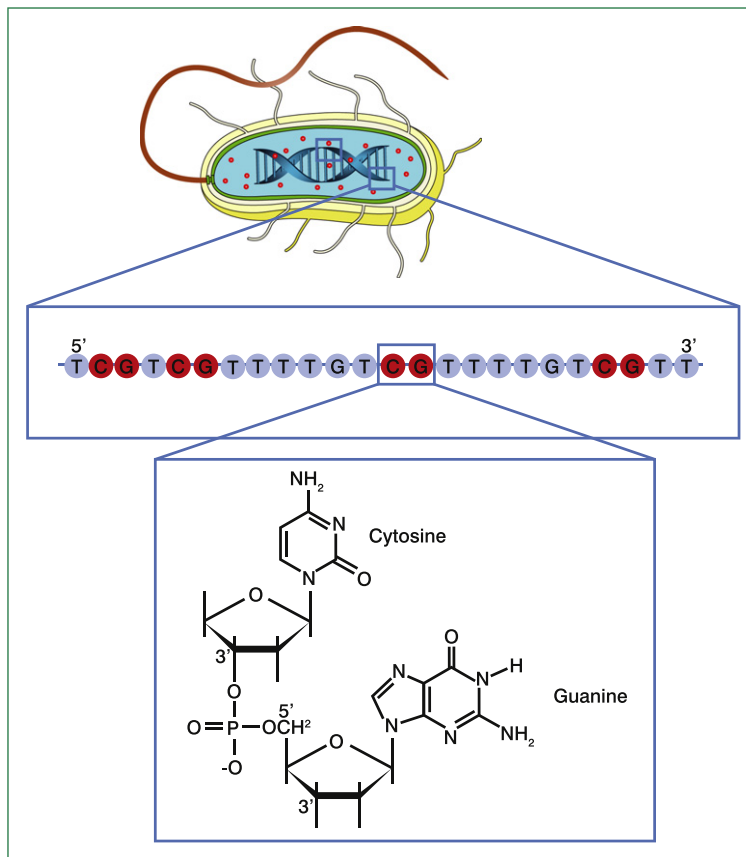
Quillaja saponaria (Figure 4.10). Saponins are used widely as emulsifiers in cosmetics as well as in the food and drink industry. The crude extract, known as Quil A, was first limited to use as an adjuvant for veterinary vaccines due to its local reactogenicity. The purified QS21 fraction derived from Quil A has potent ability to enhance
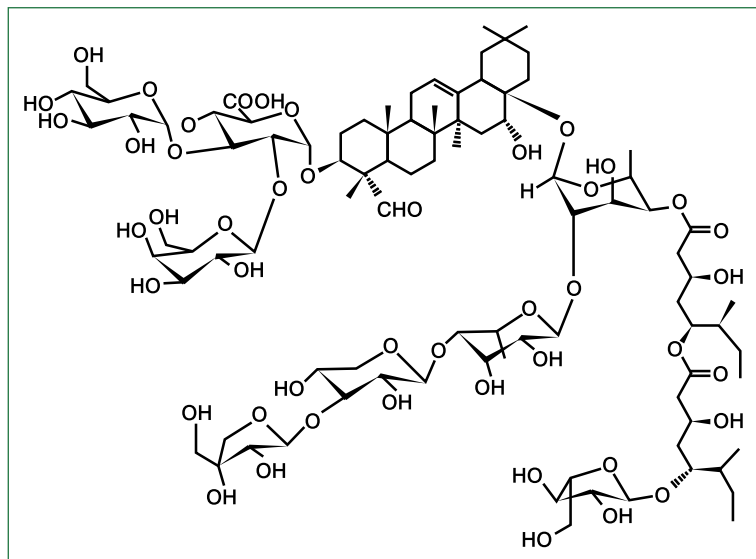
Figure 4.10 Chemical structure of QS21. QS21 is a triterpene glycoside derivative of a saponin which is extracted from the bark of the South American tree *Quillaja saponaria*. Saponins are included in many cosmetic and industrial products.

antigen presentation to APCs, especially to induce cytotoxic T-lymphocyte production when tested in animals (Newman et al., 1997). It has been shown that QS21 as a surfactant can be used to facilitate penetration of proteins through cell membranes, thus inducing intracellular immune responses. QS21 has shown an acceptable tolerability profile for use in human candidate vaccines when properly formulated with *ISCOM*™ (immune-stimulating complex consisting of cholesterol and phospholipids), or liposomes. Several clinical trials are in progress with AS01-containing candidate vaccines against infections, including HIV, tuberculosis and malaria. The active substance in the candidate malaria vaccine, currently in Phase III, is the recombinant antigen RTS,S which targets the pre-erythrocytic stage of the parasite (see *Chapter 3 — Vaccine antigens*). Protective immunity against malaria requires the specific stimulation of both humoral and CMI responses, with the goal of decreasing the number of infectious parasites available to invade the liver while also destroying any hepatocytes that become infected. The RTS,S vaccine antigen has been formulated with

several different adjuvant combinations (Kester et al., 2009). AS01 has been selected for the final formulation because it demonstrated a better immune response and showed a trend towards improved efficacy in several clinical trials compared with the other adjuvant combinations.

AS15 FOR ANTIGEN-SPECIFIC CANCER IMMUNOTHERAPEUTICS

AS15 combines the effects of four adjuvants: liposome, MPL (TLR4 agonist), CpG (TLR9 agonist) and QS21. AS15, the most complex combination of adjuvants to date, is under investigation for use in cancer immunotherapy (Brichard and Lejeune, 2007). Antigen-specific cancer immunotherapeutics (ASCI) are designed to treat cancer by targeting antigens that are selectively expressed or over-expressed by tumour cells, but not by normal cells. AS15 has been selected for use in ASCI based on its ability to induce both high antibody *titre*s and robust T-cell responses. AS15 aims to improve the immune response against the target antigen through a stronger immune activation which is sufficient to overcome tumour immuno-suppressive processes. It has been shown in clinical trials that AS15, in comparison with other adjuvant combinations, elicits the most appropriate immune response for ASCI. The melanoma antigen A3 (MAGE-A3) is the target of current ASCI applications since it is expressed by different tumours. After showing promising results in Phase II studies, MAGE-A3/AS15 is in Phase III clinical studies as cancer-specific immunotherapy against NSCLC and melanoma.

Safety profile of adjuvants

The safety profile of aluminium salt adjuvants has been well established through the use of billions of doses of aluminium-containing vaccines administered to infants, children, adolescents, adults and the elderly over more than 80 years. The safety of *MF59*™ and virosomes has been demonstrated through almost a decade of use. Innovative adjuvants to date have shown an acceptable safety profile in clinical trials across a

variety of applications and in post-licensure experience. Increased reactogenicity, especially at the injection site, is consistently found for adjuvanted vaccines compared with those that are non-adjuvanted. The vaccination-related local symptoms which are generally reported with higher frequency are mild to moderate in intensity, of short duration, and do not impact compliance with vaccination schedules. Overall, adjuvanted vaccines are considered to have a positive benefit—risk ratio that is clinically acceptable. For more detailed information on vaccine safety see *Chapter 5 — Vaccine development*.

Conclusion

More in-depth understanding and recognition of the important role of the innate immune response in regulating the induction of an adaptive response has led to a reappraisal of the role that adjuvants can play in *vaccinology* and is enabling vaccine researchers to use adjuvants to greater advantage.

Development of novel adjuvants and adjuvant combinations is likely to help to address the challenges in modern vaccinology, such as vaccines targeting complex pathogens (see *Chapter 3 — Vaccine antigens*) or vaccines for immunologically challenged subjects. In addition to their role in prophylactic vaccines, current and future adjuvants are likely to play a prominent role as immunotherapeutics, especially for cancer therapy.

The box, right, summarises the challenges of complex diseases and the needs of specific populations and how adjuvants can help to address them.

> ### How adjuvants can help to address vaccination challenges
> **Complex diseases**
> - AS01-adjuvanted RTS,S candidate malaria vaccine: immune response including strong humoral and T-cell responses together with clinical efficacy represents the first evidence that a vaccine against a parasite is feasible
> - *MF59*™- and AS03-adjuvanted pandemic influenza vaccines: antigen-sparing effect and cross-immunity observed for H5N1
>
> **Specific populations**
> - AS04-adjuvanted HBV vaccine: enhanced immune response in pre-haemodialysis and haemodialysis immunocompromised patients
> - *MF59*™-adjuvanted seasonal influenza vaccine: enhanced immune response in elderly/immunosenescent subjects

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