Chapter 17
Vaccines

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Abstract Vaccines continue to offer the key line of protection against a range of infectious diseases; however, the range of vaccines currently available is limited. One key consideration in the development of a vaccine is risk-versus-benefit, and in an environment of perceived low risk, the benefit of vaccination may not be recognised. To address this, there has been a move towards the use of subunit-based vaccines, which offer low side-effect profiles but are generally weakly immunogenic. This can be compensated for by the development of effective adjuvants. Nanotechnology offers key attributes in this field through the ability of nanoparticulates to incorporate and protect antigens from rapid degradation, combined with their potential to effectively deliver the antigens to appropriate cells within the immune system. These characteristics can be exploited in the development of new adjuvants. This chapter will outline the applications of nanosystems in vaccine formulations and consider the mechanisms of action behind a range of formulations.

17.1 Exploiting Nanotechnology in Vaccine Formulation

Infectious diseases remain among the leading causes of death worldwide. Vaccination offers one of the most effective strategies in global healthcare to address this. However, there is an on-going need to develop new and more effective vaccine formulations so as to offer protection against new emerging diseases [e.g. severe acuter respiratory syndrome (SARS) virus coronavirus, and human immunodeficiency

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virus (HIV)] and re-emerging old and/or persistent infectious diseases (e.g. Tuberculosis, malaria, and foodborne infections). As we have already seen within this book, nanotechnology can play a key role in many pharmaceutical applications, and vaccines are no exception. The application of nanoscience to vaccine formulation offers the potential to enhance the efficacy of vaccination by promoting enhanced protection and effective delivery of antigens. To effectively exploit nanotechnology in vaccine development, we must first consider the possible mechanisms which support effective immunisation.

17.1.1 Vaccines—an Introduction

The immune system comprises of many cellular and humoural components which protect the host from disease, a term which includes infection, autoimmune syndromes, injury, and mutations. To do so, the host has acquired the ability to evolve to its environment, a classic example being the gut whereby commensal microbes live in harmony with the host. The immune system has two main functions; to recognise invading pathogens and to activate mechanisms that will destroy them. Such pathogens are controlled and terminated by the innate immune response which is ready to react quickly (Fig. 17.1a). Most components of innate immunity are present before the onset of infection and constitute a set of disease-resistance mechanisms that are not specific to a particular pathogen. These mechanisms include cellular and molecular components that recognise classes of molecules as different to the frequently encountered pathogens (Goldsby et al. 2003). Phagocytic cells such as neutrophils, macrophages, in addition to pattern recognition receptors, NK cells, complement, and variety of antimicrobial compounds synthesised by the host all play important roles in innate immunity (Goldsby et al. 2003).

The adaptive immune response (Fig. 17.1b) is made up of B and T lymphocytes that have unique receptors specific to various microbial antigens (Sudhakar and Subramani 2005), in contrast to the receptors of the innate immune system which are of many different types but not specific to a particular pathogen (Parham 2009). These antigen-specific receptors are encoded by genes generated during a complex process of gene rearrangement that occurs during the course of lymphocyte development. As each B and T lymphocyte contains a unique antigenic receptor, it allows for large and diverse population of cells capable of recognising a wide spectrum of pathogens. This is termed the lymphocyte repertoire (Sudhakar and Subramani 2005). In response to an infection, lymphocytes-bearing receptors specific for the pathogen are then selected to participate in the immune response. The proliferation and differentiation of these cells, termed clonal selection and expansion, generates a large population of specific effector cells. To assist in future invasion by the same pathogen, some of the lymphocytes persist in the body and provide long-term immunological memory, thus resulting in a faster and stronger response (Parham 2009).

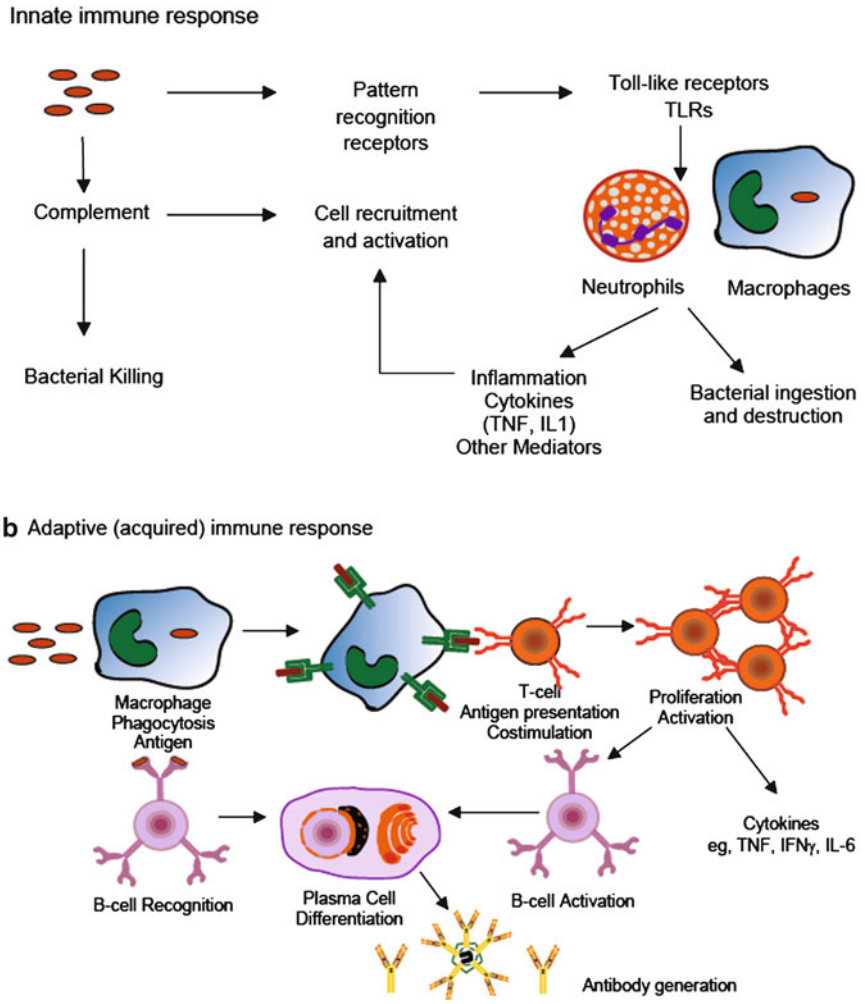

Fig. 17.1 Schematic representation of the immune response. (**a**) Innate immune response. Non-self-cells are rapidly attacked in the innate immune system. Key players in the innate system are neutrophils, macrophages, pattern recognition receptors, NK cells, and complement. The desired end result is the destruction of the foreign substance, the non-self-cell. (**b**) Adaptive immune response. The major players in this response are the B lymphocytes, T lymphocytes, and NK cells. The desired end result is destruction of the non-self-cell but through a more complex and tightly orchestrated series of events (based on Bingham 2008)

Therefore, the immune system is developed to offer a wide range of protection—however, one issue is that during an infection the body must be able to respond quickly and vigorously enough to provide the appropriate protection without the individual suffering the potentially lethal consequences of the infection. To address this vaccines have been developed; vaccines have been defined as ‘any preparation

made from a pathogen that is used for vaccination and provides protective immunity against infection with the pathogen' (Parham 2009). The ultimate goal of a vaccine is to develop long-lived immunological protection, whereby the first encounter with a pathogen is remembered and recognised by the immune system and therefore the immune system can generate a rapid, protective response against the infection.

17.1.2 The History of Vaccination

The origins of vaccination lie with smallpox, a disease which once ravaged most, if not all parts of the world. Initial descriptions of smallpox stem from as early as 1122 BC from texts originating in China. Smallpox was known to spread rapidly and resulted in disfigurement, blindness, and death. It was also known that smallpox was infectious, and early reports dating from 430 BC describe survivors of smallpox being used to treat those infected in a process known as inoculation (Gross and Sepkowitz 1998). Over many hundreds of years the concept of inoculation began to take form and involved a small swab of infectious material (otherwise known as pus) being placed on the skin of non-infected persons, and in 1,722, members of the English Royal Family were successfully immunised against smallpox using this method (Riedel 2005). However, it was the work of Edward Jenner, born in 1749 in Gloucestershire, UK, that supported the development of vaccination. Jenner, after overhearing a young dairymaid claim that she may never have smallpox as she had had cowpox, decided to further investigate this. In 1796 Jenner successfully inoculated a young boy with infectious material from a dairymaid who had cowpox lesions; 2 months later he exposed the boy to smallpox and no disease resulted. This was the beginning of what we now term vaccination.

17.2 Current Conventional Vaccines

In their traditional organisation, vaccines are grouped into four categories: killed, attenuated, toxoids, or subunit vaccines.

- *Live attenuated vaccines*
  Live attenuated vaccines consist of a live microbial agent that has mutated so that it has a reduced ability to grow in human cells and is no longer pathogenic to humans (Parham 2009). These microorganisms are still able to infect their target cells. However, infection is inefficient (mild) and there are limitations in the replication of the microorganisms. Vaccines produced in this manner include the bacillus Calmette-Guérin (BCG) and the measles, mumps, yellow fever vaccines, and rubella combination vaccine (MMR), and such vaccines are generally capable of stimulating both a humoural and cell-mediated immune response. However, there is a risk of reversion to virulence, and this type of vaccine is not considered safe for use in immunocompromised individuals.

Table 17.1 European licensed adjuvants for inclusion in vaccines

Adjuvant	Produced by	Disease
Aluminium salts	Various	Various
MF59® (squalene)	Novartis	Influenza
AS03 (squalene + tocopherol)	GSK biologicals	Influenza
AS04 (MPL + aluminium hydroxide)	GSK biologicals	HPV, HBV
Virosome	Crucell	Influenza, HAV

GSK GlaxoSmithKline, *MPL* monophosphoryl lipid A, *HPV* human papillomavirus, *HBV* hepatitis B virus, *HAV* hepatitis A virus
Adapted from (Friede 2009; Mbow et al. 2010)

- *Inactivated vaccines*
  Inactivated vaccines consist of microorganisms or viruses that have been treated with, e.g. heat or chemicals such as formaldehyde, thereby removing their ability to be infectious while retaining immunogenicity. While offering advantages in terms of safety, such vaccines are generally less effective than live attenuated vaccines, usually only stimulating humoural immunity and often requiring booster doses. Examples of these vaccines include: trivalent inactivated influenza vaccines, cholera, and hepatitis A vaccines.
- *Toxoids*
  Some microorganisms produce toxic compounds that are the responsible for causing the disease (i.e. tetanus toxin and diphtheria toxin). Toxoids are inactivated forms of these toxic compounds. In addition to being successful vaccines in their own right, toxoids may also be used to increase the immunogenicity of some other vaccines, such as the *Haemophilus influenzae* type B (Hib), which contains a polysaccharide unit from the virus conjugated to diphtheria or tetanus toxins.
- *Subunit vaccines*
  Subunit vaccines initiate strong immune responses using a small part of the organism that could include a gene from the genome. Recombinant DNA technology has greatly facilitated the development of such vaccines, a process where foreign genes are introduced into yeast or bacteria expression systems. This allows the production of large quantities of antigen that is purified and used as a vaccine (Arvin and Greenberg 2006). The principle benefits of subunit vaccines are their inability to revert to a pathogenic form, decreased toxicity, reproducible production, and improved antigen specificity; however, the immune response induced by such vaccines is short-lived and thus several boosts are required to achieve protection. For Hepatitis B virus for example, only the surface protein of the virus is used to generate the subunit vaccine.

As is highlighted in Table 17.1, there is no clear rule as to which type of vaccine (recombinant/killed/attenuated, etc.) may be superior against a certain disease. Certainly the present aim of vaccinologists is to focus on safety (including low toxicity and prevention of reversion to virulence); however, immunogenicity obviously plays a major role. With regard to smallpox, while the live attenuated composition of the vaccine led to the successful eradication of the disease, there were numerous

other factors which certainly contributed including the lack of an animal reservoir, non-zoonotic disease, stability of the vaccine formulation, and clear disease symptoms. However, it is interesting to note that the side effects from this vaccine were also sufficiently bad for the vaccination campaign in the United States to be halted in 1972, 8 years prior to the declaration by the WHO that the world was free from smallpox (Kennedy et al. 2009). Therefore, even the only vaccine to have ever led to the eradication of a disease had its faults and it is most probable that if the smallpox vaccine was still required today, it would not be used as it would fail clinical trials.

17.3 Improving Subunit Vaccine Efficiency with Adjuvants

The first marketed subunit vaccine, available in the United States in 1981, was for protection against Hepatitis B (Hilleman 2000). Therefore, in terms of arrivals onto the vaccine market, the subunit vaccines are latecomers with development rather than discovery being hindered by their poor immunogenic profile. However, they have the very important advantage over attenuated or killed vaccines in that there is no chance of reversion to a virulent form. Subunit vaccines are based on solely the antigenic epitopes originally derived from a virulent organism; there is a total loss of the molecules which would have typically alerted the host to the dangerous nature of the pathogen, in addition to the loss of particulate nature. This appears to be the downfall of subunit vaccines—they lack sufficient resemblance to pathogens. Therefore in an effort to improve the immune responses to subunit antigens, adjuvants are included in the formulation.

Adjuvants, whose name stems from the Latin ‘adjuvare’ meaning to aid, are defined as substances used in combination with a specific antigen that produce a robust immune response when compared to the antigen alone (Gupta et al. 2005; Vogel 1995). Adjuvants come in such a wide range of shapes and sizes that even though documentation on adjuvants has existed for nearly 100 years, there is still no universally approved grouping system. Figure 17.2 summarises some of the current suggestions for mechanisms of adjuvant action.

One of the first papers published by Ramon described the adjuvant effect of a range of compounds including tapioca, agar, and starch oil (Ramon 1925). Following this, the use of inorganic compounds including aluminium phosphate and aluminium hydroxide was documented (Glenny et al. 1926) and these aluminium-based compounds became the first licensed adjuvants in commercial vaccines. Until late 2009, aluminium-based compounds remained the only US-licensed adjuvants, while in Europe a wider scope of adjuvants had been recognised (e.g. Table 17.1). However, adjuvant development from bench to marketed vaccines has been slow with the first non-aluminium salt adjuvant being licensed less than 20 years ago (Mbow et al. 2010; O’Hagan and Gregorio 2009). The slow output and lack of successful licensing is due to numerous reasons including poor scale-up and inflexibility with regard to the scope of antigens with which they can be administered (O’Hagan and Gregorio 2009). However, when the varied immunising abilities of different vaccine adjuvants bearing

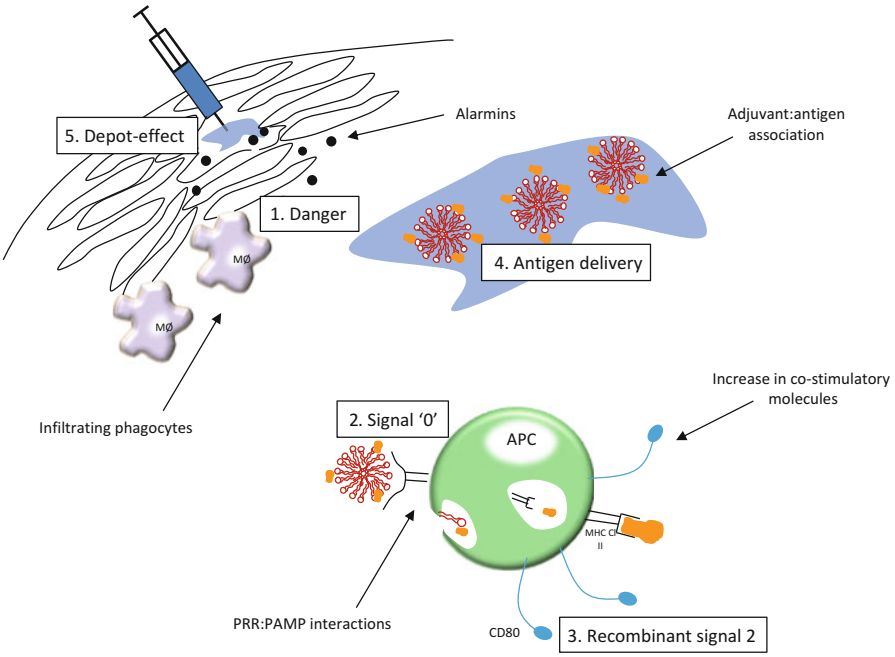

Fig. 17.2 Classification of adjuvants: the five steps of adjuvant action are described in the text. *APC* antigen-presenting cells, *MØ* macrophage, *PRR* pathogen recognition receptors, *PAMP* pathogen-associated molecular patterns

remarkably similar structure and composition are considered, these requirements are hardly surprising. For these reasons adjuvants are licensed in individual vaccines as opposed to being considered a separate entity (Fig. 17.3).

17.3.1 'Alum'—The Original Particulate Adjuvant

'Alum' is a collective term often used to refer to a group of aluminium salts including aluminium hydroxide, aluminium phosphate, and aluminium potassium sulphate. The correct term for this group of adjuvants is aluminium salts and not Alum, which correctly refers to aluminium potassium sulphate (Marrack et al. 2009). The widespread use of aluminium salts as vaccine adjuvants is due to a combined ability to (generally speaking) improve vaccine immune responses as well as provide an excellent safety profile. While occasional local reactions including inflammation, erythroma, subcutaneous nodules, and allergic reactions are reported, when the numbers of people who have been vaccinated are considered, aluminium salts are exemplary adjuvants (Clements and Griffiths 2002). The major downfall of aluminium salts is the polarised immune response which they activate being predominate activators of Th2-biased immunity, i.e. aluminium salts are ideal adjuvants in vaccines requiring

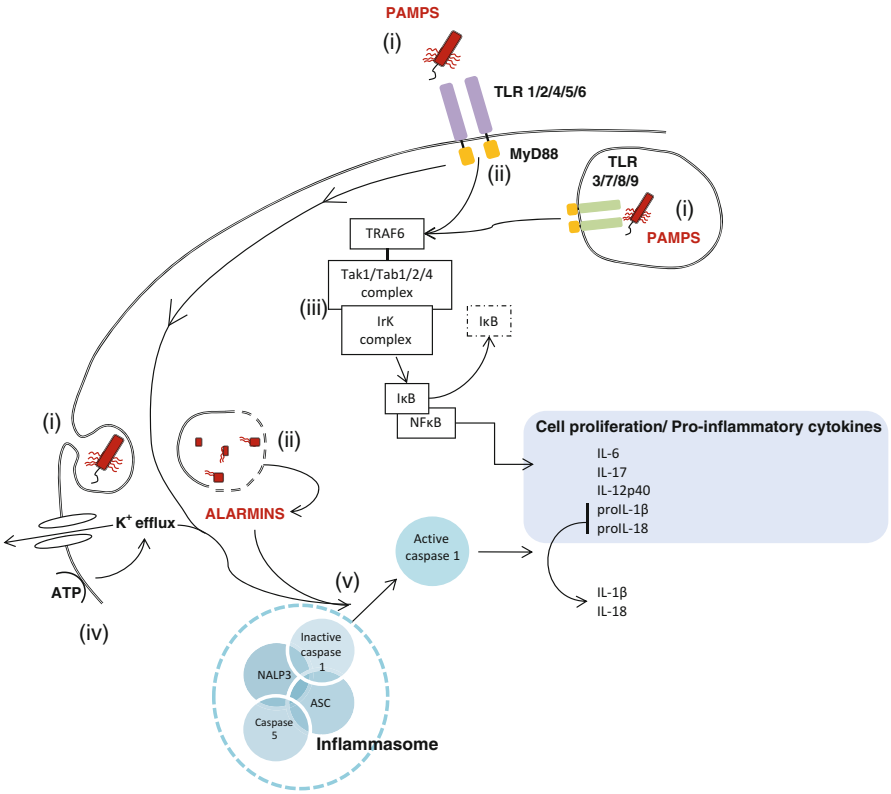

Fig. 17.3 Mechanisms of immunological action of aluminium salts: downstream signalling events after activation of Toll-like receptors (TLR) and the inflammasome. (i) Pathogens are endocytosed and activate TLRs via their pathogen-associated molecular patterns (PAMPs) resulting in the initiation of intracellular events including release of alarmins and activation of MyD88 adapter proteins respectively (ii). The TRAF6 signalling pathway becomes activated leading to cleavage of IκB and activation of the transcription factor NFκB with production of pro-inflammatory cytokines such as IL-1β and IL-18, both of which are produced with 'pro' inhibitory domains (iii). Endogenous ATP can act as an alarmin to activate the P2X7 receptor leading to potassium efflux from the cell (iv). The combination of potassium efflux and TLR activation lead to cleavage of the active caspase component of inflammasomes which is then able to cleave pro-domains of the cytokines IL-1β and IL-18 (v)

strong humoral immunity with high levels of IgG1 antibodies and cytokines such as IL-4. Examples of this include vaccines against extracellular pathogens such as parasitic diseases (e.g. leishmaniasis) or toxoid-producing pathogens (e.g. diphtheria, tetanus). Among the methods used to mediate the Th1/Th2 balance, one has involved combining aluminium salts with adjuvants known as strong stimulators of Th2 responses. Examples include aluminium hydroxide combined with cationic liposomes (Agger et al. 2008) or monophosphoryl lipid A (MPL), the latter of which is now the licensed adjuvant AS04 (GlaxoSmithKline (GSK) Biologicals).

It is only recently that the mechanisms by which aluminium salt adjuvants act have started to be uncovered, challenging the previous dogma that adjuvanticity was

simply due to longer retention of antigen at the injection site, also known as the depot-effect (Marrack et al. 2009). The importance and mechanisms of antigen association to aluminium adjuvants remains debated (e.g. Morefield et al. 2005); however, it is clear that aluminium salts prevent the rapid removal and degradation of antigen normally seen upon injection of free antigen. There is now a wide range of literature on the mechanisms of aluminium action (for in-depth reviews see (Brewer 2006; Marrack et al. 2009)) which strongly suggest a role for the inflammasome and uric acid release upon local tissue damage (Marrack et al. 2009; O'Hagan and Gregorio 2009). Briefly, injection of aluminium salts is known to cause tissue damage and cell death with release of alarmins such as endogenous uric acid, infiltration of neutrophils, and inflammatory mediators. Uric acid is capable of activating caspase 1, a component of the inflammasome, which can subsequently cleave pro-units of IL-1β and IL-18 to their active forms (Kool et al. 2008, see reference within Kennedy et al. 2009; Mariathasan 2007; Martinon et al. 2002; Monie et al. 2009) (Fig. 1.5).

17.3.2 Improving our Understanding of How Adjuvants Work

Generally speaking, adjuvants can be divided into groups depending on their physical properties, such as inorganic salts, liposomes, oil in water (o/w) emulsions, surfactants, etc. This classification poses the problem that the method in which the adjuvant acts is unknown and therefore does not help with eliminating or identifying potential nanosystems as future adjuvant candidates. Another classification which will become more complex with time is proposed by O'Hagen and De Gregorio (2009) whereby adjuvants are classed as first- or second-generation adjuvants. In this system a first-generation adjuvant refers to one of the more traditional substances normally composed of one immunostimulatory compound. These include aluminium salts, liposomes, and MF59 among others. Addition of further immunostimulatory compounds to these existing first-generation adjuvants results in a second-generation adjuvant such as the aforementioned 'Adjuvant Systems' (GSK Biologicals), IC-31® (Intercell, Austria), and ISCOMS. While the possibility exists to extend the system to third-generation adjuvants (and fourth and fifth, etc.), the system becomes increasingly awkward and does not give any indication as to how the adjuvants may work. In 2000 Virgil Schijns proposed a system whereby adjuvants can be divided into groups depending on their method of immunostimulatory action (Schijns 2000). While five groups were suggested, it is possible that further mechanisms may be deduced in the future, in addition to the difficulty in classification when one adjuvant has more than one mode of action. Finally and possibly the simplest involves a classification system based on whether the adjuvant works via TLRs or not (Mosca et al. 2008). In this instance only two groups exist (TLR-dependent and TLR-independent); however, for adjuvants containing two or more immunogenic components (such as GSK's AS04 adjuvant containing aluminium hydroxide and MPL), they may act via both TLR-dependent and TLR-independent mechanisms

further complicating matters. For the purposes of identifying the mechanisms of action of present and future adjuvants, the classification system by Schijns is the most appropriate and the basis of how these adjuvants work will be described herein.

17.3.3 Concepts of Adjuvant-Mediated Immunogenicity

According to Schijns classification, there are five methods in which adjuvants may be immunostimulatory. Highlighted in Fig. 17.1, these methods provide an ideal way to explain the immune system with relevance to systemic delivery of vaccines. Upon parental vaccine delivery there is localised tissue damage which results from the physical insertion of the needle and vaccine components into the tissue milieu. Cells will inevitably be ruptured releasing their intracellular contents including mitochondria, uric acid, and heat shock proteins (HSPs), all of which are termed 'alarmins' (Bianchi 2007). Alarmins are host-derived substances released upon non-intentional cell death (in contrast to apoptosis) and, unsurprisingly signal to the host that there is a problem such as tissue destruction or stress. This can be considered the first mechanism of adjuvant action (Shi et al. 2000) and is termed the danger signal, in reference to the 'danger model' originally described by Matzinger (1994). The danger model assumes that the host can differentiate between harmless and harmful as opposed to self and non-self [the later model originally described by Metchnikoff and thoroughly reviewed by Janeway (1992)]. Overlapping with the danger model is the concept of signal 0, the second class by which adjuvants act. Signal 0 can include alarmins but with respect to adjuvant composition and formulation the signal 0 group principally includes exogenous pathogen-associated molecular patterns (PAMPs). Importantly the signal 0 concept is the physical binding of alarmins or PAMPS [collectively termed danger-associated molecular patterns, or DAMPS (Bianchi 2007)] to pathogen recognition receptors (PRR's) and the resulting intracellular signalling cascade that results in activation of the cell (see Fig. 17.4 for an schematic of DAMPS and their TLRs). PRRs are constitutively expressed on or within cells of the innate immune system (Schijns 2000) and include, among others, TLRs, inflammasomes, integrins, c-type lectins, and antibody Fc receptors. The specific signalling cascades initiated by these DAMP-PRR interactions will be discussed later but some of the resulting actions include the production of pro-inflammatory cytokines, chemokines, and co-stimulatory molecules required for the productive activation of the T cell upon antigen presentation. In fact, antigen presentation without simultaneous co-stimulatory molecule interactions has been shown to lead to tolerance. Co-stimulatory molecules such as CD80 and CD86 are consequently very important for successful T cell activation and while being expressed constitutively on DCs, they are only expressed on other APCs including macrophages and B cells upon activation. Adjuvants which are able to activate macrophages and B cells to induce expression of CD80 or CD86, and those able to upregulate expression of the said markers on DCs can be grouped under the third adjuvantal category, 'recombinant signal 2'. The principle adjuvants in this group are cytokines which can be considered as endogenous adjuvants and

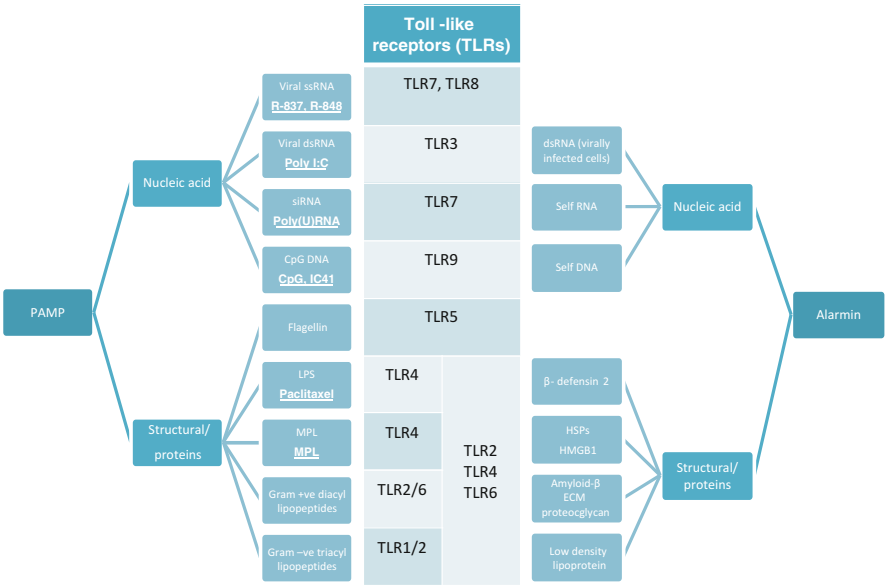

Fig. 17.4 Flow chart showing pathogen-associated molecular patterns (PAMP) and alarmins which are known to bind Toll-like receptors (TLRs). Synthetic analogues known to target specific TLRs are bold underlined. *R-837* Imiquimod, *R-838* resiquimod, *HSP* heat shock protein, *HMGB1* high motility group box 1, *ECM* extracellular matrix. Data obtained from a range of sources including (Bianchi 2007; Dockrell and Kinghorn 2001; Monie et al. 2009; O'Hagan and Gregorio 2009)

have also been administered with vaccines experimentally [see (Boyaka and McGhee 2001; Egan and Israel 2002; Heath and Playfair 1992) for review articles], as well as TLR-signalling adjuvants.

The final two adjuvantal categories relate more to the physical structure of the adjuvant and its physical localisation in vivo. As discussed previously, there is much diversity in the structures of adjuvants, although many of them are based on emulsions or vesicular structures. The benefits of vesicular structures are twofold; firstly they can be used to 'carry' antigen, either by entrapment or adsorption; secondly vesicles are naturally occurring and appropriately sized structures that can be endocytosed by cells depending on their composition and size, making them ideal intracellular delivery systems. This paradigm of assisting antigen uptake is the fourth adjuvantal category and principally concerns liposomes, nanoparticles, microspheres, virosomes, emulsions, and ISCOMs, all of which are able to package antigen into a delivery vehicle. The final concept is the idea that the antigen held at the injection site for a long period of time results in lengthy presentation of the antigen to innate immune cells. Known as the depot-effect, it refers to localisation of antigen (with or without adjuvant) at the injection site and not in the lymphoid organs (although increased presentation of antigen in the lymphoid organs may be a direct consequence of the depot-effect and is often the desired effect). The depot-effect is therefore dependent on numerous factors such as the route of injection, the tissue found at the injection site, and characteristics associated with the formulation itself such as viscosity and particulate size.

17.3.4 Defining the Ideal Adjuvant

From this analysis of different 'types' of adjuvants, it is clear that there are still many overlaps between groupings; however, the system devised by Schijns does help to define the mechanisms by which adjuvants work. With this in mind, it is possible to envisage how the perfect adjuvant would work. Firstly, it must contain a sufficient amount of PAMPs to alert the immune system of danger but without causing hyper-immunostimulation which may result in anaphylactic shock or local tissue damage by excess of inflammatory mediators. Too few PAMPs or PAMPs without sufficient toxicity may not stimulate sufficient production of pro-inflammatory molecules or chemokines which are vital to alert circulating APCs. It may just be that a certain degree of local tissue damage is also beneficial as it allows the release of alarmins, further promoting APC influx to the injection site (Shi et al. 2000). The adjuvant should also ideally stay associated with the antigen until uptaken by APCs so that the immune system can make a collective association of both components. Free antigen, in the form of nucleic material or small peptide antigens, is rapidly degraded by extracellular enzymes and many subunit protein vaccine antigens are themselves immunogenically inert. Protein and peptide antigens undergo rapid removal via the circulatory or lymphatic system and without any danger signals attached, the protein may simply be removed before antigen uptake and presentation can occur.

17.4 Nano-Enabled Vaccine Formulations

Given the key characteristics outlined above, it is no surprise that nanosystems have been extensively considered and exploited as potential vaccine adjuvants, with a wide range of nanomaterials being considered. Examples of such include nanoparticles, virosomes, immune-stimulating complexes (ISCOMS), liposomes, and bilosomes among others. By associating antigens with such nanosystems, the antigen can be protected from the extracellular milieu thereby limiting peptide/protein and nucleic antigen breakdown by enzymes, as well as preventing the rapid removal of such small compounds by the mononuclear phagocytic system (MPS).

17.4.1 Liposomes, Niosomes, and Other Vesicular-Based Adjuvants

The immunological role and adjuvant properties of liposomes were first identified by Allison and Gregoriadis (1974), where the ability of negatively charged liposomes (prepared with the inclusion of dicetyl phosphate) to deliver and potentiate immune responses against diphtheria toxoid (DT) was demonstrated. Since then the

immunological adjuvanticity of liposomes has been well recognised and liposomes have been extensively investigated as potential vaccine adjuvants for more than 20 years and for a number of antigens, including, e.g. tetanus toxoid (Davis and Gregoriadis 1987), *Leishmania major* antigen (Kahl et al. 1989), hepatitis B surface antigen (Brunei et al. 1999), DNA vaccines (Perrie et al. 2001, 2003), and tuberculosis vaccines (Davidsen et al. 2005; Smith Korsholm et al. 2007), with some liposomal-based vaccines (i.e. virosomes) having been licensed for human use (i.e. Inflexal vaccine for influenza).

Yet while there is a wealth of research dating from the 1980s and 1990s based on improving immune responses with the aid of liposomes, generally more attention was given to the ability of the formulations to effectively deliver the vaccines through enhancing protection and APC uptake of the antigen. However, given current understanding of adjuvants, more recently the focus has turned not just to delivery attributes but also immunomodulation, therefore research has focussed on combining the delivery attributes of liposomes with adjuvant properties through the inclusion of immunomodulating molecules. For example, Olsen et al. (2001) showed an increase and an induction of protective immunity against tuberculosis when isolated protein antigens, found in mycobacterial culture filtrates, were incorporated within liposomal vesicles combined with dimethyldioctadecylammonium (DDA; when hydrated in an aqueous environment this cationic lipid self-assembles into closed bilayers) (Olsen et al. 2001). Yet these identified proteins possess low inherent immunogenicity when injected alone (Andersen 1994). The authors reached a conclusion that in order for immune protection to remain high over an extended period, a depot must have been formed at the injection site by DDA (Holten-Andersen et al. 2004; Olsen et al. 2001). Holten-Andersen and co-workers conclude DDA may act to increase antigen and immunostimulator uptake into APC when it forms the depot.

The immunostimulatory properties of cationic lipids were originally documented in a screening study by Gall (1966). Within this study a wide range of compounds were investigated for their ability to adjuvant diphtheria or tetanus toxoids in guinea pigs. Compounds included non-ionic, anionic, and cationic surface-acting agents, amines, guanidines, benzamidines, thioureas, thiosemicarbazides, thiosemicarbazones, thiouroniums, and various nitrogenous bases. With regard to the group of surface-acting agents, or more commonly termed surfactants, Gall observed increased adjuvanticity for those expressing cationic quaternary ammonium head groups and long alkyl chains (Gall 1966). Within this group was DDA, a synthetic amphiphile, which contains a quaternary ammonium group with two 18-carbon-long alkyl chains forming the hydrophobic moiety and two methyl groups, which together with the ammonium group form the polar head group (Fig. 17.5). The positively charged head group carries a monovalent counter ion, typically bromide or chloride. Due to its amphiphilic character DDA can form liposomal structures when dispersed in aqueous media at temperatures above its gel-to-liquid phase transition temperature (~47 °C) (Davidsen et al. 2005). DDA is known to induce cell-mediated immunity and delayed-type hypersensitivity (Snippe et al. 1982), and along with its cationic nature and surfactant properties, has been shown to be an effective adjuvant

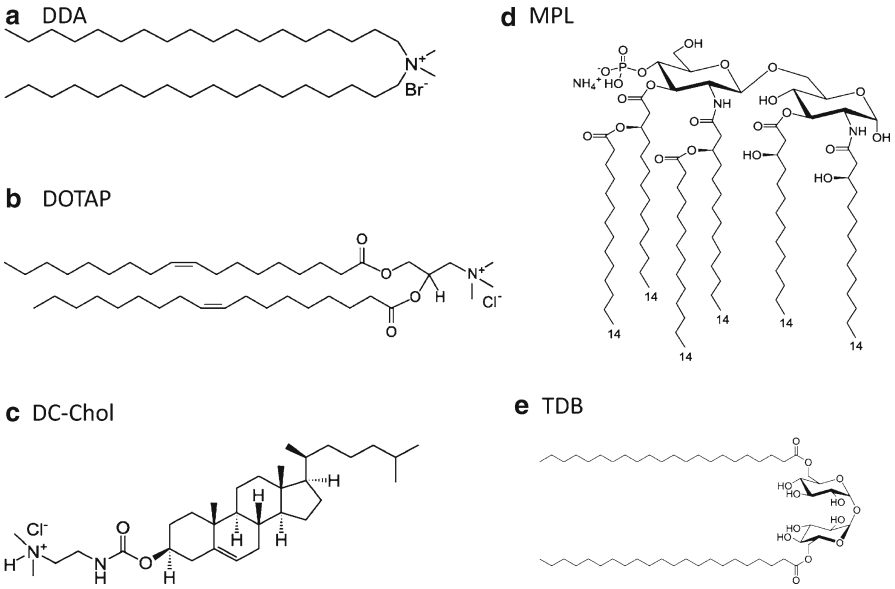

Fig. 17.5 Examples of lipids currently investigated in potential liposomal adjuvant systems (images courtesy of Avanti Polar lipids inc., http://avantilipids.com/)

in numerous applications, including mucosal immunisation (Klinguer et al. 2001), gene delivery (Esposito et al. 1999), and subunit vaccine delivery (Lindblad et al. 1997; Brandt et al. 2000; Holten-Andersen et al. 2004; Rosenkrands et al. 2005).

The mechanism of action behind the adjuvant effect of DDA has been attributed to its positive surface charge and its ability to associate antigens (Hilgers et al. 1985). This was recently confirmed and further elaborated by using ovalbumin (OVA) as a model antigen (Korsholm et al. 2007). Stimulation of immature bone marrow-derived dendritic cells (BMDCs) with fluorescently labelled OVA showed that adsorption of OVA onto DDA enhanced the cellular acquisition of the antigen. Further inhibition of active cellular processes by OVA stimulation at 4 °C or by the addition of cytochalasin D reduced the cellular uptake, suggesting that active actin-dependent endocytosis is the predominant uptake mechanism (Korsholm et al. 2007). DDA-mediated OVA uptake was further associated with a functional enhancement of the APCs. This was shown by measuring the increase in IFN-gamma production and cellular proliferation of purified autologous DO11.10 T-cells transgenic for a T-cell receptor recognising a major histocompatibility complex (MHC) class II–restricted OVA-epitope (OVA323-339). Both proliferation and IFN-gamma production were increased upon interaction with either murine BMDCs or purified B-cells, stimulated with OVA adsorbed to DDA (Korsholm et al. 2007; Christensen et al. 2009). More recent studies replacing DDA with a neutral lipid, DSPC, further demonstrate the role of the cationic lipid in the liposomal adjuvant

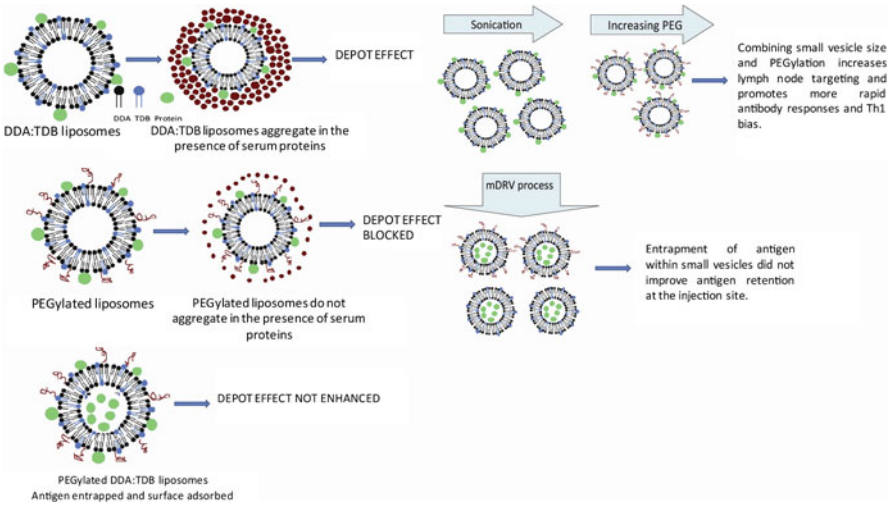

Fig. 17.6 Summary of the outcomes of manipulation of DDA:TDB liposomes in terms of vesicle size, surface pegylation, and antigen location (Kaur et al. 2012a, b)

by showing that neutralisation of the surface charge of the liposomes changes the biodistribution profile and diminishes their immunogenicity (Henriksen-Lacey et al. 2010).

The role of charge in the depot-effect of cationic liposomes has been further demonstrated by masking of the cationic charge in combination with manipulation of vesicle size and antigen location (Fig. 17.6); results from our laboratory have shown that PEGylation of DDA-based liposomes (which contained an immunostimulatory lipid trehalose dibenate) with polyethylene glycol (PEG) at 25 mol% was able to significantly inhibit the formation of a liposome depot and also severely limit the retention of antigen at the site, resulting in a faster drainage of the liposomes from the site of injection (SOI). This change in biodistribution profile was reflected in the immunisation response, where lower levels of IgG2b antibody and IFN-γ and higher levels of IL-5 cytokine were found. Additional studies investigated the impact of a combination of reduced vesicle size and surface pegylation on the biodistribution and adjuvanticity of the formulations, in a bid to further manipulate the pharmacokinetic profiles of these adjuvants. From these biodistribution studies, it was found that with small unilamellar vesicles, 10 % PEGylation of the formulation could influence liposome retention at the injection site after 4 days, while higher levels (25 mol%) of PEG blocked the formation of a depot and promoted clearance to the draining lymph nodes. Interestingly, while the use of 10 % PEG in the small unilamellar vesicles did not block the formation of a depot at the SOI, it did result in earlier antibody response rates and switch the type of T cell responses from a Th1 to a Th2 bias, suggesting that the presence of PEG in the formulation not only controls the biodistribution of the vaccine, but also results in different types of interactions with innate immune cells (Kaur et al. 2012a, b).

Table 17.2 Clinically approved virosome products

Product name		Formulation	Clinical indications
Epaxal	Inactivated Hepatitis A virus	These vesicles are known as virosomes as the bilayer structures are built from phosphatidylcholne, cephalin, and purified influenza virus surface antigens. The subunit antigens are incorporated within the bilayer lipid membrane and the vesicles are ~150 nm	Hepatitis A vaccine
Inflexal V	Influenza haemagglutinin glycoprotein and neuraminidase	Similar to Epaxal, these are virosomes prepared from phosphatidylcholine. The subunit antigens are incorporated within the bilayer lipid membrane	Influenza vaccine

Yet it is important to note that the efficacy of DDA may not be purely electrostatically driven as substitution of DDA with other cationic lipids including 3_-[*N*-(*N*′,*N*′-dimethylaminoethane)carbomyl] cholesterol (DC-Chol; Fig. 17.5) and 1,2-dioleoyl-3-trimethylammonium propane (DOTAP; Fig. 17.5) were considered: While all three cationic liposomes facilitated increased antigen presentation by antigen-presenting cells, the monocyte infiltration to the SOI and the production of IFN-γ upon antigen recall was markedly higher for DDA and DC-Chol-based liposomes which exhibited a longer retention profile at the SOI. A long-term retention and slow release of liposome and vaccine antigen from the injection site hence appears to favour a stronger Th1 immune response (Henriksen-Lacey et al. 2011). Similarly a modification of the hydrophobic backbone of DDA to a lower transition temperature lipid (dimethyldioctadecylammonium bromide; DODA) demonstrated that the antigen would more readily dissociate from the less rigid bilayer, DODA-based liposomes and these liposomes were also rapidly removed from the SOI. This resulted in lower up-regulation of co-stimulatory CD40 and CD86 molecules on adjuvant-positive antigen-presenting cells (Christensen et al. 2012).

Furthermore, the adjuvant properties of these DDA systems can be additionally supplemented by the use of a second lipid which can act as an immunomodulator. Table 17.2 highlights example immunomodulators that can be incorporated into liposomal delivery systems. For example, α,α′-trehalose 6,6′-dibehenate (TDB; Fig. 17.5) is a synthetic analogue of trehalose 6,6′-dimycolate (TDM) with two saturated fatty acid chains of 22 carbons (behenyl), each replacing the branched mycobacterial mycolic acids of >70 carbons. These two behenyl chains are linked by ester bonds to carbon number 6 of each of the two glucopyranose rings making up the trehalose head group. TDB has been shown to retain much of the bioactivity of the native form, while showing less toxicity as a result of the shorter fatty acid chains (Pimm et al. 1979; Olds et al. 1980). The combination of the DDA with TDB was first studied by Holten-Andersen et al. (2004). Using ESAT-6 as a possible TB antigen they investigated the ability of seven different immunostimulators to increase the protective efficacy of DDA, which included four mycobacteria-derived immunostimulators.

DDA combined with MPL and/or TDB induced an effective IFN-gamma response and protection in mice was equivalent to that provided by BCG vaccination (Christensen et al. 2009). The adjuvant activity of DDA:TDB when combined with Ag85B-ESAT-6 was also compared to aluminium hydroxide, an adjuvant approved for human use (Davidsen et al. 2005). $CD4^+$ T cells in mice secreted high levels of IFN-gamma and low levels of interleukin-5 (IL-5) in response to DDA:TDB, whereas the opposite pattern was observed for aluminium hydroxide (Davidsen et al. 2005; Christensen et al. 2009). Although high levels of IgG1 antibody titres were seen with both DDA:TDB-adjuvated vaccine and aluminium hydroxide-adjuvated vaccine, higher levels of IgG2 antibody titres were seen with DDA:TDB (Davidsen et al. 2005; Agger et al. 2008). DDA:TDB has also been shown to induce a multi-functional $CD4^+$ T-cell populations expressing several cytokines, mainly tumour necrosis factor-alpha (TNF-alpha)$^+$, IL-2^+, and IFN-gamma$^+$, TNF-alpha$^+$, IL-2^+. In mice such a population is maintained for at least 1 year and thus are long-lived (Lindenstrom et al. 2009).

In addition to liposomes there are a wide range of alternative vesicle constructs and many of these have been investigated as potential adjuvants; these include niosomes (e.g. Baillie et al. 1985), surfactant polymers (e.g. polymersomes (Okada et al. 1995, see reference within Mann et al. 2006)), vesicles incorporating bile salts to improve stability (e.g. bilosomes (Conacher et al. 2001)), or virus components (e.g. virosomes (Almeida et al. 1975)) to name but a few. Many of these systems use alternatives to phospholipids to circumvent potential issues related to storage instabilities and cost (e.g. synthetic-based systems), others to improve stability within harsh biological environments (e.g. bilosomes and polymersomes), or alternatively to modulate the properties of the vesicles in terms of immunological efficacy (e.g. virosomes).

In terms of niosome use for antigen delivery, the combination of 1-monopalmitoyl glycerol, cholesterol, and dicetyl phosphate (DCP) is often employed (Bramwell and Perrie 2005). The inclusion of DCP within these systems helps vesicle formation and is also reported to enhance stability due to the electrostatic repulsive forces between the vesicles which restrict aggregation (Bayindir and Yuksel 2010). The anionic nature of the vesicles has been reported to aid in uptake when delivering antigens via the oral route (Eldridge et al. 1990). Niosomes prepared from 1-monopalmitoyl glycerol, cholesterol, and DCP at a 5:4:1 M ratio incorporating bovine serum albumin (Brewer and Alexander 1992), ovalbumin (Brewer et al. 1996, see reference within Bramwell and Perrie (2005)), or a synthetic peptide containing a known T-cell epitope (Brewer et al. 1996) were shown to stimulate higher levels of IgG2a compared with Freud's complete adjuvant, but the vesicle formulations were shown to be weak stimulators for IgG1. In addition, the adjuvant activity of niosomes was wholly dependent on the model antigen being entrapped within the vesicles; mixing free antigen with the preformed vesicles was unable to elicit a significant immune responses (Brewer and Alexander 1992). This was attributed to the ability of niosomes to retain the antigen for a prolonged period and promoting APC uptake through active or passive targeting to cells (Brewer and Alexander 1992; Conacher et al. 2001). 1-monopalmitoyl glycerol (MPG)-based niosomes have also been considered for the delivery of DNA vaccines. MPG-based niosomes

incorporating cationic surfactants (DC-Chol) rather than anionic surfactants have been shown to offer an increased stability and increased plasmid DNA retention in the presence of competitive anions when compared to similarly formulated PC-based liposomes and engender transgene-specific immune responses comparable with their liposomal counterparts (Obrenovic et al. 1998; Perrie et al. 2002, 2004).

As with the liposome systems a range of immunostimulatory agents have been considered in the design of niosomes as vaccine adjuvants. For example, MPG-based vesicles incorporating both DDA and TDB were developed by Vangala et al. (2006), which resulted in an increase in the vesicle size due to the hydrophilicity of the surfactants, without altering the zeta potential of the vesicles compared to DDA:TDB vesicles. These systems were used to deliver two malarial antigens (Merozoite surface protein 1 (MSP1) and glutamate rich protein (GLURP)), and the MPG-based vesicles, in comparison to DDA liposomes, gave similarly strong Th2 humoural responses when analysing IgG1 titres; however, the MPG-based vesicles also showed high IgG2b titres unlike the DDA:TDB systems (Vangala et al. 2006).

In a further modification of non-ionic-based vesicles, bile acids have been included in the formulation. These systems, known as bilosomes, have been developed to promote the oral delivery of vaccines by offering protection to antigens from the enzymes present in the GIT and acting as immunological adjuvants. This is achieved by incorporating bile salts such as sodium deoxycholate into the formulation thereby increasing the stability of the carrier, thus preventing premature release of the antigen via the oral route. It has been proposed that by incorporating bile salts into the vesicles this offers resistance against degradation and disruption from the digestive enzymes, therefore making the formulation more stable (Schubert et al. 1983). Studies using bilosomes incorporating several antigens have proven to be successful in various animal models, e.g. the A/panama (Mann et al. 2004), tetanus toxoid (Mann et al. 2006), and hepatitis B (Shukla et al. 2010).

17.4.2 Virosomes

Virosomes, in terms of general structural attributes, resemble liposomal systems and are often considered within the general area of liposomes. They are unilamellar vesicles (with a mean diameter <150 nm) built from phospholipids, but in addition, virosomes incorporate functional viral envelope glycoproteins, such as influenza haemagglutinin. This promotes heamaglutinin-receptor binding, cell fusion, and immunostimulation. Of all the variations of liposomal systems discussed, currently only virosomes have been developed as clinical products. Two examples of virosome-based vaccines are Epaxal® and Inflexal® (Table 17.3), which are licensed in over 40 countries for clinical use.

Table 17.3 A selection of immunomodulators which can enhance the immunogenicity of liposomal systems

Immunomodulator	Description
MPL (monophosphoryl lipid A)	Induces the synthesis and secretion of various cytokines and is effective at potentiating mucosal and systemic immune responses to the incorporating antigen. This adjuvant has no observed side effects, other than minor irritation at the injection site Thoelen et al. (1998)
MPD (muramyl dipeptide)	Derived from bacterial cell walls and activates macrophages thus regulates the immune system Murata et al. (1997)
TDM (trehalose 6,6′-dimycolate)	Cord factor, which is a glycoprotein present on the cell membrane surface of *M. tuberculosis*. Activates macrophages and synthesis of cytokines, to drive a Th1 immune response. It is extremely toxic as it induces hypersensitivity granulomas, complex inflammatory events, and apoptosis Yamagami et al. (2001)
TDB (trehalose 6,6′-dibehenate)	An analogue of trehalose 6,6′-dimycolate (TDM) but consists of a shorter fatty acid chains, therefore is considered to be less toxic Davidsen et al. (2005). Very immunogenic as a co-adjuvant for eliciting protective immunity against tuberculosis Holten-Andersen et al. (2004); Davidsen et al. (2005)

17.4.3 Immune-Stimulating Complexes

Immune-stimulating complex particles (ISCOMs) were initially described by Morein et al. (1978) as novel structures that facilitated antigenic presentation of membrane proteins. ISCOMs are cage-like particles approximately 40 nm in size that incorporate protein antigen through hydrophobic interactions and due to their particulate nature (Myschik et al. 2006). ISCOMs generally consist of a mixture of Quil A saponins, cholesterol, and phospholipids. Therefore, while this system contains lipids, their structural attributes are very different from those of liposomes, and it is the addition of the saponin to the phosophipid/cholesterol mixture that drives the change in structure of these nanosystems. ISCOMs can also be prepared to offer a cationic charge when DC–cholesterol replaces cholesterol, or the substitution of PC with dioleoyl-trimethyl-ammonium-propane. The cationic complexes formed are similar to the classical anionic ISCOMs and allow a more diverse range of antigens to be used in their formulation (Lendemans et al. 2005, see reference within Lendemans et al. 2007). Orally, ISCOMs have shown promising systemic immune responses by eliciting Th1, Th2, and MHC-restricted cytotoxic T-cell responses in addition to local induction of IgA (Mowat et al. 1993). As a result, the introduction of immunostimulatory agents has been shown to be useful to enhance immune responses to sub-unit vaccines and offer a promising platform for further studies.

17.4.4 Solid Nanoparticles as Adjuvants

Solid nanoparticles have also been extensively investigated for their potential as vaccines adjuvants. Like the vesicle type systems already described, they can be

prepared in a range of sizes, with a choice of surface characteristics and include a selection of immunomodulators. Polymeric nanoparticles are generally formulated from natural or synthetic polymers with the most commonly studied polymers being those which are biodegradable such as poly(lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA), poly(caprolactone) (PCL), and polysaccharides (particularly chitosan). The advantage of these polymers is that they are well characterised and used in a range of clinical products, particularly PLGA. Alternatively, nanoparticles can be prepared from solid (high melting point) lipids dispersed in an aqueous phase. Examples of lipids used can include solid triglycerides, saturated phospholipids, and fatty acids which are well tolerated by the body. Due to their composition, they are sometimes described as 'solidified' o/w emulsions in which the oil globule is replaced by solidified lipids. Much like the solid polymeric nanoparticles, solid lipid nanoparticles can be used as vaccine adjuvants by the antigen being incorporated within the lipid matrix of the particle or by attaching it to the lipid nanoparticle surface. Lipid particles normally start from around 50 nm in size and can be prepared in a large-scale by homogenisation to disperse the lipid into an aqueous environment.

The use of polymer-based particulates as vaccine adjuvants has been strongly investigated, but it has been more recent work that has refocused investigations into the potential advantage of using these systems in the nano range. For example, work by Stano et al. (2012) demonstrated that by increasing the size of their polypropylene sulphide nanoparticles from 30 to 200 nm, the antigen was more effectively delivered into both MHC class I and MHC class II-presentation pathways. Enhancing the targeting of nanoparticles to dendritic cells has also been considered, e.g. the addition of a recombinant fusion protein to the surface of PLGA nanoparticles was shown to promote a twofold increase in DC uptake in vitro, and in vivo studies demonstrated these formulations promoted enhanced antigen-specific IgG and IgG subclasses, and higher cytokine responses.

17.4.5 Emulsion-Based Adjuvants

While not all emulsion-based systems fall within the nanoscience definition, it is useful to consider the role of emulsions in general in vaccine formulation. In 1997 the first emulsion adjuvant MF59® (Novartis, Italy) was licensed in Europe in the influenza vaccine Fluad® (O'Hagan and Gregorio 2009). MF59® is an oil-in-water (o/w) emulsion composed of 5 % squalene (naturally occurring oil) combined with surfactants sorbitan trioleate (Span 85) and poly(oxyethylene) sorbitan monooleate (Tween 80). Despite concerns regarding its safety due to the occurrence of autoimmune dysfunctions in rats (Carlson et al. 2000), the adjuvant has an established safety profile in humans of good-health and immunocompromised populations (Black et al. 2010; Donatoa et al. 1999). It is interesting to note that MF59® is not the first emulsion to be licensed for human use; the well-known experimental adjuvant Incomplete Freund's adjuvant (ICF) (Freund et al. 1948) was once used in

human influenza vaccines (Chang et al. 1998). In contrast to MF59®, ICF is a water in oil (w/o) emulsion composed of a light mineral oil (such as Bayol F) and the emulsifier mannide monooleate (Aracel A™), combined in a 9:1 volume ratio (Lindblad 2000). While it has been withdrawn from human use due to occasional serious local reactions, it remains an experimental gold-standard adjuvant in immunisation protocols. The success of MF59® has been attributed to the rendering of soluble antigen to particulate form, improved cell recruitment to the injection site, and antigen uptake with transport to local lymph nodes (Mosca et al. 2008). Similar to aluminium salts, no direct activation of dendritic cells has been noted, although improved trafficking and antigen uptake by macrophages and DCs, respectively, has been noted, and expression of soluble activation factors may indeed lead to indirect DC activation (Mosca et al. 2008).

17.4.6 The Influence of Vaccine Formulation and Delivery Route on Vaccine Performance

In terms of potential advantages of these solid nanoparticles over the bilayer type systems, there has been few direct comparisons made, as it is difficult to control the number of different parameters between the systems. Similarly little work has been undertaken to understand the impact of the route of administration of such these adjuvants on the type and strength of immune response promoted. However, in a recent multi-centre study, the difference in immune responses generated in mice vaccinated by the subcutaneous, intradermal, intramuscular, and intralymphatic routes was considered with ovalbumin-loaded liposomes, *N*-trimethyl chitosan (TMC) nanoparticles, and poly(lactide-co-glycolide) (PLGA) microparticles, all with and without specifically selected immune-response modifiers were directly compared (Mohanan et al. 2010). Interestingly, neither the route of administration nor the presence of immunomodulators within the formulations made a notable difference to induced IgG1 antibody responses. However, the administration route had a strong impact both on the kinetics and magnitude of the IgG2a response: a single intralymphatic administration of all the evaluated vaccine formulations (liposomes, nanoparticles, and microspheres) generated a strong IgG2a response, whereas subcutaneously, only the adjuvanted nanoparticles were able to promote notable IgG2a responses, and the intradermal and intramuscular routes generated intermediate IgG2a responses (Mohanan et al. 2010). The benefit of the intralymphatic administration route for eliciting a Th1-type response was confirmed in terms of IFN-gamma production. This study demonstrated that the IgG2a associated with Th1-type immune responses is sensitive to the route of administration, whereas IgG1 response associated with Th2- type immune responses was relatively insensitive to the administration route of the particulate delivery systems. Therefore, consideration of the vaccine formulation in combination with the route of administration should be considered when planning and interpreting preclinical research or development on vaccine delivery systems.

17.5 Clinically Relevant Nanosystem-Based Vaccines

Given the range of clinically approved nanotechnology-based pharmaceutical products already available, it is interesting to note that currently only virosomal vaccines [Epaxal® and Inflexal® V (Crucell)] are clinically approved for use. However, until the desirable characteristics for a nanosystem-based vaccine adjuvant are fully elucidated, it is difficult to identify key attributes we should be focusing on other than final in vivo performance, which is a lengthy and expensive marker to rely on. Similarly there continues to be issues regarding regulation. For example the WHO stipulates that liposomal adjuvants must be licensed as a vaccine formulation, and not as an adjuvant which could be combined with various antigens post-licensing. This adds further complications to the development and licensing processes. However, progress continues with cationic nanosystems such as the DDA:TDB formulation developed by Staten Serum, Institute, and the cationic Adjuvant IC31® (Intracell, Austria).

17.6 Concluding Remarks

Overall, liposomes offer a strong potential as vaccine adjuvants by combining the ability to deliver antigens to the correct cells, and also appropriately interact and stimulate such cells. While progress remains limited to-date, new advances in the understanding of effective vaccine systems and in the production and regulation of liposomes in a cost-effective manner should enhance their progress into the clinical setting.

Problem Box

1. Identify the various vaccine types currently clinically available. Discuss their respective advantages and disadvantages.

   Answer: Vaccines can be divided into three basic groups: (1) live vaccines, (2) inactivated vaccines, (3) toxoids and subunit systems.

   Live attenuated vaccines consist of live microorganisms or viruses that have mutated so that it has a reduced ability to grow in human cells. They are still able to infect their target cells, but the infection is mild and replication is limited. These vaccines generally give good protection, but there is a risk of reversion to virulence, and this type of vaccine is not considered safe for use in immunocompromised individuals.

   Inactivated Vaccines consist of microorganisms or viruses that have their ability to be infectious removed while retaining immunogenicity. In terms of advantages they tend to have improved safety profiles compared

(continued)

Problem Box (continued)

with live system, they are generally less effective than live attenuated vaccines, usually only stimulating humoural immunity and often requiring booster doses.

The third group consists of part of the infectious agent, such as excreted toxoids or subunit proteins. Subunit vaccines initiate strong immune responses using a small part of the organism. The advantage of subunit vaccines is their good safety profile; however, the immune response induced by such vaccines is short-lived and thus several boosts are required to achieve protection.

2. Describe the structural attributes of virosomes and discuss, with the use of examples, how these are able to be used as vaccines.

Answer: Virosomes are bilayer vesicles around 150 nm in size. They are prepared from phospholipids and incorporate functional viral envelop glycoproteins, such as influenza haemagglutinin. This promotes heamaglutinin-receptor binding, cell fusion, and immunostimulation. For example, Epaxal is a virosome vaccine clinically indicated for Hepatitis A immunisation. By incorporating the subunit antigen for Hepatitis A, the antigen can be protected from the extracellular milieu, thereby limiting antigen breakdown by enzymes, as well as preventing the rapid removal of such small compounds by the MPS. This ensures the antigen carried within the virosome delivery system to the appropriate immunological cells, and subsequently the antigen is taken by the cells due to the action of the glycoproteins.

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