

Chapter 2

Classification of Vaccines

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2.1 Introduction

The introduction of human vaccines has had a tremendous impact on global health by dramatically reducing the mortality and morbidity caused by infectious diseases, and next to the wider availability of potable water, it is considered the most cost-effective and successful medical intervention ever introduced. Vaccines have inevitably prevented disease, complications, and the death of millions of infants and children by protecting against many deadly infectious diseases (Bloom et al. 2005; Ehreth 2003).

Although vaccines have mainly demonstrated their value to human society during the past century, the principle of vaccination has been used in China and India for more than a thousand years as the practice of variolation, where individuals were inoculated with live and virulent smallpox virus to achieve protection against a later encounter. Although the procedure did lead to protection, it was not without the risk of death or causing an epidemic. However, Edward Jenner is generally honored for the pioneering development of the first vaccine more than 200 years ago by demonstrating that exposure of humans to cowpox virus induced cross-protective immunity towards smallpox (Riedel 2005). The word vaccine was in fact coined by Jenner, and is derived from the Latin word *vacca*, which means cow.

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Subsequently, the development of vaccines have for more than a century been based on Louis Pasteur's principle of isolating, purifying, and injecting the causative microorganisms in order to induce protective immunity (Rappuoli 2007). After World War II more systematic childhood vaccination programs became a widespread tool for improving public health (Bloom et al. 2005). The mortality caused by serious and life-threatening diseases has been dramatically reduced as a result of these successful global childhood vaccination programs, and the introduction of vaccines has led to the eradication of smallpox and near eradication of infectious diseases such as polio (Ehret 2003; Rappuoli 2007). The World Health Organization (WHO) currently recommends routine immunization against 12 different diseases (Table 2.1). Furthermore, additional vaccines are recommended for populations at high risk or regions with special needs.

Despite this true medical success story, current vaccination efforts do face a number of obstacles. Three million people are estimated to die annually from vaccine-preventable illnesses, and infectious diseases still remain the leading cause of death worldwide for several reasons. The rapid progress towards universal vaccination coverage in the 1970s and 1980s has slowed in the past decade, and several childhood illnesses have started to re-emerge as a result of inefficient vaccine coverage. This may be due to public perception of vaccination, where an individual may find it rational to refuse vaccination in order to avoid the possible side effects, or due to political reasons. The consequence has been the reemergence of diseases such as measles and pertussis in certain industrialized countries and of polio in certain developing countries (Bloom et al. 2005).

Infectious disease-caused mortality can also be explained by lack of efficacious vaccines where conventional vaccinology has failed due to factors such as antigenic drift, and by the existence of more difficult target diseases, for example, tuberculosis (TB), human deficiency virus-acquired immune deficiency syndrome (HIV-AIDS), and malaria. Antigenic drifts represent a challenge for vaccine development,

Table 2.1 WHO recommendations for routine immunization (WHO 2012)

Disease/antigen	Age group
Bacillus Calmette-Guérin (BCG)	Children
Hepatitis B	Children (+adolescents/adults in high risk groups)
Polio	Children
Diphtheria	Children, adolescents, and adults
Tetanus	Children, adolescents, and adults
Pertussis	Children
<i>Haemophilus influenzae</i> type B	Children
Pneumococcal	Children
Rotavirus	Children
Measles	Children
Rubella	Children
Human papilloma virus (HPV)	Adolescent girls

and the success stories in vaccinology arise to a large extent from development of vaccines against pathogens with no or little antigenic drift, for example, vaccines against diphtheria and tetanus, where there is no antigenic drift in the target toxin antigen. Antigenic shift can result in changes in surface antigens and the influenza virus is an example of a pathogen where such changes occur annually. This antigenic variability is overcome by altering the vaccine on a yearly basis. However, pathogens where antigens change faster, e.g., human immunodeficiency virus (HIV), are more difficult to approach by conventional vaccinology. To date, conventional vaccinology has been most successful in vaccines against pathogens for which protection is antibody mediated. The difficult vaccine targets represent to a large extent pathogens for which antibodies cannot provide sufficient protection (Rappuoli 2007). An example is the intracellular pathogen *Mycobacterium tuberculosis*. In 2012, 8.6 million people were infected with *M. tuberculosis* and approximately 1.3 million people died from TB (WHO, Fact sheet 104, 2012). Numbers like these put great emphasis on the acute need for new prophylactic as well as therapeutic vaccines against global killers like TB, malaria, HIV-AIDS, and cancer. However not only new vaccines are needed since improvements to conventional vaccines could have a tremendous impact on vulnerable population groups such as the elderly, since this population is immunologically hyporesponsive. Several vaccines approved for human use are listed in Table 2.2.

2.2 Classification of Vaccines

Traditionally vaccines have been based on live attenuated pathogens, whole inactivated organisms or inactivated bacterial toxins and are most often sufficiently immunogenic. Traditional vaccines based on the whole-cell concept possess intrinsic immune stimulatory capacity, which is adequate for the induction of long-lived protective immunity. However, a great disadvantage related to this approach is that these live systems have associated adverse effects that in some cases are mild but can be severe or even fatal in others (Huang et al. 2004). Safety is of major concern in vaccine development and limits the use of the traditional approach in the development of new vaccines as traditional vaccines may cause disease in immunocompromised hosts or revert back to virulence (Robinson and Amara 2005). With these issues, new parenteral vaccines are unlikely to be live attenuated vaccines.

In light of these limitations, new strategies for vaccine development are emerging, and vaccine development is moving away from the whole-cell based approach of live attenuated or inactivated vaccines and towards the safer split and subunit vaccine technology. The field of vaccinology has undergone tremendous breakthroughs over the past 30–40 years. An important contribution to these breakthroughs is provided by the introduction of recombinant DNA technology, which solved the problem of antigen manufacturing. Also the development of conjugate vaccines, subunit vaccines, and the non-replicating recombinant virus-like particles (VLPs) has had an enormous impact on vaccine development and success (Rappuoli 2007).

Table 2.2 Vaccines licensed for human use (non-exhaustive list)

Trade name	Disease target	Causative agent	Vaccine type	Vaccine components	Adjuvant/ comment
AGRIFLU®—Novartis Vaccines and Diagnostics, Inc. (various others)	Influenza	Influenza virus	Inactivated	Trivalent for influenza type A and B (whole virus based)	None
BCG vaccine “SSI”	Tuberculosis	<i>Mycobacterium tuberculosis</i>	Live attenuated	Bacillus Calmette Guérins (BCG), Danish strain 1331	None
BioThrax® (Emergent Biodefense Operations Lansing, Inc.)	Anthrax	<i>Bacillus anthracis</i>	Subunit vaccine	Cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of <i>Bacillus anthracis</i> and proteins, including the 83 kDa protective antigen (PA) protein, released during the growth period	Alum
Cervarix® (GlaxoSmithKline)	Cervical cancer	Human papillomavirus (HPV)	Subunit—virus like particles	Recombinant L1 protein, the major antigenic protein of the capsid, of oncogenic HPV types 16 and 18	AS04
COMVAX® (Merck & Co.)	Hib-induced diseases (pneumonia, meningitis) Hepatitis B	<i>Haemophilus influenzae</i> type B Hepatitis B virus	Conjugate vaccine	Haemophilus b conjugate (meningococcal protein conjugate) and Hepatitis B (recombinant) vaccine	Alum
FluMist® Quadrivalent (MedImmune, LLC)	Influenza	Influenza virus	Live inactivated	Quadrivalent vaccine for administration by intranasal spray. FluMist Quadrivalent contains four vaccine virus strains: an A/H1N1 strain, an A/H3N2 strain, and two B strains Cold adapted, temperature sensitive, and attenuated	None
Gardasil® (Merck & Co.)	Cervical cancer	Human papillomavirus (HPV)	Subunit—virus like particles	Recombinant quadrivalent vaccine prepared from the purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV Types 6, 11, 16, and 18	Alum

Havrix® (GlaxoSmithKline)	Hepatitis A	Hepatitis A virus	Inactivated virus	The virus (strain HM175) is propagated in MRC-5 human diploid cells. Treatment with formalin ensures viral inactivation	Alum
Influenza A (H1N1) 2009 Monovalent Vaccine Sanofi Pasteur, Inc.	Influenza	Influenza virus	Inactivated (against influenza disease caused by pandemic (H1N1) 2009 virus)	Monovalent split vaccine whole cell approach	None
IPOL® Sanofi Pasteur	Polio	Poliovirus (type 1, 2 and 3)	Live inactivated	Inactivated at +37 °C for at least 12 days with 1:4,000 formalin	None
Infanrix® (GlaxoSmithKline)	Diphtheria	<i>Corynebacterium diphtheriae</i>	Subunit	Toxoid of diphtheria and tetanus and the acellular pertussis antigens (inactivated pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin)	Alum
	Tetanus	<i>Clostridium tetani</i>			
	Pertussis	<i>Bordetella pertussis</i>			
Pediarix® (GlaxoSmithKline)	Diphtheria	<i>Corynebacterium diphtheria</i>	Subunit	As Infarix in combination with HBsAg and type 1, 2, and 3 polio viruses Inactivated with formaldehyde	Alum
	Tetanus	<i>Clostridium tetani</i>			
	Pertussis	<i>Bordetella pertussis</i>			
	Polio	Poliovirus			
Recombivax HB® (Merck & Co.)	Hepatitis B	Hepatitis B virus	Subunit viral vaccine	Derived from hepatitis B surface antigen (HBsAg) Oka/Merck strain of VZV	Alum
	Hepatitis B	Hepatitis B virus			
	Herpes Zoster	Herpes Zoster virus			
Zostavax® (Merck & Co.)	Herpes Zoster	Herpes Zoster virus	Live attenuated		

2.2.1 *Live Attenuated Vaccines*

Conventional vaccines have been based on live attenuated pathogens, and contain laboratory-weakened versions of the original pathogen. The rationale for using live attenuated vaccines is that they mimic the natural infection, which results in an effective vaccination strategy. The advantage of this type of vaccine is that both a strong cellular and an antibody response are produced. Usually, long-term protection is also achieved, and a single inoculation is often sufficient. The attenuation of the microorganism results in a non-pathogenic microorganism, which still possesses all the pathogenic features as the original microorganism (Clem 2011).

Attenuation can be achieved via different approaches. Edward Jenner's approach was to use a virus pathogenic in a different host but not pathogenic to humans, as he isolated pus from cows with cowpox, and this provided the basis for his smallpox vaccine (Riedel 2005). Naturally occurring attenuated strains can also be used, exemplified by the use of type 2 poliovirus. Attenuation is also possible by applying harsh conditions on a virulent virus strain (e.g., cold adaption of influenza virus).

The Bacillus Calmette Guérin (BCG) vaccine against TB is an example of an attenuated live vaccine. The currently used vaccine strains are all descendants of the original *M. bovis* isolate that Calmette and Guérin passaged through many cycles. Further passages, under different laboratory conditions, have resulted in a variety of new BCG strains with phenotypic and genotypic difference.

One such strain is the 1331 strain produced at the Danish Serum Institute (WHO 2004). As adults with lung TB are the major source of disease transmission, BCG vaccination of children has had very limited influence on the global epidemic. Another very important limitation of BCG is the lack of effect in the two billion individuals already infected with TB, which underlines the need for the development of new TB vaccines (WHO 2004).

Another example of an attenuated live viral vaccine is the measles, mumps, and rubella vaccine (MMR). This vaccine has been available in the United States since 1971 (Ravanfar et al. 2009). Priorix® is a marketed MMR vaccine produced by GlaxoSmithKline. The vaccine contains attenuated MMR viruses. Each of these attenuated virus strains, measles (the Schwarz strain), mumps (the RIT 4385 strain), and rubella (the Wistar RA 27/3 strain) is obtained separately by propagation in chick embryo tissue cultures (mumps and measles) or MRC5 human diploid cells (rubella) (Wellington and Goa 2003).

2.2.2 *Inactivated Vaccines*

The main advantage of killed or inactivated vaccines over attenuated vaccines is safety. Since these vaccines are based on killed/inactivated pathogens, the concerns regarding reverting back to virulence are obviated. However, this also constitutes a huge disadvantage since the lack of replication results in a fast clearance from the

body leading to a decreased efficacy, as compared to the live vaccines. Killed/inactivated vaccines do, however, give rise to a more complex or greater inflammatory immune response in comparison to the newer subunit vaccines due to the fact that most of the pathogenic components are preserved.

Inactivated vaccines are used widely. An example of such a vaccine is the Hepatitis A vaccine Epaxal® from Crucell. This vaccine is based on a hepatitis A virus (strain RG-SB) which is inactivated by formalin treatment. The inactivated vaccine is adsorbed onto a virosome formulation, which constitutes the adjuvant system (Bovier 2008).

2.2.3 Subunit Vaccines

Subunit vaccines are, by definition, vaccine agents that comprise one or more components of a pathogen rather than the entire pathogen. Subunit vaccines are composed of one or several recombinant peptides/proteins or polysaccharides normally present in the structure of the target pathogen (Dudek et al. 2010). In terms of safety and cost of production, these vaccines offer considerable advantages over the traditional vaccines, as these are composed of very well-defined and highly pure components. This approach results in a more appealing safety profile due to the lack of replication and the removal of material that may initiate unwanted host responses (Robinson and Amara 2005).

For bacterial subunit vaccines, two main types exist. The first type is the toxoid vaccines which are generated against bacteria where toxins are the main disease-causing agents. The toxins are inactivated by converting the toxins into detoxified versions (toxoids), for instance by treatment with formaldehyde. These toxoids can then safely be used for vaccination purposes. The close resemblance of the toxoid to the toxin enables the immune system to neutralize and fight the natural toxins via generation of anti-toxoid antibodies. Examples of toxoid vaccines are the different vaccines against diphtheria, tetanus, and pertussis. The second major group of bacterial subunit vaccines is based on the capsular polysaccharides of encapsulated bacteria. There are several examples of vaccines of this type, including vaccines against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b (Hib). A variation of this is the conjugate vaccine, which is created by covalently attaching an antigen (often the bacterial polysaccharides) to a carrier protein, e.g., tetanus toxoid, resulting in the generation of more efficacious vaccines. Common virus subunit vaccines are the split virus vaccines where the structure of the viruses has been disrupted, resulting in a mixture of the various viral components.

Alternatively, subunit vaccines may consist of one or more viral or bacterial proteins, or peptide fragments of these. In some cases, such antigens might be sufficiently immunogenic by themselves. This is the case for the subunit vaccine for influenza comprising the two purified surface antigens hemagglutinin (HA) and neuraminidase (NA). These two proteins are isolated for the seasonal flu vaccine from three selected virus strains and combined in a trivalent vaccine, with or without an

adjuvant. Also for the hepatitis B vaccine, the surface antigen, HBsAg, is sufficiently immunogenic, and a vaccine based on recombinant HBsAg was the first genetically engineered vaccine product produced commercially and used worldwide.

However, in many cases the highly purified subunit antigens lack many of the intrinsic pathogenic features which render these protein-based antigens weakly immunogenic by themselves and co-administration of adjuvants is often required. The addition of adjuvants not only enables the induction of an effective immune response, but also provides the potential to modulate the immune response (Reed et al. 2009; O'Hagan 2001). The use of adjuvants can also allow for a dose-sparing effect or can reduce the number of required administrations.

2.2.3.1 Adjuvants

A vaccine adjuvant is defined as a component that potentiates the immune response to an antigen and/or modulates it towards a desired immune response. The term adjuvant is derived from the Latin word *adjuvare*, which means to help. The most commonly used adjuvants are the aluminum salts commonly, although incorrectly, referred to as alum (Chap. 3). The adjuvant effect of alum was discovered by Glenney in 1926, and alum has now been utilized for more than 70 years in vaccines (Glenney et al. 1926). For many years alum was the only adjuvant approved worldwide and it has been used in large numbers of vaccines for human use (Clements and Griffiths 2002). Formulation is achieved by adsorption of antigen onto highly charged aluminum particles (Reed et al. 2009).

In recent years, there has been substantial progress in the discovery of new efficient adjuvants for subunit vaccines [reviewed by (Foged 2011)], and a handful of these have been marketed as components of approved licensed vaccines. Examples of adjuvants are emulsions, liposomes, polymeric nanoparticles, immune-stimulating complexes (ISCOMs), and VLPs, which are described in the following chapters.

Adjuvants can broadly be classified into delivery systems and immunopotentiating compounds, generally pathogen-associated molecular patterns (PAMPs) such as the toll-like receptor (TLR) ligands. The function of delivery systems is to effectively deliver the vaccine components to the target antigen-presenting cells (APCs) and thereby enhance the amount of antigen reaching the cells or tissue responsible for induction of immune responses. Delivery systems are often particulate in nature and mimic nature in terms of size and shape resulting in a delivery system with similar dimensions as a given pathogen, which is a natural target for APCs. The combination of delivery systems and immunopotentiators has great potential due to concomitant enhanced antigen delivery and potent stimulation of innate immunity [reviewed by (Reed et al. 2009, 2013)].

Thus adjuvants are a heterogeneous group of compounds that can have many different functions, i.e., depot or targeting functions and immunostimulatory or immunomodulatory functions (Guy 2007). Adjuvants utilize very different mechanisms in order to potentiate an immune response: (a) depot effect; (b) up-regulation of cytokines and chemokines; (c) cellular recruitment at the site of injection; (d) increased antigen uptake and presentation to APCs; (e) activation and maturation of

APCs and migration to the draining lymph nodes; and (f) activation of the inflammasome [reviewed by (Awate et al. 2013)]. Understanding of the adjuvant mechanism of action can be utilized to develop vaccines with a very specific and tailored effect. The mechanism behind adjuvanticity is however in many cases poorly understood since immune responses to vaccines involve a very complex cascade of events and the isolated effect of an adjuvant can be very difficult to dissect.

The antigen can be associated to a delivery system by surface adsorption or encapsulation, depending on the mode of preparation. In this sense, delivery systems provide the potential to control antigen kinetics and dynamics. This is done (a) by stabilizing as well as protecting the antigen from degradation; (b) by inhibiting/delaying clearance of the antigen from the injection site; (c) targeting and also carrying the antigen to the APCs; (d) prolonging the time of exposure of antigen to the immune cells; (e) enhancing the antigen uptake in the APCs; and (f) controlling the antigen release and intracellular trafficking (reviewed by Foged 2011; O'Hagan and De Gregorio 2009).

Immunopotentiators function via direct activation of the innate immune system by interacting with the APCs through pattern recognition receptors (PRRs) (O'Hagan and Valiante 2003). Examples of such immunopotentiators are ligands of innate immune receptors, the TLRs, NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and RIGI-like receptors (RLRs) [reviewed by (Reed et al. 2013; Foged 2011; Guy 2007)]. A wide variety of PAMPs are recognized through TLRs, examples thereof are lipopolysaccharide (LPS) and its derivatives which are recognized through TLR4, peptidoglycans from Gram-positive bacteria and lipopeptides are recognized through TLR2, RNA is recognized through TLR3, bacterial flagellin through TLR5, single-stranded RNA and imidazoquinolines signal through TLR7 and TLR8, and unmethylated CpG motifs in bacterial DNA are recognized through TLR9 (Gay and Gangloff 2007; Medzhitov 2001).

A growing body of preclinical and clinical data demonstrates that TLR agonists are potent vaccine adjuvants and provide the opportunity for tailoring and modulating the immune response against a vaccine by inducing distinct cytokine profiles (Duthie et al. 2011). Monophosphoryl lipid A (MPL) is the most studied TLR agonist for vaccination purposes. MPL is derived from LPS which is found in the cell wall of Gram-negative bacteria (Casella and Mitchell 2008). The adjuvant formulation AS04 from GlaxoSmithKline is based on MPL adsorbed to alum (Garçon 2010) and is approved for the hepatitis B vaccine Fendrix™ (Garçon et al. 2007) and the HPV vaccine Cervarix™ in combination with VLPs (Schwarz 2009; Romanowski et al. 2009). In addition new and synthetic TLR agonists are being developed and the availability of such immunopotentiators has expanded.

Hence rational development and formulation of adjuvant systems can result in a wide variety of ways to modulate the immune response in a desired direction.

The non-TLRs are not as well described as the TLRs and include intracellular innate receptors such as the RLRs, the soluble NLRs, and CLRs. The surface-expressed CLRs include the mannose receptor and DC-SIGN that are able to bind a wide range of viruses, bacteria, and fungi through recognition of sugar moieties (Guy 2007).

Adjuvant systems are defined as functional excipients and are in that sense components of a specific vaccine. Table 2.3 lists adjuvant delivery systems used in

vaccines approved for human use. The aluminum salts are described further in Chap. 3 of this book, the oil-in-water emulsions MF59 and AS03 are described in Chap. 4, and VLPs are discussed in Chap. 9.

In order to achieve the optimal immunological effect, an adjuvant appropriate for the formulation must be considered. The choice of formulation is in turn dependent upon the choice of antigenic components, the type of immune response that is needed, the optimal/desired route of administration, any potential adverse effects, and the stability of the vaccine. These factors must be considered in the early phases of development. Also the adjuvant must be chemically as well as physically stable in order to face the quality control criteria (see Chap. 19) which ensures reproducible manufacturing as well as activity (Reed et al. 2009).

The inclusion of adjuvants in vaccine formulations should be justified. Efficacy, safety, and tolerability are the most important factors for vaccine development. The use of adjuvants should therefore be considered in relation to the target population and should be selected based on a risk/benefit ratio. For example, a higher risk is more acceptable for cancer patients than for healthy children.

2.2.4 DNA Vaccines

DNA vaccines represent a new generation of vaccines that are attractive due to their simplicity in addition to several other advantages they have over conventional vaccines. The principle underlying DNA vaccination is to induce immunity by transiently transfecting host cells with plasmid DNA (pDNA) encoding antigen, as opposed to injecting antigen in the form of a peptide or protein. Upon DNA vaccination, host cells produce the protein (antigen) encoded by the DNA and immunity against this particular protein is subsequently induced (Bins et al. 2013; Senovilla et al. 2013). The great advantages associated with DNA vaccines are that they can be manufactured relatively easily at low costs, and both humoral and cellular immune responses can be elicited. In addition, pDNA is fairly stable at room temperature (Bins et al. 2013), which renders the normally required cold chain redundant for DNA vaccine storage. This is certainly of high importance for the effectiveness of vaccine programs in developing countries.

As yet no DNA vaccines have been approved for human use. Several clinical trials are being conducted at this point in time for different cancers and HIV-AIDS. Some DNA vaccines are approved/registered for veterinary use (Bins et al. 2013; Senovilla et al. 2013).

2.2.5 Dendritic Cell-Based Vaccines

Another type of vaccination strategy is based on dendritic cells (DCs). The function of these cells is to acquire, process and present antigens to T-cells, and provide the stimulatory signals and cytokines required to induce T-cell proliferation and differentiation into effector cells (Chap. 1). Therefore, a much-studied vaccination

Table 2.3 Adjuvant delivery systems used in vaccines approved for human use

Delivery system	Adjuvant name	Vaccine	Disease target	Company	Reference
Mineral salt	Aluminum hydroxide/phosphate	Various	Various, e.g., Diphtheria, tetanus, hepatitis		Clements and Griffiths (2002)
Oil-in-water emulsion	MF59	Fluad®	Influenza	Novartis	Schultze et al. (2008) and Podda (2001)
		Focetria®			Banzhoff et al. (2009) and Gasparini et al. (2010)
		Aflunov®			Galli et al. (2009a) and Galli et al. (2009b)
Oil-in-water emulsion	AS03	Arepanrix® Prepandrix® Pandemrix®	Influenza	GlaxoSmith Kline	Roman et al. (2010) Walker and Faust (2010)
Water-in-oil emulsion	Montanide	Cima Vax EGT™	Cancer	Bioven	Rodriguez et al. (2010)
Virosomes		Exapal®	Hepatitis A	Crucell	Bovier (2008)
		Inflexal V®	Influenza	Crucell	Gluck and Metcalfe (2002)
		Invivac™	Influenza	Solvay	de Bruijn et al. (2006)
VLPs adsorbed onto alum		Gardasil®	HPV	Merck	Schiller et al. (2008)
MPL adsorbed onto alum	AS04	Fendrix®	Hepatitis B	GlaxoSmith Kline	Garcon et al. (2007)
VPL + MPL adsorbed onto alum		Cervarix®	HPV	GlaxoSmith Kline	Schwarz (2009)

MPL monophosphoryl lipid A, *VLP* virus-like particle

strategy is to load *in vitro*-generated DCs with antigens and infuse them into a patient so as to elicit T-cell-mediated responses, particularly in the context of cancer where DC function *in vivo* is often blunted or subverted by factors released by the tumor (Chap. 13). While preclinical studies have repeatedly shown that DC-based vaccines can delay or prevent tumor progression, human clinical trials have been disappointing in comparison, offering only marginal benefit for patients. There is therefore still a need to improve the stimulatory capacity of the injected cells, and strategies for how to achieve this are discussed further in Chap. 13.

2.3 Pharmaceutical and Delivery Challenges for the Development of Subunit Vaccines

Research in the field of modern vaccinology is to a large extent conducted in the absence of knowledge of how the physicochemical properties of the subunit formulations impact the efficacy, safety, and mechanism of action (Mortellaro and Ricciardi-Castagnoli 2011). In order to move towards a more rational process regarding vaccine development it is of crucial importance to increase understanding of vaccine formulation, which is a great challenge since vaccines are often very complex systems (Reed et al. 2009). An in-depth understanding of the physicochemical properties and what effect production and biological processes impose on safety and efficacy is desirable during development of subunit vaccines, also from a stability and quality control point of view. Therefore, there are a substantial number of pharmaceutical challenges associated with the subunit vaccine development process. With these complex systems a tremendous amount of work on development, formulation, and characterization is needed. Also the regulatory challenges facing scientists who research and develop subunit vaccines are of great importance for the successful development of subunit vaccines. The pharmaceutical analysis and quality control of vaccines are described further in Chaps. 19–21 of this book.

A crucial aspect in addressing the challenges in vaccine development is vaccine delivery, which encompasses (a) administration of the vaccine formulation to specific sites of the body and (b) delivery of the antigen to, and activation of, relevant cells of the immune system. Administration of vaccine formulations to specific sites of the body can be achieved by various routes, and the most commonly used routes have been intramuscular (i.m.) and subcutaneous (s.c.) injection. During the past decades, much effort has been devoted to exploring the use of minimally invasive or noninvasive administration routes, such as nasal delivery, pulmonary delivery, transcutaneous delivery, oral delivery, and sublingual/buccal delivery. Such alternative routes of administration might allow for easier and more convenient administration, e.g., needle-free approaches, and might eventually result in increased vaccine coverage by increasing the willingness of the public to be vaccinated. In addition, the use of alternative administration routes might affect the quality of the immune response. One example is mucosal vaccination. Most pathogens access the body through the mucosal membranes. Therefore, effective vaccines that protect at these sites are much needed. However, despite early success with the live attenuated oral polio

vaccine, only a few new mucosal vaccines have been approved for human use. This is partly due to problems with developing safe and effective mucosal adjuvants.

Each of these immunization routes requires specially designed formulations (e.g., suspensions, emulsions, powders, tablets) and specially designed delivery devices (such as microneedles, nasal sprayers, and pulmonary inhalers). To license a product for vaccination applying alternative administration routes, the combination of formulation and device should be licensed as a whole. For this reason, formulation development and development of a suitable device should go hand in hand. In Chaps. 14–18, different administration routes are discussed together with formulations and devices used specifically for these routes.

Finally, the development of stable vaccine formulations is important to consider, in particular the development of thermostable vaccines that can be distributed independently of the expensive cold chain are highly in demand for the developing countries. Processes for drying of vaccines such as spray drying, spray freeze drying, and supercritical fluid technology are further described for pulmonary formulations in Chap. 16.

2.4 Conclusions

Prophylactic vaccination is the medical intervention with by far the largest impact on public health and has greatly reduced the incidences of bacterial and viral infections. Despite this the field of vaccinology faces a number of challenges, and there is still an unmet medical need for new vaccines due to the existence of a number of infectious diseases for which no effective vaccine is available (e.g., HIV-AIDS, malaria), or for which existing vaccines provide insufficient immunity (e.g., TB) or are unaffordable for those most in need (e.g., Pneumococcal disease). Conventional vaccines include the live, attenuated, or inactivated whole organism vaccines. Novel vaccine development strategies aim towards more safe, efficient, and stable vaccines in the future. New generation vaccines are usually of the subunit vaccine type, which are based on highly purified recombinant or synthetic antigens. A number of adjuvant technologies are used to enhance efficacy and there are efforts ongoing to explore the usage of noninvasive administration routes. This poses special demands in terms of formulation development and device technology for optimizing the delivery of antigens and immunopotentiators to the immune system.

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