Nanotechnology in Vaccine Delivery

45

Martin J. D'Souza, Suprita A. Tawde, Archana Akalkotkar, Lipika Chablani, Marissa D'Souza, and Maurizio Chiriva-Internati

Contents

Abstract

Immunotherapy has been a trusted therapy for centuries to eliminate infectious diseases. However, the successful immunotherapy depends on several factors such as nature of pathogen, vaccine delivery system, route of administration, and immune system of the host. With the advances in nanotechnology, immunotherapy is now targeting different challenging disorders including cancer as well as infectious diseases. Along with the evolution of several adjuvants to enhance immune response to vaccines, nanotechnology plays an important role by acting as self-adjuvant in form of particles.

45.1 Introduction

Advances in nanotechnology have led to innumerable ways for prevention or treatment of various diseases. Its impact on immunotherapy potentiates the vaccine delivery and efficacy.

Immunotherapy is a specialized way of eliminating diseases, where it prepares the immune system to combat the attack of foreign antigens (in case of infectious diseases) or self-antigens (in case of cancer). It has been proved very well for centuries that immunotherapy has been a cost-effective tool to prevent the disease.

With the evolution of different challenging diseases, there is an urgent need of vaccine development against them to save lives of millions all

M.J. D'Souza, PhD (✉) • A. Akalkotkar
M. D'Souza
Vaccine Nanotechnology Laboratory,
Department of Pharmaceutical Sciences,
College of Pharmacy and Health Sciences,
Mercer University, Atlanta, GA, USA
e-mail: dsouza_mj@mercer.edu

S.A. Tawde, PhD
Akorn Inc, Vernon Hills, IL 60031, USA

L. Chablani, PhD
Department of Pharmaceutical Sciences,
St. John Fisher College, Wegmans School of Pharmacy,
Rochester, NY 14618, USA

M. Chiriva-Internati, PhD
Texas Tech University Health Science Center,
School of Medicine, Lubbock, TX 79430, USA

M. Giese (ed.), *Molecular Vaccines Volume 2*,
DOI 10.1007/978-3-319-00978-0_19,

throughout the world. Moreover, in case of existing vaccines, there is still a need to address issues with respect to safety, effectiveness, ease of administration, time of preparation, and, most importantly, the cost.

Recent developments in immunology and molecular biology explore new vaccine materials and aim at triggering memory response to vaccines, which protect host's immune system against the disease attack for a longer period of time. Vaccine efficacy depends on its ability to induce memory T-cells and B-cells through Th1 and Th2 immune pathways, respectively. Conventional vaccine materials include whole foreign organism vaccine (live/attenuated/killed/lysate), cellular fragments of pathogens such as bacterial polysaccharides, and bacterial toxins [1]. On the other hand, development of recombinant technology and RT-PCR allows to obtain specific antigen expression or synthetic peptide on larger scale and to use as vaccines. DNA vaccines are recently developed type of immunotherapy which has shown encouraging results in some clinical trials [2–4].

There are two major approaches for vaccination: prophylactic or therapeutic. Prophylactic vaccines find their applications in the prevention of viral, bacterial, or parasitic infectious diseases such as influenza, HIV, tuberculosis, malaria, pneumonia, polio, and smallpox, which are caused by foreign antigens. However, in case of cancer which is caused by self-altered cells, vaccine formulation is a challenging task as it requires immune response against self-cell antigens without causing autoimmune response. There are very few prophylactic cancer vaccines available on market such as Gardasil® (Merck) and Cervarix® (GSK) vaccine for human papillomavirus infection causing cervical cancer. Prophylactic cancer vaccines can prevent the tumor development based on the use of overexpressed or mutated proteins, mutated oncogenic growth factor receptors, heat-shock proteins, or other tumor-associated antigens [5]. In case of therapeutic approach, vaccines are given in order to trigger immune response against existing residual tumor cells mostly in combination with surgery or chemotherapy and thus aiming at preventing or prolonging the relapse [6]. Currently, there is only one therapeutic cancer vaccine, Provenge® (Dendreon), approved recently by FDA for treatment of prostate cancer. On the other hand, studies are being carried out for melanoma and colorectal cancer [7]. Various other clinical trials have been reported utilizing DNA/dendritic cell (DC)/viral vector-based vaccines depicting the continuous growth in the field of cancer immunotherapy [8, 9].

Vaccine efficacy depends mainly on the immunogenicity of antigen. It can further be enhanced by the use of vaccine adjuvants which activate immune cells. Various adjuvants are being explored for their effectiveness to trigger humoral, cellular, and/or mucosal immunity against several antigens. Humoral immune response was found to be elicited mostly with the use of protein adjuvants. Cytotoxic T-cell responses were found to be triggered by ISCOMs, Montanide™, Montanide ISA720, ISA 51, and viral vectors. MF59 and MPL® (monophosphoryl lipid) were shown to enhance Th1 responses. Viruslike particles, nondegradable nanoparticles, and liposomes produced cellular immunity. Douglas et al. incorporated Montanide ISA720 as an adjuvant to obtain both T-cell and B-cell response equal or higher than the response obtained with viral or protein adjuvants alone against Plasmodium falciparum MSP1. In case of commercially available cancer vaccines, monophosphoryl lipid A (MPL) is being used in Cervarix® as a TLR-4-targeted adjuvant, while Gardasil® contains alum as an adjuvant. Compound AS04 (a combination of alum and monophosphoryl lipid A) has also been used in human vaccines against hepatitis B virus.

Adjuvants which are approved for human use include alum, compound AS04 (a combination of alum and monophosphoryl lipid A), AS03, and MF59. Among these, alum is used in many vaccines such as HAV, HBV, HPV, diphtheria, tetanus, Haemophilus influenzae type B, and pneumococcal conjugate vaccines. However, alum is a poor adjuvant for triggering Th1 response. A list of adjuvants tested in animal models includes bacterial toxins such as cholera toxin, heat-labile enterotoxin of E. coli, nontoxic B subunit of cholera toxin, Toll-like receptor

(TLR) 9 agonist, and cytosine phosphoguanosine (CpG) dinucleotides [10]. Montanide, PLG, flagellin, QS21, AS01, AS02, RC529, ISCOM, IC31, CpG, MF59 with MTP-PE, immunostimulatory sequences (ISS), and 1018 ISS are some of the adjuvants which are in clinical trials against various disorders such as malaria, cancer, flu, hepatitis B, hepatitis C, HIV, and TB [11]. Heffernan et al. found that the co-formulation of chitosan and IL-12 induced Th1, IgG2a, and IgG2b antibody immune response to a model protein vaccine. Denisov et al. evaluated various adjuvants (larifan, polyoxidonium, natrium thiosulphate, TNF-β, and Ribi adjuvant system) for their ability to enhance immune responses to the live brucellosis vaccine, Brucella abortus strain 82-PS (penicillin sensitive) in guinea pigs, and they found that the highest protection was offered by combining TNF-β or polyoxidonium with S82-PS. The recent findings by Chen et al. inferred that a compound 3′ 5′-cyclic diguanylic acid (c-di-GMP), which is a bacterial intracellular signaling molecule, can act as a vaccine adjuvant and has shown immunostimulatory properties. In a study by Skountzou et al., bacterial flagellins from

Escherichia coli and *Salmonella*, coadministered intranasally with inactivated A/PR/8/34 (PR8) virus, were found to be enhancing the efficacy of influenza vaccines in mice. Thus, they can be termed as good candidates as mucosal vaccine adjuvants to improve protection against influenza epidemics as well as other infectious diseases. On the other hand, cancer vaccine efficacy has also been enhanced by the use of cytokines as adjuvants such as interleukins, IL-2, IL-12, and GM-CSF [12–15].

Another way of enhancing vaccine efficacy is with the use of nanotechnology. Nanotechnology has been explored for its different applications in delivering small molecules, proteins, and peptides. Recently, vaccine delivery has been achieved through various pharmaceutical approaches to establish enhanced efficacy and ease of delivery and to address the issues related to stability. Vaccine material has been formulated into nanocarriers such as liposomes, polymeric nanoparticles, ISCOMs, dendrimers, micelles, VLPs, and carbon nanotubes.

45.2 Need for Particulate Vaccines

Currently, there are no particulate vaccines available in the market, but extensive research is going on in this field that would eventually bring particulate vaccine approach from bench to clinical interphase. Nanovaccine against notorious diseases is an attractive option as it can elicit both humoral and cellular immunity [16]. Nanotechnology has also proven to offer mucosal immunity which can be targeted for infectious diseases caused by mucosal entry of pathogen [17]. These nanovectors bear the advantage of being similar to a pathogen in terms of size; thus, they are efficiently recognized by antigen-presenting cells (APCs) of skilled immune system [18]. Further, they will be drained into the nearby lymph nodes where they can activate the immune cells of the body. These immune cells are drained towards the epithelial gatekeeper cells receiving various chemokine signals [19].

In contrast to natural infections, vaccines alone are incapable of producing a high antibody response [20]. The approach of using nanoparticles as vaccines which can incorporate multiple antigens in a single entity will lead to an enhanced humoral response as well as provide cellular immunity [16, 21–24]. Uddin, Lai, and Yeboah et al. have successfully formulated and tested oral vaccines using the particulate vaccine delivery system for typhoid, melanoma, and tuberculosis, respectively [25–27]. In all these studies, significantly higher mucosal and serum antibody titers (IgA and IgG) were obtained for orally administered particulate vaccine than those observed for the oral solution vaccine. The duration of antigen presentation also plays an important role to enhance the immune response [28]. The release of antigen must be in a pulsatile fashion to decrease the number of booster doses required. The persistence of antigens can be obtained only if the particles are remaining intact and are protected from degradation in the harsh acidic gastric conditions.

Also, it is possible to modify the outer surface of the nanoparticles to increase its uptake by the APCs. It can be conjugated with either an immunostimulatory or targeting moiety; else the

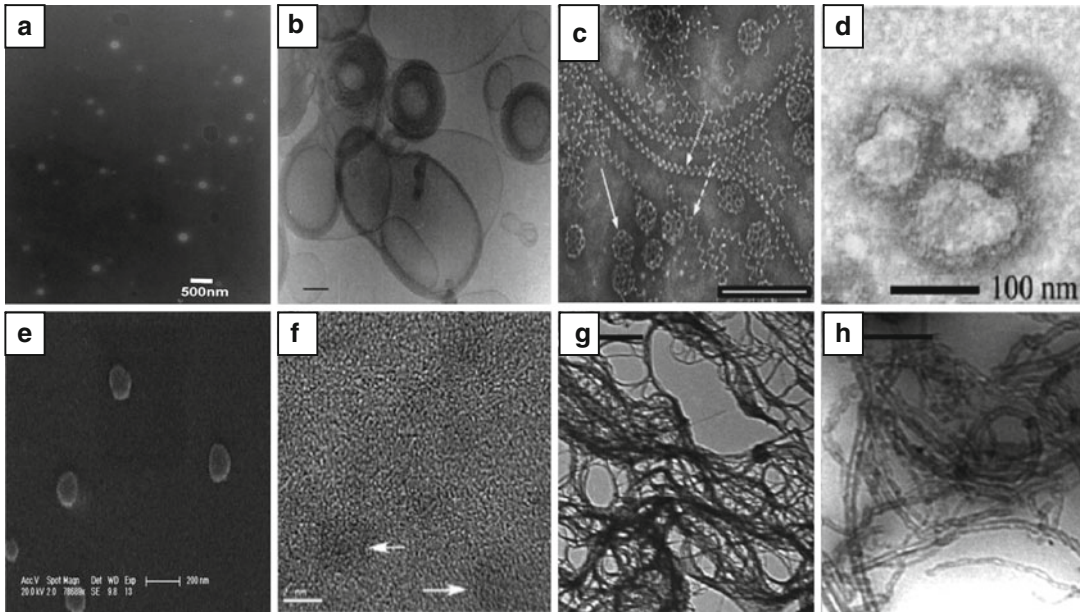

Fig. 45.1 Different nanocarriers for vaccine delivery: (**a**) TEM image of PEG-PLGA nanoparticle (scale bar corresponds to 500 nm) (Reproduced from Bharali et al. [34] with permission from Elsevier). (**b**) Cryo-EM image of cationic liposomes entrapping DNA (scale bar corresponds to 200 nm) (Reproduced from Perrie et al. [35] with permission from Elsevier). (**c**) TEM image of ISCOMs of different types such as typical cage-like (*solid arrow*), helices (*dashed arrow*), and double helices (*dotted arrows*) (scale bar corresponds to 200 nm) (Reproduced from Sun et al. [36] with permission from Elsevier). (**d**) EM image of influenza H1N1 viruslike particles (scale corresponds to 100 nm) (Reproduced from Quan et al. [37], open-access article). (**e**) SEM image of PEG-PEI-PBLG copolymeric micelles (scale corresponds to 200 nm) (Reproduced from Tian et al. [38] with permission from Elsevier). (**f**) TEM image of PAMAM dendrimer (Reproduced from Jackson et al. [39] with permission from ACS). (G, H) TEM images of single-walled (**g**) and multi-walled (**h**) carbon nanotubes (scale bar corresponds to 1 **μ**m and 250 nm, respectively) (Reproduced from Klumpp et al. [40] with permission from Elsevier)

inherent property of the surface itself can be modified. Surface charge also plays a vital role in uptake of the particles and affects the levels of immune response. It is shown that cationic particles are promising for uptake into macrophages and dendritic cells (DCs) [29].

Another useful property of nanoparticles is incorporation of various immunopotentiators to enhance the immune response to a further extent. This also includes targeting ligands, which can help to minimize the adverse effects of the vaccines. Some of the examples of these targeting ligands include aleuria aurantia lectin (AAL), ulex europaeus agglutinin 1 lectin (UEA-I), and wheat germ agglutinin (WGA) which act as targeting ligand to the M-cells present in Peyer's patches. This will eventually help to increase the uptake of particles through small intestine and bypass oral tolerance [30, 31]. Various co-stimulatory molecules like interleukins or cytokines can be included in the formulation to increase the immune response. It has been shown that DCs have receptors for both IL-2 and IL-12; hence, they have the capacity to present exogenous antigens and activate both MHC class I (cross-presentation) and MHC class II pathways by vaccination [32, 33].

This review aims to discuss the role of these nanocarriers as potential vaccine delivery vehicles as shown in Fig. 45.1. A brief description of each one is as follows:

45.3 Polymeric Nanoparticles

Polymeric nanoparticles (as shown in Fig. 45.1a) as vaccine delivery vehicles have been explored widely as they can act as adjuvants themselves.

Polymeric nanoparticles can offer the protection to proteins and peptides against gastric degradation upon oral administration and therefore vaccines are definitely one of the major applications for such particles [41]. The particles of size less than 1 μm offer adaptive immunity by facilitating their targeted uptake and extended presentation by APCs [42]. Nevertheless, the immune response also depends on rate of dissolution, surface morphology, charge, and size [43].

Oral administration is the most preferred route of administration as it is more patient compliant. Intestinal uptake of these particles is the key factor for determining the efficiency of oral vaccines. The usage of nanoparticles versus the use of microparticles as vaccine carriers for oral delivery is always debatable. There are conflicting reports as to which size can be considered as the optimum size range for eliciting a stronger and lasting immune response [44].

In this study by Desai et al., it was shown that particles of 100 nm showed increased uptake across the intestine in a rat model when compared to particles of 500, 1, and 10 μm size. These particles were prepared of polylactic/polyglycolic acid copolymer (50:50). Conventional nanoparticles are susceptible for entrapment in mucus due to steric as well as adhesive interactions. These interactions can be overwhelmed by tailoring the size of nanoparticles, which allows the particles to diffuse through mucus [45]. Here, Primard et al. reported that nanoparticles of size greater than 300 nm are less effective to move across the mucus lining of the intestine, when given orally. Therefore, particles in size range 200–250 nm were found to be taken up in M-cells of Peyer's patch of small intestine.

In contrast, a study conducted by Gutierro et al. showed that 1,000 nm particles of bovine serum albumin as a model protein incorporated in PLGA elicited higher IgG response when compared to 200 and 500 nm particles, and the immune response induced by 200 and 500 nm particles was comparable to each other by oral and subcutaneous route of administration [46].

However, in a contradictory study by Wendorf et al., poly(lactide-co-glycolide) nanoparticles of size 110 and 800–900 nm were compared for their efficacy and were found to be offering comparable immune response [47]. In a study by van den Berg J. et al., cationic nanoparticles containing DNA vaccines were evaluated via dermal route. It was seen that these cationic nanoparticles blocked vaccination-induced antigen expression in mice and ex vivo human skin due to immobilization of the nanoparticles in extracelvlular matrix caused by electrostatic interactions. Therefore, shielding the surface charge of the nanoparticles by PEGylation improved in vivo antigen expression [48]. Polylactic acid is one of the widely used biodegradable polymers in vaccine delivery. However, the use is restricted due to hydrophobic nature and generation of acidic microenvironment upon its degradation, rendering it unfavorable to the encapsulated antigen. In a study by Jain et al., PEG-derivatized block copolymers of PLA were used for development of nanoparticles encapsulating HBsAg for mucosal vaccination against hepatitis B. These polymers were found to produce better sIgA mucosal immune response [49], while in case of cancer, T-cell immune response can also be altered with the use of nanoparticles [50]. Several other polymers have been tried to formulate vaccine nanoparticles as listed in Table 45.1. Interesting uptake study performed by Primard et al. showed the poly(lactic acid) nanoparticles traversed from intestinal mucosa to Peyer's patch and then interacted with underlying B-cells and dendritic cells upon oral administration [45]. Due to all these advantages of polymeric nanoparticles, they remain a potential vaccine delivery system.

45.4 Liposomes

Although there are various nanocarriers available for vaccine delivery, liposomes play a prominent role as drug and potential vaccine delivery vehicles. Liposomes were introduced by Bangham et al. in 1960s [51], almost a decade later Allison et al. elicited their role as an immunological adjuvant [52], and since then, many studies have been done to exploit this approach. These are nanostructures (as shown in Fig. 45.1b) composed of phospholipids having a capacity to encapsulate both hydrophilic and hydrophobic

Table 45.1 Summary of current polymeric nanoparticles under research for various vaccines tested using animal models

Polymer used	Size (nm)	Charge (mV)	Vaccine preparation	Immune response	Route	Method of preparation	Animal model	Reference
Methoxypolyethylene glycol–poly(lactide-co-glycolide)	150–200	NA	Recombinant hepatitis B surface antigen (HBsAg)	Anti-HBs antibodies	IP	Double emulsion/ solvent evaporation	BALB/cNCr mice	[34]
An inner hard core of poly(methyl-methacrylate) (PMMA) and hydrophilic tentacular shell and poly(ethylene-glycol) chains	960 ± 38	32.2 ± 0.6	HIV-1 Tat	Humoral, cellular responses, Th1-type T cell responses and CTLs	IM	Emulsion polymerization	BALB/c (H2kd) mice	[53]
Poly(γ-glutamic acid)	200	NA	gp120 of HIV-1	Efficiently taken up by dendritic cells, CD8+ T cell responses, not effective for protection After the challenge inoculation with SHIV	IN, SC	Solvent evaporation	Rhesus macaques	[54]
Inner core of poly(methyl-methacrylate) (PMMA) and hydrophilic outer shell of Eudragit L100-55	NA	NA	HIV-1 Tat	Long-lasting cellular and humoral responses	IM, SC, IN	Emulsion polymerization	BALB/c mice	[55]
Polypropylene sulfide	50	NA	Ovalbumin as a model antigen	Cytotoxic T lymphocytic responses in lung and spleen tissues, as well as humoral response in mucosal airways	IN	NA	C57BL/6 mice	[56]
Polypropylene sulfide	NA	NA	Ovalbumin as a model antigen	Cytotoxic and helper T cell responses	ID	Emulsion polymerization	C57Bl/6 mice	[57]
N-trimethyl chitosan and poly(lactic-co-glycolic acid)	500	24.5 ± 0.90	Ovalbumin as a model antigen	Humoral immune response, mucosal response in case of intranasal administration	IM, IN	Emulsification/ solvent extraction	Balb/c mice.	[58]
Polysaccharide chitosan	160–200	6–10	Recombinant hepatitis B surface antigen (rHBsAg)	Anti-HBsAg IgG levels	IM	Mild ionic gelation technique	BALB/cmice	[59]

IM intramuscular, *SC* subcutaneous, *IP* intraperitoneal, *ID* intradermal, *IN* intranasal, *NA* not available

drugs as well as vaccine antigens of various origins. They not only act as carriers to protect these bioactive moieties but also possess immunogenic properties, thus acting as a potential adjuvant [35, 60–62]. Conventional liposomes have been unsuccessful as vaccine particles due to their rapid clearance from the body because of their uptake by reticuloendothelial system [63], although, with the advent of stealth/PEGylated liposomes, increased half-life of these circulating nanocarriers has been achieved [64]. Doxil®, a PEGylated liposome of doxorubicin, is a marketed product utilizing this application and is used for the treatment of cancers. Other liposomal marketed formulations include Ambisome® (Gilead), Myocet® (Elan), and Depocyt® (SkyePharma).

To enhance the immunogenicity of these carriers, various other approaches have been employed. Mohammed et al. describe the use of cationic liposomes leading to improved stability and sustained immunological effects against Mycobacterium tuberculosis [65]. Further the use of adjuvants incorporated in the liposome has been explored to provide immune-stimulant effect; recently, the efficacy of monophosphoryl lipid A integrated dimethyldioctadecylammonium (DDA) and trehalose 6,6′-dibehenate (TDB) liposomes has been shown to induce cellular immunity along with the humoral response [66, 67]. Altin et al. further review the use of liposomes and plasma membrane vesicles (PMV) as a carrier for targeted delivery of antigens [63]. There are various other forms of liposomes which have been found to be promising as antigen carriers such as virosomes, archaesomes, and proteosomes [68–70].

Apart from imparting immunomodulatory properties, the physical properties of these nanocarriers are also important to act as a potent vaccine delivery vehicle. Xiang et al. discuss the role of size in development of particulate vaccines and describe various particle size range and their respective uptake mechanism; this can be useful as smaller liposomes mimic the uptake mechanisms of viruses whereas larger liposomes can follow a pathway as used by the bacteria [43]. As discussed previously, surface charge of these species also dictates their efficacy as a particulate vaccine; for example, cationic liposomes have shown better efficacy than others [71]. Such modifications in physical properties, use of immunoadjuvants, and stealth properties of these carriers potentiate their use as a particulate vaccine [72]. Considering the success of liposomal products in the market, it is promising to have a liposomal vaccine soon.

45.5 Immunostimulatory Complexes (ISCOMs)

ISCOMs (immunostimulatory complexes), as shown in Fig. 45.1c, are particulate vaccine nanocarriers of 40 nm size which are made up of cholesterol, phospholipid, and saponin along with antigen/s. However, ISCOMATRIX™ is now available without antigen and having the same composition as ISCOMs. This matrix provides incorporation of antigen which can be used as ISCOMATRIX™ vaccine with similar immunostimulatory activity as seen with ISCOMs. The immunostimulatory property is imparted to these complexes due to Quil A which is a purified less toxic extract from Quillaja saponin. These complexes have been reported to produce immune responses against variety of antigens such as viral, bacterial, parasitic, or tumor antigens [73, 74].

Some researchers have tried to enhance the immunostimulatory properties of these complexes by varying or replacing some of the components such as phospholipids or Quil A [36]. Several ISCOM™ and ISCOMATRIX™ vaccines have shown to induce humoral and cellular response in animal models (as shown in Table 45.2). These systems can access both the MHC I and MHC II pathways and act as a potent immunomodulator of both the innate and adaptive immune systems. Intranasal delivery of influenza ISCOMATRIX™ vaccine in humans has shown to induce systemic and mucosal responses, and therefore the ISCOMATRIX™ adjuvant can be used as a mucosal adjuvant [75]. Antigen-specific CTL, T-helper cells, and antibodies can be induced by ISCOM and ISCOMATRIX™

Table 45.2 Summary of various Immunostimulatory Complexes (ISCOMs) based vaccines under research using animal models

Vaccine delivery system	Vaccine preparation	Immune response	Route of administration	Animal model	Reference
ISCOM	Influenza viruses, H3N2	Humoral and cellular immunity	NA	*Cynomolgus Macaques*	[76]
ISCOM	Used as a adjuvant for human norovirus GII.4 HS66 strain vaccine	Th2 biased responses with significantly elevated IgM, IgA and IgG antibody-secreting cells	Oral/IN	Gnotobiotic pigs	[77]
ISCOM	Avian influenza A viruses of the H5N1 subtype	Strong antibody responses	IM	Roosters	[78]
ISCOM	A/PR8/34 Influenza virus	Strong mucosal as well as systemic antibody and cytotoxic T-lymphocyte responses	IN	BALB/c mice	[79]
ISCOM	Virosomal influenza A H5N1	Th1 CD4+ cells and strong antibody responses	IM	BALB/c mice	[80]
ISCOMATRIX™	MEM influenza antigen	Mucosal and serum antibody response	IN	BALB/c mice	[81]
ISCOMATRIX™ and ISCOM™	*H. pylori*	Reduction in *H. pylori* colonization	IN/SC	mice	[82]
ISCOM	Recombinant NcSRS2, of the intracellular protozoan parasite *Neospora caninum*	*N. caninum* specific antibodies and cellular response	SC	BALB/c mice	[83]

IM intramuscular, *SC* subcutaneous, *IN* intranasal, *NA* not available

vaccines for cancer and infectious diseases [36]. Another modification of ISCOM with regard to charge resulted in cationic ISCOM derivatives (PLUSCOMs), which offered high anionic antigen loading and therefore enhanced T-cell response in comparison to classic anionic ISCOMs against a model protein antigen (ovalbumin) [84]. Moreover, these complexes can reduce the dose of antigen required to induce immune response [85]. Table 45.2 lists different ISCOMs and ISCOMATRIX™ which have been studied in vivo against various infections.

45.6 Virus-like Particles

Along with a range of nanocarriers available for the vaccine delivery, viruslike particles (VLPs), as shown in Fig. 45.1d, are one of the most potent ones [86]. As the name indicates, these are particles resembling size range of a virus from 22 to 150 nm and contain self-assembled envelopes/proteins of various viruses. As they lack the genetic material, they are regarded noninfectious. Noad et al. detail that for more than 30 different infectious viruses, VLPs have been produced, eliciting the need of this approach [87]. Due to various advantages of this delivery system, currently there are VLP-based vaccines commercially available against two diseases—HBV and HPV [88, 89]. Also, various clinical trials are in progress utilizing this particulate delivery system. Recently, Buonaguro et al. discussed the role of VLPs as particulate vaccines, their contribution to current vaccines and clinical trials, and also the immune response elicited by these particles [90]. Also, a detailed review by Grgacic et al. describes the role of VLPs as vaccine particles to elicit immune response [91]. Structurally, VLPs can be defined as enveloped or non-enveloped depending

upon the presence or absence of their lipid envelope, surrounding the capsid protein.

VLPs of human papillomaviruses (HPV) are a good example of single-capsid non-enveloped VLPs consisting of L1 as major capsid protein. These VLPs can be produced in yeast (Gardasil) as well as in insect cells infected with baculovirus (Cervarix). Schiller et al. review the clinical trials performed using these HPV-VLPs and describe the efficacy of these systems against HPV [92]. The review also emphasizes that there are limited safety issues related to the vaccine as seen during the clinical trials.

On the other hand, various enveloped VLPs are available against influenza A, hepatitis B, hepatitis C, and several retroviruses. Recently, Kang et al. showed the possibility of influenza A vaccination through transdermal route using VLP-coated microneedle, thus enhancing the compliance towards these nanocarriers [93]. Considering the wide applications of these VLPs and their success as a commercial particulate vaccine, they continue to remain potential nanocarriers for future vaccines [37, 94–99].

45.7 Polymeric Micelles

Polymeric micelles (as shown in Fig. 45.1e) are a well-organized nano-sized assembly of synthetic polymers. These fall in the category of association colloids that are formed spontaneously when the amphiphilic molecules or hydrophilic regions are maintained at an appropriate concentration and temperature [38, 100]. They are not held together by covalent bonds and hence can be dissociated easily. This property of micelles can be exploited as per their applications [17]. They have shown high stability in vitro as well as in vivo [101]. Physical and chemical properties of polymeric micelles can be manipulated by selection of suitable hydrophilic and hydrophobic polymers [102]. In a study by Morein et al., a 30S protein subunit micellar vaccine induced a detectable antibody titer as well as protective immunity in a challenge study against pneumonia caused by the PI-3 virus [103]. Prabakaran et al. performed similar studies where they used soya phosphatidylcholine micelles against H5N1 infection [104]. Higher levels of serum IgG, mucosal IgA, and HI titers were observed when compared to the free antigen. Hence, micelles can serve as a promising carrier for vaccine antigens.

45.8 Dendrimers

Dendrimers (as shown in Fig. 45.1f) are highly branched, monodispersed polymeric nanoparticles. Dendrimers are composed of three different components: an initiator core, branches, and terminal functional groups. The initiator core is the main component of dendrimers and the branches extend in the outer directions. The terminal groups can be modified based on charge/hydrophilic/lipophilic properties [105]. They are similar to polymeric micelles but are linked covalently unlike micelles and thus have more stronger bonds and do not tend to dissociate easily [106]. The external surface can be easily modified and alterations of the internal cavity make dendrimers a promising carrier for various biomedical and industrial applications [107]. Recent work by Baker et al. involves coupling of various functional molecules including sensing units, MRI contrast agents, triggering devices, and targeting molecules to the surface of a generation 5 dendritic polymer (MW 25,000 Da, diameter 5 nm) [108]. A specific class of dendrimers called as multiple antigenic peptide (MAP) systems has been used widely for the vaccine purposes. MAP-based malaria vaccine has been tested in phase I clinical trials [109–111]. Having the potential to enter the clinical trials, these delivery systems are expected to be available on market shortly. Table 45.3 lists some of the dendrimeric systems currently under research.

45.9 Carbon Nanotubes

Recently, inorganic nanomaterials such as nanocrystals, nanowires, and nanotubes have been receiving an increasing amount of attention for vaccine delivery. Carbon nanotubes (as shown in

Table 45.3 Summary of various dendrimer based vaccines under research in animal models

Polymer used	Size (nm)	Charge (mV)	Vaccine preparation	Immune response	Route of administration	Method of preparation	Animal model	Reference
Polyetherimine (PETIM)	NA	NA	PETIM-pDNA complexes against rabies	virus neutralizing antibody responses	IM	NA	Swiss albino mice	[112]
Poly(propylene-imine)	NA	21.3 ± 0.33	Plasmid DNA encoding pRc/CMV-HBs[S]	Total IgG and its subclasses – IgG1, IgG2a, IgG2b	IM	Complexation	Female Balb/c mice	[113]
Polyacrylate core with a minimal B-cell epitope J14	20	−16	J 14 B-cell epitope	IgG subclass Ab response – IgG1, IgG2b, and IgG3	SC	Dialysis followed by "click" reaction	Murine model	[114]

IM intramuscular, *SC* subcutaneous, *NA* not available

Fig. 45.1g–h) are explored as a vehicle for vaccines because of their capacity to link to an antigen while maintaining their conformation and thus inducing antigen-specific antibody response. They can also be modified in a non-immunogenic material [115]. Functionalized carbon nanotubes can be used as nanovectors for the delivery of antigen/s by forming covalent bonds or supramolecular assemblies based on non-covalent interactions [40]. Though carbon nanotubes remain an area of interest for current researchers, still extensive work is required before they can enter the clinical trials.

45.10 Challenges and Future Directions

With a wide range of nanocarriers available for vaccine delivery, nanotechnology not only gets the well-deserved limelight but also attracts attention of regulatory bodies and bears certain challenges that need to be considered before marketing these nanocarriers.

45.10.1 Advantages and Disadvantages of Nanoparticles as Vaccines

Nanovaccines have its own pros and cons as a delivery system. They are made up of biodegradable polymers and hence are considered safe for administration. Nanoparticulate vaccine can be administered easily by different routes such as parenteral, oral, transdermal, nasal, and even pulmonary route. Thus, being noninvasive, delivery systems other than parenteral allow pain-free delivery of vaccines over conventional vaccines [116]. They can trigger the immune system efficiently as described earlier. Moreover, release of antigen at a controlled rate and time in a desirable fashion can be achieved by nanoparticles [117].

The cost of production and storage of these vaccines is a basic concern. But the reproducibility of nanovaccines is a greater question [70]. On the other hand, nanoparticles of size larger than 300 nm are reported to be less efficient to traverse across the mucosal lining of intestine and hence result in lower particle uptake through Peyer's patches in the intestine and lesser immune response for vaccine particles [45]. Thus, size and charge of the nanoparticles play a critical role in determining the efficacy of vaccine formulation. Therefore, the reproducibility of vaccines during manufacturing should be ensured, which needs critical evaluation of the particles. Another issue is to address the sterilization performed by nonthermal methods needs to be taken care of [118]. Also, small nanoparticles are cleared rapidly from the body, whereas the larger aggregates might get accumulated in the organs and cause toxicity issues.

The "nano" size which makes these carriers so promising is also the reason behind the concerns of these delivery systems. Researchers propose that the smaller the carrier, the better it functions and remains protected by the body's RES system; also ways have been devised to impart stealth properties to these carriers to avoid their uptake by such phagocytic cells. Although all these properties make the nanocarrier a potential delivery system, it also makes it harder to be cleared from the body, thus adding to "nanotoxicity." Comparatively extensive studies have been done to determine the toxicity profile of nano-sized molecules than nanocarriers. Little is known about the toxic effects of such nanocarriers which have been used for vaccine delivery. Even though the use of these carriers remains questionable, various researches are being done to answer these concerns and regulatory authorities remain to be a part of these hassles.

Declaration of Interest As indicated in the affiliations, Suprita A. Tawde, Archana Akalkotkar, and Lipika Chablani were graduate students and Marissa D'Souza was a summer research student working under Prof. Dr. Martin J. D'Souza in the Vaccine Nanotechnology Laboratory, Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, Georgia.

References

1. Heffernan, M.J., Zaharoff, D.A., Fallon, J.K., Schlom, J., Greiner, J.W.: In vivo efficacy of a chitosan/IL-12 adjuvant system for protein-based vaccines. Biomaterials **32**, 926–932 (2011)
2. Guimaraes-Walker, A., et al.: Lessons from IAVI-006, a phase I clinical trial to evaluate the safety and immunogenicity of the pTHr.HIVA DNA and MVA. HIVA vaccines in a prime-boost strategy to induce HIV-1 specific T-cell responses in healthy volunteers. Vaccine **26**, 6671–6677 (2008)
3. Beckett, C.G., et al.: Evaluation of a prototype dengue-1 DNA vaccine in a Phase 1 clinical trial. Vaccine **29**(5), 960–968 (2011)
4. Martin, J.E., et al.: A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. Vaccine **26**, 6338–6343 (2008)
5. Finn, O.J., Forni, G.: Prophylactic cancer vaccines. Curr. Opin. Immunol. **14**, 172–177 (2002)
6. Arlen, P.M., Madan, R.A., Hodge, J.W., Schlom, J., Gulley, J.L.: Combining vaccines with conventional therapies for cancer. Update. Cancer. Ther. **2**, 33–39 (2007)
7. Tartaglia, J., et al.: Therapeutic vaccines against melanoma and colorectal cancer. Vaccine **19**, 2571–2575 (2001)
8. Anderson, R.J., Schneider, J.: Plasmid DNA and viral vector-based vaccines for the treatment of cancer. Vaccine **25**(Suppl 2), B24–B34 (2007)
9. Sheng, W.Y., Huang, L.: Cancer immunotherapy and nanomedicine. Pharm. Res. **28**(2), 200–214 (2011)
10. Harandi, A.M., Medaglini, D., Shattock, R.J.: Vaccine adjuvants: a priority for vaccine research. Vaccine **28**, 2363–2366 (2010)
11. Mbow, M.L., De Gregorio, E., Valiante, N.M., Rappuoli, R.: New adjuvants for human vaccines. Curr. Opin. Immunol. **22**, 411–416 (2010)
12. Nguyen, C.L., et al.: Mechanisms of enhanced antigen-specific T cell response following vaccination with a novel peptide-based cancer vaccine and systemic interleukin-2 (IL-2). Vaccine **21**, 2318–2328 (2003)
13. Toubaji, A., et al.: The combination of GM-CSF and IL-2 as local adjuvant shows synergy in enhancing peptide vaccines and provides long term tumor protection. Vaccine **25**, 5882–5891 (2007)
14. Yin, W., et al.: A novel therapeutic vaccine of GM-CSF/TNFalpha surface-modified RM-1 cells against the orthotopic prostatic cancer. Vaccine **28**, 4937–4944 (2010)
15. Germann, T., Rude, E., Schmitt, E.: The influence of IL12 on the development of Th1 and Th2 cells and its adjuvant effect for humoral immune responses. Res. Immunol. **146**, 481–486 (1995)
16. Fifis, T., et al.: Size-dependent immunogenicity: therapeutic and protective properties of nano-vaccines against tumors. J. Immunol. **173**, 3148–3154 (2004)
17. Chadwick, S., Kriegel, C., Amiji, M.: Nanotechnology solutions for mucosal immunization. Adv. Drug Deliv. Rev. **62**, 394–407 (2010)
18. Randolph, G.J., Inaba, K., Robbiani, D.F., Steinman, R.M., Muller, W.A.: Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. Immunity **11**, 753–761 (1999)
19. Malik, B., Goyal, A.K., Mangal, S., Zakir, F., Vyas, S.P.: Implication of gut immunology in the design of oral vaccines. Curr. Mol. Med. **10**, 47–70 (2010)
20. Ada, G.: Vaccines and vaccination. N. Engl. J. Med. **345**, 1042–1053 (2001)
21. Fehr, T., Skrastina, D., Pumpens, P., Zinkernagel, R.M.: T cell-independent type I antibody response against B cell epitopes expressed repetitively on recombinant virus particles. Proc. Natl. Acad. Sci. U.S.A. **95**, 9477–9481 (1998)
22. Bachmann, M.F., et al.: The influence of antigen organization on B cell responsiveness. Science **262**, 1448–1451 (1993)
23. Bachmann, M.F., Zinkernagel, R.M.: Neutralizing antiviral B cell responses. Annu. Rev. Immunol. **15**, 235–270 (1997)
24. O'Hagan, D.T., Singh, M., Ulmer, J.B.: Microparticle-based technologies for vaccines. Methods **40**, 10–19 (2006)
25. Uddin, A.N., Bejugam, N.K., Gayakwad, S.G., Akther, P., D'Souza, M.J.: Oral delivery of gastro-resistant microencapsulated typhoid vaccine. J. Drug Target. **17**, 553–560 (2009)
26. Yeboah, K.G., D'Souza, M.J.: Evaluation of albumin microspheres as oral delivery system for Mycobacterium tuberculosis vaccines. J. Microencapsul. **26**, 166–179 (2009)
27. Lai, Y.H., D'Souza, M.J.: Formulation and evaluation of an oral melanoma vaccine. J. Microencapsul. **24**, 235–252 (2007)
28. Storni, T., Ruedl, C., Renner, W.A., Bachmann, M.F.: Innate immunity together with duration of antigen persistence regulate effector T cell induction. J. Immunol. **171**, 795–801 (2003)
29. Thiele, L., Merkle, H.P., Walter, E.: Phagocytosis and phagosomal fate of surface-modified microparticles in dendritic cells and macrophages. Pharm. Res. **20**, 221–228 (2003)
30. Akande, J., et al.: Targeted delivery of antigens to the gut-associated lymphoid tissues: 2. Ex vivo evaluation of lectin-labelled albumin microspheres for targeted delivery of antigens to the M-cells of the Peyer's patches. J. Microencapsul. **27**, 325–336 (2010)
31. Lai, Y.H., D'Souza, M.J.: Microparticle transport in the human intestinal M cell model. J. Drug Target. **16**, 36–42 (2008)
32. Pulendran, B., Banchereau, J., Maraskovsky, E., Maliszewski, C.: Modulating the immune response with dendritic cells and their growth factors. Trends Immunol. **22**, 41–47 (2001)
33. Banchereau, J., Steinman, R.M.: Dendritic cells and the control of immunity. Nature **392**, 245–252 (1998)

34. Bharali, D.J., Pradhan, V., Elkin, G., Qi, W., Hutson, A., Mousa, S.A., Thanavala, Y.: Novel nanoparticles for the delivery of recombinant hepatitis B vaccine. Nanomedicine **4**, 311–317 (2008)
35. Perrie, Y., Mohammed, A.R., Kirby, D.J., McNeil, S.E., Bramwell, V.W.: Vaccine adjuvant systems: enhancing the efficacy of sub-unit protein antigens. Int. J. Pharm. **364**, 272–280 (2008)
36. Sun, H.X., Xie, Y., Ye, Y.P.: ISCOMs and ISCOMATRIX. Vaccine **27**, 4388–4401 (2009)
37. Quan, F.S., Vunnava, A., Compans, R.W., Kang, S.M.: Virus-like particle vaccine protects against 2009 H1N1 pandemic influenza virus in mice. PLoS One **5**, e9161 (2010)
38. Tian, H.Y., et al.: Biodegradable cationic PEG-PEI-PBLG hyperbranched block copolymer: synthesis and micelle characterization. Biomaterials **26**, 4209–4217 (2005)
39. Jackson, C.L., et al.: Visualization of dendrimer molecules by transmission electron microscopy (TEM): staining methods and cryo-TEM of vitrified solutions. Macromolecules **31**, 6259–6265 (1998)
40. Klumpp, C., Kostarelos, K., Prato, M., Bianco, A.: Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. Biochim. Biophys. Acta **1758**, 404–412 (2006)
41. des Rieux, A., Fievez, V., Garinot, M., Schneider, Y.J., Preat, V.: Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J. Control. Release **116**, 1–27 (2006)
42. Rice-Ficht, A.C., Arenas-Gamboa, A.M., Kahl-McDonagh, M.M., Ficht, T.A.: Polymeric particles in vaccine delivery. Curr. Opin. Microbiol. **13**, 106–112 (2010)
43. Xiang, S.D., et al.: Pathogen recognition and development of particulate vaccines: does size matter? Methods **40**, 1–9 (2006)
44. Desai, M.P., Labhasetwar, V., Amidon, G.L., Levy, R.J.: Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm. Res. **13**, 1838–1845 (1996)
45. Primard, C., et al.: Traffic of poly(lactic acid) nanoparticulate vaccine vehicle from intestinal mucus to sub-epithelial immune competent cells. Biomaterials **31**, 6060–6068 (2010)
46. Gutierro, I., Hernandez, R.M., Igartua, M., Gascon, A.R., Pedraz, J.L.: Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. Vaccine **21**, 67–77 (2002). S0264410X02004358 [pii]
47. Wendorf, J., et al.: A comparison of anionic nanoparticles and microparticles as vaccine delivery systems. Hum. Vaccin. **4**, 44–49 (2008)
48. van den Berg, J.H., et al.: Shielding the cationic charge of nanoparticle-formulated dermal DNA vaccines is essential for antigen expression and immunogenicity. J. Control. Release **141**, 234–240 (2010)
49. Jain, A.K., et al.: Synthesis, characterization and evaluation of novel triblock copolymer based nanoparticles for vaccine delivery against hepatitis B. J. Control. Release **136**, 161–169 (2009)
50. Demento, S., Steenblock, E.R., Fahmy, T.M.: Biomimetic approaches to modulating the T cell immune response with nano- and micro- particles. Conf. Proc. IEEE Eng. Med. Biol. Soc. **2009**, 1161–1166 (2009)
51. Bangham, A.D., Standish, M.M., Miller, N.: Cation permeability of phospholipid model membranes: effect of narcotics. Nature **208**, 1295–1297 (1965)
52. Allison, A.G., Gregoriadis, G.: Liposomes as immunological adjuvants. Nature **252**, 252 (1974)
53. Castaldello, A., Brocca-Cofano, E., Voltan, R., Triulzi, C., Altavilla, G., Laus, M., Sparnacci, K., Ballestri, M., Tondelli, L., Fortini, C., Gavioli, R., Ensoli, B., Caputo, A.: DNA prime and protein boost immunization with innovative polymeric cationic core-shell nanoparticles elicits broad immune responses and strongly enhance cellular responses of HIV-1 tat DNA vaccination. Vaccine **24**, 5655–5669 (2006)
54. Himeno, A., Akagi, T., Uto, T., Wang, X., Baba, M., Ibuki, K., Matsuyama, M., Horiike, M., Igarashi, T., Miura, T., Akashi, M.: Evaluation of the immune response and protective effects of rhesus macaques vaccinated with biodegradable nanoparticles carrying gp120 of human immunodeficiency virus. Vaccine **28**, 5377–5385 (2010)
55. Caputo, A., Castaldello, A., Brocca-Cofano, E., Voltan, R., Bortolazzi, F., Altavilla, G., Sparnacci, K., Laus, M., Tondelli, L., Gavioli, R., Ensoli, B.: Induction of humoral and enhanced cellular immune responses by novel core-shell nanosphere- and microsphere-based vaccine formulations following systemic and mucosal administration. Vaccine **27**, 3605–3615 (2009)
56. Stano, A., van der Vlies, A.J., Martino, M.M., Swartz, M.A., Hubbell, J.A., Simeoni, E.: PPS nanoparticles as versatile delivery system to induce systemic and broad mucosal immunity after intranasal administration. Vaccine **29**(4), 804–812 (2011)
57. Hirosue, S., Kourtis, I.C., van der Vlies, A.J., Hubbell, J.A., Swartz, M.A.: Antigen delivery to dendritic cells by poly(propylene sulfide) nanoparticles with disulfide conjugated peptides: cross-presentation and T cell activation. Vaccine **28**, 7897–7906 (2010)
58. Slutter, B., Bal, S., Keijzer, C., Mallants, R., Hagenaars, N., Que, I., Kaijzel, E., van Eden, W., Augustijns, P., Lowik, C., Bouwstra, J., Broere, F., Jiskoot, W.: Nasal vaccination with N-trimethyl chitosan and PLGA based nanoparticles: nanoparticle characteristics determine quality and strength of the antibody response in mice against the encapsulated antigen. Vaccine **28**, 6282–6291 (2010)
59. Prego, C., Paolicelli, P., Diaz, B., Vicente, S., Sanchez, A., Gonzalez-Fernandez, A., Alonso, M.J.: Chitosan-based nanoparticles for improving immunization against hepatitis B infection. Vaccine **28**, 2607–2614 (2010)

60. Gregoriadis, G.: Liposomes as immunoadjuvants and vaccine carriers: antigen entrapment. Immunomethods **4**, 210–216 (1994)
61. Wang, D., et al.: Liposomal oral DNA vaccine (mycobacterium DNA) elicits immune response. Vaccine **28**, 3134–3142 (2010)
62. Karkada, M., Weir, G.M., Quinton, T., Fuentes-Ortega, A., Mansour, M.: A liposome-based platform, VacciMax, and its modified water-free platform DepoVax enhance efficacy of in vivo nucleic acid delivery. Vaccine **28**, 6176–6182 (2010)
63. Altin, J.G., Parish, C.R.: Liposomal vaccines–targeting the delivery of antigen. Methods **40**, 39–52 (2006)
64. Immordino, M.L., Dosio, F., Cattel, L.: Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int. J. Nanomedicine **1**, 297–315 (2006)
65. Mohammed, A.R., Bramwell, V.W., Kirby, D.J., McNeil, S.E., Perrie, Y.: Increased potential of a cationic liposome-based delivery system: enhancing stability and sustained immunological activity in pre-clinical development. Eur. J. Pharm. Biopharm. **76**(3), 404–412 (2010)
66. Nordly, P., Agger, E.M., Andersen, P., Nielsen, H.M., Foged, C.: Incorporation of the TLR4 agonist monophosphoryl lipid a into the bilayer of DDA/TDB liposomes: physico-chemical characterization and induction of CD8(+) T-cell responses in vivo. Pharm. Res. **28**(3), 553–562 (2011)
67. Henriksen-Lacey, M., et al.: Liposomes based on dimethyldioctadecylammonium promote a depot effect and enhance immunogenicity of soluble antigen. J. Control. Release **142**, 180–186 (2010)
68. Gasparini, R., Lai, P.: Utility of virosomal adjuvated influenza vaccines: a review of the literature. J. Prev. Med. Hyg. **51**, 1–6 (2010)
69. Patel, G.B., Zhou, H., KuoLee, R., Chen, W.: Archaeosomes as adjuvants for combination vaccines. J. Liposome Res. **14**, 191–202 (2004)
70. Sharma, S., Mukkur, T.K., Benson, H.A., Chen, Y.: Pharmaceutical aspects of intranasal delivery of vaccines using particulate systems. J. Pharm. Sci. **98**, 812–843 (2009)
71. Henriksen-Lacey, M., et al.: Liposomal cationic charge and antigen adsorption are important properties for the efficient deposition of antigen at the injection site and ability of the vaccine to induce a CMI response. J. Control. Release **145**, 102–108 (2010)
72. Zhong, Z., et al.: A novel liposomal vaccine improves humoral immunity and prevents tumor pulmonary metastasis in mice. Int. J. Pharm. **399**, 156–162 (2010)
73. Pearse, M.J., Drane, D.: ISCOMATRIX adjuvant: a potent inducer of humoral and cellular immune responses. Vaccine **22**, 2391–2395 (2004)
74. Pearse, M.J., Drane, D.: ISCOMATRIX adjuvant for antigen delivery. Adv. Drug Deliv. Rev. **57**, 465–474 (2005)
75. Drane, D., Pearse, M.J.: Immunopotentiators in modern vaccines, pp. 191–215. Elsevier Academic Press, Massachusetts, USA (2006)
76. Rimmelzwaan, G.F., Baars, M., van Amerongen, G., van Beek, R., Osterhaus, A.D.: A single dose of an ISCOM influenza vaccine induces long-lasting protective immunity against homologous challenge infection but fails to protect Cynomolgus macaques against distant drift variants of influenza A (H3N2) viruses. Vaccine **20**, 158–163 (2001)
77. Souza, M., Costantini, V., Azevedo, M.S., Saif, L.J.: A human norovirus-like particle vaccine adjuvanted with ISCOM or mLT induces cytokine and antibody responses and protection to the homologous GII.4 human norovirus in a gnotobiotic pig disease model. Vaccine **25**, 8448–8459 (2007)
78. Rimmelzwaan, G.F., Claas, E.C., van Amerongen, G., de Jong, J.C., Osterhaus, A.D.: ISCOM vaccine induced protection against a lethal challenge with a human H5N1 influenza virus. Vaccine **17**, 1355–1358 (1999)
79. Sjolander, S., Drane, D., Davis, R., Beezum, L., Pearse, M., Cox, J.: Intranasal immunisation with influenza-ISCOM induces strong mucosal as well as systemic antibody and cytotoxic T-lymphocyte responses. Vaccine **19**, 4072–4080 (2001)
80. Madhun, A.S., Haaheim, L.R., Nilsen, M.V., Cox, R.J.: Intramuscular Matrix-M-adjuvanted virosomal H5N1 vaccine induces high frequencies of multifunctional Th1 CD4+ cells and strong antibody responses in mice. Vaccine **27**, 7367–7376 (2009)
81. Sanders, M.T., Deliyannis, G., Pearse, M.J., McNamara, M.K., Brown, L.E.: Single dose intranasal immunization with ISCOMATRIX vaccines to elicit antibody-mediated clearance of influenza virus requires delivery to the lower respiratory tract. Vaccine **27**, 2475–2482 (2009)
82. Skene, C.D., Doidge, C., Sutton, P.: Evaluation of ISCOMATRIX and ISCOM vaccines for immunisation against Helicobacter pylori. Vaccine **26**, 3880–3884 (2008)
83. Pinitkiatisakul, S., Friedman, M., Wikman, M., Mattsson, J.G., Lovgren-Bengtsson, K., Stahl, S., Lunden, A.: Immunogenicity and protective effect against murine cerebral neosporosis of recombinant NcSRS2 in different iscom formulations. Vaccine **25**, 3658–3668 (2007)
84. McBurney, W.T., et al.: In vivo activity of cationic immune stimulating complexes (PLUSCOMs). Vaccine **26**, 4549–4556 (2008)
85. Boyle, J., et al.: The utility of ISCOMATRIX adjuvant for dose reduction of antigen for vaccines requiring antibody responses. Vaccine **25**, 2541–2544 (2007)
86. Plummer, E.M., Manchester, M: Viral nanoparticles and virus-like particles: platforms for contemporary vaccine design. *WIREs* Nanomed. Nanobiotechnol. **3**, 174–196 (2011). doi:10.1002/wnan.119
87. Noad, R., Roy, P.: Virus-like particles as immunogens. Trends Microbiol. **11**, 438–444 (2003)

88. Campo, M.S., Roden, R.B.: Papillomavirus prophylactic vaccines: established successes, new approaches. J. Virol. **84**, 1214–1220 (2010)
89. Ludwig, C., Wagner, R.: Virus-like particles-universal molecular toolboxes. Curr. Opin. Biotechnol. **18**, 537–545 (2007)
90. Buonaguro, L., Tornesello, M.L., Buonaguro, F.M.: Virus-like particles as particulate vaccines. Curr. HIV Res. **8**, 299–309 (2010)
91. Grgacic, E.V., Anderson, D.A.: Virus-like particles: passport to immune recognition. Methods **40**, 60–65 (2006)
92. Schiller, J.T., Castellsague, X., Villa, L.L., Hildesheim, A.: An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. Vaccine **26**(Suppl 10), K53–K61 (2008)
93. Pearton, M., et al.: Influenza virus-like particles coated onto microneedles can elicit stimulatory effects on Langerhans cells in human skin. Vaccine **28**, 6104–6113 (2010)
94. Akahata, W., et al.: A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. Nat. Med. **16**, 334–338 (2010)
95. Quan, F.S., Huang, C., Compans, R.W., Kang, S.M.: Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. J. Virol. **81**, 3514–3524 (2007)
96. Krammer, F., et al.: Influenza virus-like particles as an antigen-carrier platform for the ESAT-6 epitope of Mycobacterium tuberculosis. J. Virol. Methods **167**, 17–22 (2010)
97. Song, J.M., et al.: Protective immunity against H5N1 influenza virus by a single dose vaccination with virus-like particles. Virology **405**, 165–175 (2010)
98. Kang, S.M., et al.: Induction of long-term protective immune responses by influenza H5N1 virus-like particles. PLoS One **4**, e4667 (2009)
99. Muratori, C., Bona, R., Federico, M.: Lentivirus-based virus-like particles as a new protein delivery tool. Methods Mol. Biol. **614**, 111–124 (2010)
100. Torchilin, V.P.: Structure and design of polymeric surfactant-based drug delivery systems. J. Control. Release **73**, 137–172 (2001)
101. Torchilin, V.P.: Micellar nanocarriers: pharmaceutical perspectives. Pharm. Res. **24**, 1–16 (2007)
102. O'Reilly, R.K.: Spherical polymer micelles: nanosized reaction vessels? Philos. Transact. A Math. Phys. Eng. Sci. **365**, 2863–2878 (2007)
103. Morein, B., Sharp, M., Sundquist, B., Simons, K.: Protein subunit vaccines of parainfluenza type 3 virus: immunogenic effect in lambs and mice. J. Gen. Virol. **64**(Pt 7), 1557–1569 (1983)
104. Prabakaran, M., et al.: Reverse micelle-encapsulated recombinant baculovirus as an oral vaccine against H5N1 infection in mice. Antiviral Res. **86**, 180–187 (2010)
105. Bharali, D.J., Khalil, M., Gurbuz, M., Simone, T.M., Mousa, S.A.: Nanoparticles and cancer therapy: a concise review with emphasis on dendrimers. Int. J. Nanomedicine **4**, 1–7 (2009)
106. Patri, A.K., Majoros, I.J., Baker, J.R.: Dendritic polymer macromolecular carriers for drug delivery. Curr. Opin. Chem. Biol. **6**, 466–471 (2002)
107. Klajnert, B., Bryszewska, M.: Dendrimers: properties and applications. Acta Biochim. Pol. **48**, 199–208 (2001)
108. Baker, J. R., Jr.: Dendrimer-based nanoparticles for cancer therapy. Hematology. Am. Soc. Hematol. Educ. Program. **1**, 708–719 (2009)
109. Moreno, C.A., et al.: Preclinical evaluation of a synthetic Plasmodium falciparum MAP malaria vaccine in Aotus monkeys and mice. Vaccine **18**, 89–99 (1999)
110. Nardin, E.H., et al.: A totally synthetic polyoxime malaria vaccine containing Plasmodium falciparum B cell and universal T cell epitopes elicits immune responses in volunteers of diverse HLA types. J. Immunol. **166**, 481–489 (2001)
111. Nardin, E.H., et al.: Synthetic malaria peptide vaccine elicits high levels of antibodies in vaccinees of defined HLA genotypes. J. Infect. Dis. **182**, 1486–1496 (2000)
112. Shampur, M., Padinjarenmattathil, U., Desai, A., Narayanaswamy, J.: Development and immunogenicity of a novel polyetherimine (PETIM) dendrimer based nanoformulated DNA rabies vaccine. Int. J. Infect. Dis. **14**(Suppl 1), E453 (2010). Elsevier Science
113. Dutta, T., Garg, M., Jain, N.K.: Poly(propyleneimine) dendrimer and dendrosome mediated genetic immunization against hepatitis B. Vaccine **26**, 3389–3394 (2008)
114. Skwarczynski, M., Zaman, M., Urbani, C.N., Lin, I.C., Jia, Z., Batzloff, M.R., Good, M.F., Monteiro, M.J., Toth, I.: Polyacrylate dendrimer nanoparticles: a self-adjuvanting vaccine delivery system. Angew. Chem. Int. Ed. Engl. **49**, 5742–5745 (2010)
115. in het Panhuis, M.: Vaccine delivery by carbon nanotubes. Chem. Biol. **10**, 897–898 (2003)
116. Kendall, M.: Engineering of needle-free physical methods to target epidermal cells for DNA vaccination. Vaccine **24**, 4651–4656 (2006). doi:10.1016/j.vaccine.2005.08.066. S0264-410X(05)00841-8 [pii]
117. Kersten, G., Hirschberg, H.: Antigen delivery systems. Expert Rev. Vaccines **3**, 453–462 (2004). doi:10.1586/14760584.3.4.453. ERV030423 [pii]
118. Nandedkar, T.D.: Nanovaccines: recent developments in vaccination. J. Biosci. **34**, 995–1003 (2009)