Nanoparticles for mucosal vaccine delivery

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25.1   Introduction

The mucus membranes lining the surfaces of the gastrointestinal, respiratory, and genitourinary tracts and the ocular and ear cavity cover a very large surface area (approximately $400\,\text{m}^2$) of the human body. Due to its close proximity to the external environment, the mucosa is exposed continuously to foreign substances and infectious agents. Approximately 70% of all pathogens are estimated to enter the host through the mucosa [1]. These surfaces constitute sites of extensive immunological activity. Continuous immunological monitoring conveys information to the highly specialized innate and adaptive mucosal immune system to counteract potential insults from the environment and to protect the host. Mucosal vaccines exploit the intense immunological activity associated with this administration route, and they protect against several mucosally transmitted bacterial and viral diseases [2]. To date, only a few (primarily oral) mucosal vaccines have been approved for human use, and they include the Sabin polio vaccine, vaccines against rotavirus, *Salmonella typhi*, and cholera, and a nasal influenza vaccine [2] (Table 25.1). These vaccines are all conventional vaccines, that is, they consist of whole pathogenic organisms, which are either killed or live attenuated. Hence, they are associated with several disadvantages, for example, tedious and expensive production, quality control and distribution, and risk of reversion to virulence. In contrast, subunit vaccines represent safer alternatives because they are based on one or several highly purified antigens of the pathogenic organisms, for example, peptides, proteins, polysaccharides, or DNA/RNA. However, the increased purity and safety are often accompanied with loss of immunogenicity, which may require addition of an adjuvant. Hence, applying subunit vaccines for mucosal immunization may overcome many of the drawbacks of conventional vaccines, but it usually necessitates the use of strong adjuvants. Adjuvants constitute a large group of structurally diverse compounds, which can generally be classified as being delivery systems or immunopotentiators, respectively, while some adjuvants possess both properties [16]. Delivery systems may enhance the immune response against an antigen via a number of different mechanisms, for example, by protecting the antigen against degradation and sustaining the release of the codelivered antigen, eventually resulting in improved vaccine efficacy. Immunopotentiators activate the immune system by being ligands for pattern-recognition receptors (PRRs) expressed by antigen-presenting cells (APCs). The advantages of administering vaccines mucosally are (i) that dual immunity is

Table 25.1 Licensed mucosal vaccines.

Disease/ pathogen	Trade name	Manufacturer	Administration route/number of doses	Composition	Correlate(s) of protection	Reference
Cholera	Vaxchora	PaxVax, Redwood City, CA, USA	Oral, single dose	Live-attenuated, lyophilized CVD 103-HgR	Vibriocidal antibodies	[3]
	Dukoral	Valneva Sweden AB, Solna	Oral, two-three doses	Heat- or formalin-inactivated *Vibrio cholerae* 01 Inaba and Ogawa, classic and El Tor strains, ca. $1.25 \times 10^{11} + 1\,mg$ recombinant cholera toxin B	Antitoxin and cholera toxin B-specific IgA	[4]
	ShanChol	Shantha Biotecnics Ltd., India, Hyderabad	Oral, two-three doses	Heat- or formalin-inactivated *Vibrio cholerae* 01 Inaba and Ogawa, classic and El Tor strains + formalin-killed O139 bacteria	Vibriocidal and LPS-specific antibodies	[5]
	Euvichol/ Euvichol-Plus	EuBiologics Co., Ltd., Seoul, Republic of Korea	Oral, two-three doses	Heat- or formalin-inactivated *Vibrio cholerae* 01 Inaba and Ogawa, classic and El Tor strains + formalin-killed O139 bacteria	Vibriocidal and LPS-specific antibodies	[6]
Typhoid fever	Vivotif	PaxVax, Redwood City, CA, USA	Oral, three-four doses	Live-attenuated *Salmonella typhi* Ty21a2	Mucosal IgA, systemic IgG, LPS-specific antibodies	[7]
Poliovirus	Multiple	Multiple	Oral, three doses	Monovalent, bivalent, and trivalent vaccines	Mucosal IgA, systemic IgG	[8]

Rotavirus	Rotarix	GlaxoSmithKline, Wavre, Belgium	Oral, three doses	Live-attenuated monovalent human rotavirus RIX4414 strain, specificity G1P	Mucosal IgA, systemic IgG	[9]
	Rota Teq	Merck, West Point, PA, USA	Oral, three doses	Live-attenuated pentavalent rotavirus (four human strains express G1, G2, G3, G4, and P7) from bovine rotavirus. Fifth virus expresses P1A from human and G6 from bovine rotavirus	Mucosal IgA, systemic IgG	[10]
	Rotavac	Bharat Biotech, Hyderabad, India	Oral, three doses	Live-attenuated monovalent human rotavirus 116E, specificity G9P	Serum IgA	[11]
	Rotasiil	Serum Institute of India Pvt. Ltd., Pune, India	Oral, three doses	Live-attenuated bovine-human rotavirus reassortant (G1, G2, G3, G4, and G9)	Serum IgA	[12]
Influenza virus	FluMist	MedImmune LLC, Gaithersburg, MD, USA	Nasal, one-two doses	Live virus strain mix of one A/H1N1 strain, one A/H3N2 strain, and one B strain	Mucosal IgA, systemic IgG, possibly T cells	[13]
	FluMist Quadrivalent	MedImmune LLC, Gaithersburg, MD, USA	Nasal, one-two doses	Live virus strain mix of one A/H1N1 strain, one A/H3N2 strain, and two B strains	Mucosal IgA, systemic IgG, possibly T cells	[14]
	Nasovac-S	Serum Institute of India Pvt. Ltd., Pune, India	Nasal, one-two doses	Live-attenuated virus strains of A/H1N1, A/H3N2, and Type B influenza virus	Mucosal IgA, systemic IgG, possibly T cells	[15]

HN, hemagglutinin and neuraminidase; *Ig*, immunoglobulin; *LPS*, lipopolysaccharide.

induced at mucosal and serosal sites and (ii) that immune responses are elicited both at local and distal mucosal surfaces. Moreover, mucosal vaccines possess many advantages compared with injectable vaccines, for example, ease of administration, improved patient compliance and lower costs, circumventing needle stick injuries and needle disposal, and the opportunity for mass immunization [2, 17, 18]. Table 25.2 summarizes examples of mucosal vaccine candidates in clinical trials. No mucosal adjuvants are yet available for human use, but toxin-based adjuvants are currently tested in clinical trials (Table 25.2). The purpose of this chapter is to review the current knowledge on the nanotechnology-based vaccines, which are investigated for mucosal immunization.

25.2   Organization of the mucosal immune system

The organization and function of the mucosal immune system differs from that of the systemic immune system. Hence, understanding the mucosal immune system is important in the design of novel vaccines and vaccine delivery strategies against mucosal infections. The mucosal immune system is composed of an integrated network of immune tissues and cells, constituting approximately 80% of the total immune cells of the body [19]. This myriad of immune cells is assembled in or migrates between well-organized structures, which are collectively referred to as mucosa-associated lymphoid tissue (MALT). It covers regions of the gastrointestinal tract (gut-associated lymphoid tissue [GALT]), the respiratory tract (bronchus-associated lymphoid tissue [BALT]), and the nasal cavity (nasopharynx-associated lymphoid tissue [NALT]), and it represents the largest lymphoid organ system [20]. The mucosal immune system provides three major functions: (i) detection and inhibition of the first entry of pathogenic microbes or harmful substances, (ii) prevention of uptake of ingested or inhaled antigens, and (iii) prevention of development of deleterious immune responses, if the antigens nevertheless get access to host cells. Among the immune cells, professional APCs, including dendritic cells (DCs), B cells, and macrophages, are key immune cells that surveil the mucosal surfaces. Displaying specific functions and subsets, APCs initiate adaptive immune responses and mediate vaccine-induced immunity at the mucosa. Microfold (M) cells also play a role in the transport of antigens across epithelia by transcytosis. Antigens are subsequently delivered to DCs priming the differentiation of T-cell subsets, which in turn interact with B cells, eventually resulting in the production of antibodies at mucosal sites [21, 22].

The mucosal immune system can generally be categorized into two separate compartments—inductive sites and effector sites—based on their anatomical localization and functional properties (Fig. 25.1). At the inductive sites, priming of initial immune responses takes place after antigen uptake, which leads to immune cell activation. Upon activation, antibodies and other components of the immune system exert their specific functions at the effector sites. The MALT and the associated draining lymph nodes comprise the main inductive sites for mucosal immune responses. The lamina propria of the gastrointestinal tract, the upper respiratory tract, and the female reproductive tract and the acinar regions of exocrine glands (e.g., the mammary, lacrimal, and salivary glands)

Table 25.2 Examples of mucosal vaccines in clinical trials.

Study title	Pathogen/ condition	Vaccine	Administration route	Status	Phase	Clinical trials (government identifier)
Evaluation of the efficacy and safety of MV130 in chronic obstructive pulmonary disease (COPD)	Recurrent respiratory tract infection in COPD patients	MV130 (Bactek), polyvalent bacterial preparation	Sublingual	Active, not recruiting	II/III	NCT01842360
Evaluation of the efficacy and safety of MV140 (MV140)	Recurrent urinary tract infection	MV140 (Uromune), polybacterial vaccine	Sublingual	Recruiting	III	NCT02543827
A study assessing colonization and immunogenicity after nasal inoculation with *Neisseria lactamica* and eradication on day 4 or 14	Meningitis	*Neisseria lactamica*	Nasal	Recruiting	Not applicable	NCT03549325
Safety and immunogenicity of the respiratory syncytial virus (RSV) RSV D46cpΔM2–2 vaccine in RSV-seropositive children and RSV-seronegative infants and children	RSV infection	D46cpΔM2-2 vaccine	Nasal	Active, not recruiting	I	NCT02601612
Evaluating the infectivity, safety, and immunogenicity of the recombinant live-attenuated RSV vaccines RSV ΔNS2/Δ1313/I1314L or RSV 276 in RSV-seronegative infants 6–24 months of age	RSV infection	RSV ΔNS2/Δ1313/ I1314L and RSV 276	Nasal	Recruiting	I	NCT03227029

Table 25.2 Continued

Study title	Pathogen/ condition	Vaccine	Administration route	Status	Phase	Clinical trials (government identifier)
Evaluating the infectivity, safety, and immunogenicity of a RSV vaccine (RSV 6120/ΔNS2/1030s) in RSV-seropositive children and RSV-seronegative infants and children	RSV infection	RSV 6120/ΔNS2/1030s	Nasal	Recruiting	I	NCT03387137
Phase I clinical trial of the safety and immunogenicity of an adenovirus-based TB vaccine administered by aerosol	Tuberculosis	Ad5Ag85A	Intrapulmonary	Recruiting	I	NCT02337270
Immunogenicity of coadministered oral polio vaccine and oral cholera vaccine	Poliomyelitis and cholera	Live-attenuated poliovirus and bivalent killed whole-cell cholera vaccine	Oral	Recruiting	III	NCT03581734
Monovalent oral poliovirus vaccine type 1 intestinal and humoral immunity study (mOPV1)	Poliomyelitis	Mono- and bivalent polio vaccine	Oral	Recruiting	IV	NCT03722004
Trail to evaluate the immune effects of primary and booster immunizations with poliovirus vaccine	Poliomyelitis	Bivalent polio vaccine	Oral	Recruiting	III	NCT03821441
Phase I novel live-attenuated serotype 2 oral polio vaccine study in IPV-primed adults (nOPV2M4a)	Poliomyelitis	nOPV2M4a	Oral	Active, not recruiting	I	NCT03430349
A study to evaluate the safety and immunogenicity of novel oral polio vaccine	Poliomyelitis	nOPV2	Oral	Recruiting	II	NCT03554798

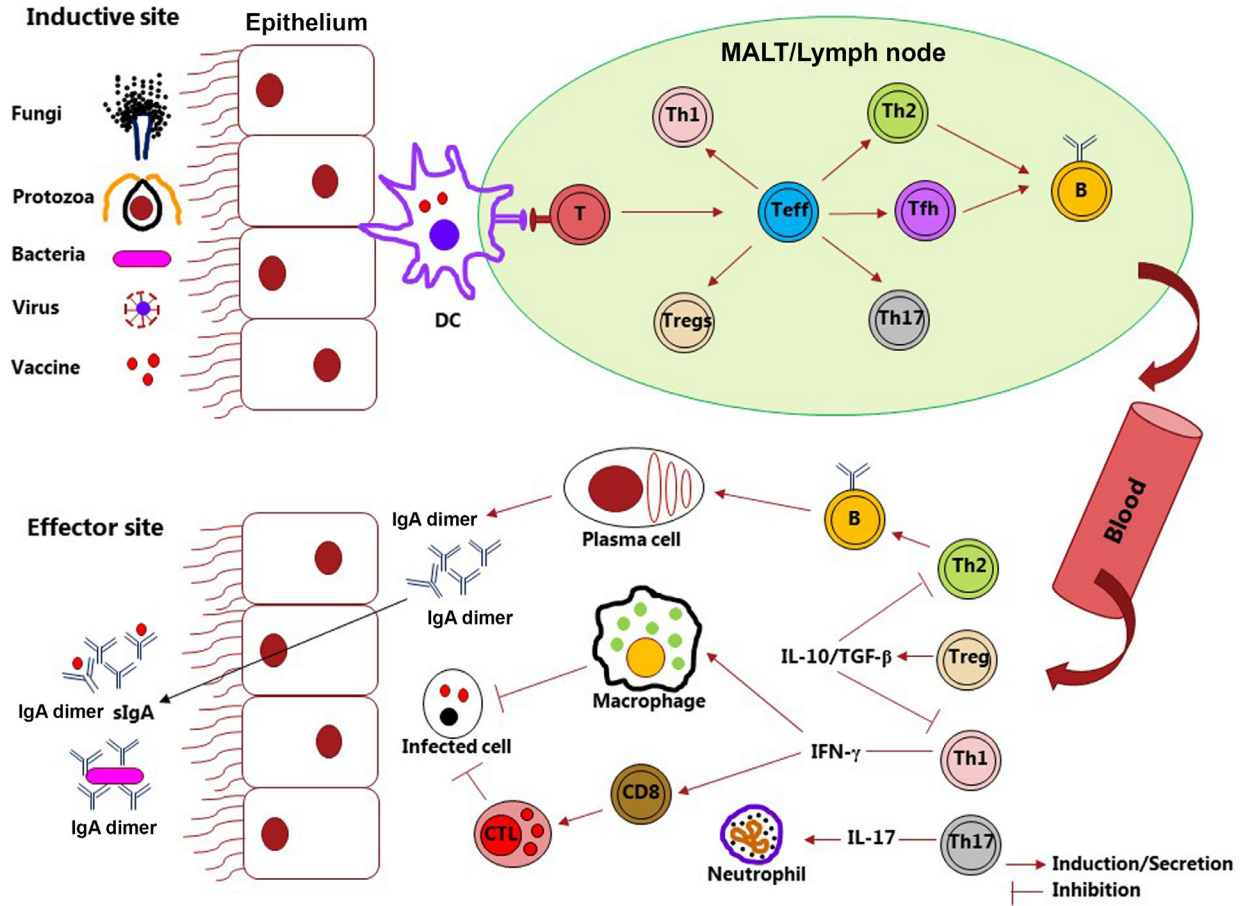|						
Extended dose intervals with oral cholera vaccine in Cameroon	Cholera	Killed whole-cell cholera vaccine	Oral	Active, not recruiting	II	NCT03719066
Effect of extended dose intervals on the immune response to oral cholera vaccine	Cholera	Cholera vaccine	Oral	Recruiting	IV	NCT03373669
Immunologic responses to a live-attenuated oral cholera vaccine	Cholera	Vaxchora	Oral	Recruiting	II	NCT03251495
A phase II dose-ranging study of oral RV3-BB rotavirus vaccine	Rotavirus	RV3-BB	Oral	Active, not recruiting	II	NCT03483116
A double-blind placebo-controlled dose escalating study to evaluate the safety and immunogenicity of dmLT by oral, sublingual, and intradermal vaccination in adults residing in an endemic area	Enterotoxigenic *Escherichia coli*	Double mutant heat-labile toxin LT(R192G/L211A) (dmLT)	Oral, sublingual	Recruiting	I	NCT03548064
Cross-reactive immunity elicited by oral and parenteral typhoid vaccines (Ty21a-ASC)	*Salmonella typhi*	Vivotif	Oral	Recruiting	IV	NCT02121145
Study to evaluate the safety and immunogenicity of orally administered HIV vaccine in healthy, HIV-uninfected adult participants	HIV	Ad4-mgag and Ad4-EnvC150	Oral	Active, not recruiting	I	NCT02771730
Multiple myeloma trial of orally administered *Salmonella*-based survivin vaccine (MAPSS)	Multiple myeloma	CVD908ssb-TXSVN	Oral	Not yet recruiting	I	NCT03762291

Fig. 25.1 See the legend on opposite page

serve as effector sites. The inductive sites provide a constant source of memory B and T cells that subsequently migrate to the mucosal effector sites via the lymphatic system, and they constitute the cellular basis for mucosal immune responses [23].

The MALT contains T- and B-cell regions and a subepithelial area rich in professional APCs, which initiate antigen-specific immune responses. M cells and epithelial cells (ECs) covering the MALT and the underlying lymphoid cells play key roles in the initiation of mucosal immune responses. M cells take up antigens from the lumen of the mucosa and transfer them to the underlying DCs, which in turn carry the antigens to the inductive sites. At the inductive sites, activation of antigen-specific B and T cells takes place. This is followed by clonal expansion and differentiation into effector B and T cells, which subsequently migrate via the lymphatics and the bloodstream into the mucosal effector sites [24]. Several T-cell subsets, including Th1, Th2, Th17, and Tregs, play important roles in regulating mucosal immune responses [25, 26]. The lamina propria serves as an effector site, and it contains antigen-specific mucosal effector cells, for example, IgA-producing plasma cells and memory B and T cells [27]. Adaptive mucosal immune responses result from $CD4^+$ T-cell help provided by $CD4^+$ Th1 or $CD4^+$ Th2 cells, which support the development of IgA-producing plasma cells. Subsequently, IgA is transported across the ECs by polymeric Ig receptors, eventually resulting in secretory IgA (sIgA) antibodies, which have specificities for various antigens encountered at

Fig. 25.1 Immunological events at inductive and effector sites of the common mucosal immune system. Inductive and effector sites are physically separated but function in concert to protect the vast mucosal surface areas. Inductive sites are organized lymphoid tissues, and they include nasopharynx-associated, bronchus-associated, gut-associated, conjunctiva-associated, lacrimal-associated, larynx-associated, salivary duct-associated, skin-associated, vulvovaginal-associated, and rectal-associated lymphoid tissue. Effector sites represent the mucosa at the corresponding inductive sites, and they are primarily structured in the form of lymphoid follicles or tonsils. Pathogens are taken up by dendritic cells (DCs) or other types of antigen-presenting cells at the inductive sites. Pathogens ingested by DCs trigger their activation by binding to pattern-recognition receptors. Activated DCs can prime T cells present in mucosa-associated lymphoid tissue (MALT), or they migrate to the draining lymph nodes. Activated $CD4^+$ T cells differentiate into various effector $CD4^+$ T cell subsets (Teff): T helper 1 (Th1), Th2, Th17, regulatory T cells (Tregs), or follicular helper T cells (Tfh). Th2 and Tfh cells activate B cells via production of cytokines, and they induce B-cell differentiation into plasma cells, antibody class switching, and affinity maturation of antibodies, eventually leading to the development of long-lived plasma cells and memory B cells. Effector T and B cells migrate via the systemic circulation and enter the effector sites. Th1 cells activate macrophages and $CD8^+$ T cells via secretion of interferon (IFN-γ). Activated macrophages can directly engulf live pathogens and pathogens destroyed by cytotoxic T cells (CTL). Th17 cells activated by IL-23 secrete IL-17 and IL-22, which drive the production of antimicrobial peptides and the recruitment and activation of neutrophils. Tregs regulate immune responses and maintain self-tolerance by negatively regulating Th1 and Th2 cells, for example, by producing the cytokines IL-10 and transforming growth factor (TGF-β). Terminally differentiated plasma cells secrete dimeric IgA, which is subsequently transported across the epithelial cells and released as secretory IgA (sIgA). The main functions of sIgA are to prevent attachment of pathogens to epithelial cells, to neutralize viral proteins, and to remove antigens infecting the mucosa.

mucosal inductive sites, and they prevent the attachment and colonization of pathogens at mucosal surfaces [25, 28]. Other important effector mechanisms, which contribute to the host defense against pathogens at the lamina propria, include locally produced IgM and IgG and mucosal cytotoxic T lymphocytes (CTL) [29].

25.3 Routes of mucosal vaccination

In addition to the advantages of mucosal vaccination discussed above, mucosal vaccination can also induce immunity at sites distal to that of vaccine administration. This is due to the existence of an integrated cross-communication pathway of lymphoid tissues composed of inductive and effector sites, which is referred to as the common mucosal immune system (CMIS). Key effector molecules of the CMIS include IgA antibodies and cytokines, chemokines, and their corresponding receptors [25]. Mucosal vaccines can be administered via oral, nasal, pulmonary, rectal, vaginal, ocular, sublingual, or transcutaneous routes. However, only the oral and nasal routes are applied clinically for the licensed mucosal vaccines [2]. Key benefits of oral immunization include ease of immunization and delivery of particulate antigens, which induce substantial antibody responses not only in the small intestine (mostly the proximal intestine) and the ascending colon but also in the mammary and salivary glands via the CMIS [30]. Disadvantages of the oral route of immunization include exposure of the antigens to the acidic environment in the stomach and enzymatic degradation in the gastrointestinal tract. Nasal immunization results in induction of antibody responses in the mucosa of the upper airways and in saliva and nasal secretions. The nasal administration route is less hostile for antigens than oral administration, and vaccination via this route has been shown to result in faster induction of immunity and long-term memory than oral immunization due to the dense DC population present in the NALT [31, 32]. Moreover, nasal immunization also results in induction of substantial IgA and IgG responses in the cervicovaginal mucosa via the CMIS, and this is exploited for vaccination against sexually transmitted diseases like chlamydia and herpes [33, 34]. In addition to the oral and nasal routes, the pulmonary, rectal, vaginal, ocular, sublingual, and transcutaneous routes are under investigation for mucosal vaccine administration. Rectal and vaginal immunization have been shown to evoke strong local antibody responses but weak responses at distal sites of the CMIS, and they are associated with poor patient compliance [35]. The sublingual route is widely used to administer sublingual immunotherapy (SLIT) for treatment of type 1 (allergic) hypersensitivity, and T-cell proliferation and allergen-specific IgA are induced when this route of administration is applied [36]. In addition, the use of this route for vaccination against bacterial and viral pathogens has been explored in several studies [37–39]. The pulmonary administration route has gained increasing attention for vaccine delivery because of the large surface area of the lungs and the APC-rich pulmonary mucosa, and it has been investigated for vaccination against a number of respiratory pathogens [40–43]. The ocular conjunctiva represents an alternative mucosal immunization route against bacterial [44, 45] and viral infection [46], and ocular administration results in induction of immune responses locally and in the vaginal mucosa via the CMIS [44]. Transcutaneous immunization is an attractive administration route for mucosal vaccination due to the presence of a dense population

of DCs in the epidermis and dermis layers of the skin, which allow for efficient antigen uptake and subsequent induction of immune responses. Techniques used for transcutaneous vaccine delivery include iontophoresis [47], elastic liposomes [48], and microneedle patches [49].

25.4 Barriers to mucosal vaccine delivery

Commonly used vaccination methods, that is, injections, target the systemic immune system and elicit only weak mucosal immune responses. In contrast, vaccine administration directly onto mucosal sites has been shown to efficiently potentiate both mucosal and systemic immunity. However, a number of challenges are associated with successful induction of immunity in the mucosa. One of them is that vaccine antigens delivered through the mucosa tend to become diluted in mucosal secretions, which may limit effective deposition onto the mucosal epithelia [50]. In addition, antigens delivered through the mucosa have a tendency to be captured within the mucus and subsequently be degraded by proteases or nucleases [50]. The acidic environment of the gastrointestinal tract is also a barrier for successful oral immunization. Mucosal tissues are abundantly colonized by commensal microbes, which can significantly influence mucosal immune regulation and serve as a barrier to optimal mucosal immunity [51]. Mucosal or oral tolerance represents another major challenge for induction of protective immunity upon mucosal immunization [52].

Subunit vaccine formulations containing peptides, proteins, DNA/RNA, or polysaccharides are prone to degradation and may lose their biological effect during passage through the mucosa. Hence, they need adequate protection for optimal efficacy. It is now well recognized that mucosal vaccines might be more efficacious, if they mimic the physicochemical properties of opportunistic pathogens, in particular with respect to shape, charge, and size [50, 53]. Therefore an effective vaccine design and delivery strategy for mucosal immunization should (i) overcome mucosal barriers, (ii) target mucosal APCs or M cells for adequate antigen processing and presentation and T- or B-cell activation, and (iii) modulate the kinetics of antigen and adjuvant presentation for induction of protective immunological memory responses. Nanotechnology-based drug delivery systems are capable of traversing physiological barriers of the mucosa, can efficiently target immune cells, and control antigen kinetics. Hence, these approaches provide great opportunities for the design and delivery of mucosal vaccines [53].

25.5 Nanotechnology-based solutions for mucosal vaccination

Nanotechnology-based applications are attractive for targeted delivery of vaccine antigens across the mucosal surfaces. Using nanotechnology, the solubility, stability, and surface properties of vaccine antigens and/or adjuvants can readily be tailored, and hence, nanoparticle-based drug delivery systems have recently generated great interest in the field of vaccinology [53]. Vaccine antigens can either be encapsulated in or

surface-adsorbed to nanoparticles. By encapsulation, nanoparticles permit a method of delivering antigens, which may otherwise degrade fast upon injection or induce a brief, local immune response. Conjugation of antigens onto nanoparticles allows presentation of antigens to APCs in a similar way as during a natural infection and may induce a similar immune response. A size in the nanoscale region provides a high surface area-to-volume ratio and a high diffusion rate, which is suitable for the delivery of biologics including vaccine antigens to mucosal sites like the eye, nasal or lung airway, or the gut mucosa. Additional advantages of nanoparticle-based delivery systems, as compared with conventional systems, include (i) local and targeted delivery of antigens; (ii) improved antigen presentation and processing; (iii) increased and sustained antigen concentration at the mucosa; (iv) enhanced bioavailability; and (v) immunomodulation, that is, either immunostimulation via proinflammatory cytokines or immunosuppression via antiinflammatory cytokines.

Many different types of nanoparticle-based delivery systems have been investigated for vaccine delivery to mucosal surfaces. These include liposomes, polymeric nanoparticles, lipid-polymer hybrid nanoparticles, emulsions, virus-like particles (VLPs), dendrimers, and immunostimulatory complexes (ISCOMs) (Table 25.3). Design and development of an efficient and safe delivery system does not only require an understanding of the used (bio)material but also the cargo (antigen + adjuvant), the target, and the desired immunological outcome [54]. Nanoparticles must be prepared from safe materials that are pure, nonreactive, and biocompatible [55], and they should render optimal encapsulation or conjugation, stability, and permeability and sensitivity to the local environment of the mucosa to the delivered antigen [54]. In addition, nanoparticles must protect the antigen from the harsh pH conditions or enzymatic activity of the mucosa, which can otherwise result in antigen degradation [56], and deliver the antigen to the right APCs for effective activation of the immune system [57]. Therefore an effective generation of an immune response against a vaccine antigen requires knowledge about antigen uptake and processing, release kinetics, and the mechanisms of generation of mucosal immunity, which should be integrated in the design of the nanoparticle carrier [57].

25.6 Physicochemical properties that influence the biological performance of nanoparticles

A number of physicochemical properties influence the interaction between antigen-loaded nanoparticles and immune cells and hence the subsequent immunological outcome. These properties can either trigger or block immune pathways, and they can affect the stability, efficacy, and safety of the vaccine [53, 109, 110]. Important physicochemical parameters include shape, size, hydrophobicity, surface charge, colloidal stability, solid-state characteristics, and bioadhesive properties. Particle shape plays a key role for antigen presentation by APCs and uptake by other immune cells and for the intracellular processing of particles. Ellipsoidal particles have been show to display improved pharmacokinetics and ability to induce T-cell responses, as compared with spherical particles, due to an enhanced circulation time upon intravenous

Table 25.3 Examples of nanoparticulate drug delivery systems.

Drug delivery system	Composition	Antigen/pathogen	Mucosal route	Animal model	Immunity type	References
Lipid-based nanoparticles						
Liposomes	DOTAP and dimethylaminoethyl carbamate; DDA and TDB (CAF01); CAF01 and CpG; phosphatidylcholine, cholesterol, and chitosan; DPPC, DPPS, and cholesterol; DOPC and cholesterol	*Streptococcus pneumonia*; *Leishmania amazonensis* antigens; streptococcal C5a peptidase, Ag85B; influenza A virus; *Salmonella enteritidis*; H56	Nasal, oral, sublingual, pulmonary	Mice	IgA, IgG, Th17; IFN-γ; IgA and Th7; Th1; IgA and IgG	[41, 58–64]
ICMVs	DOPC, MPB, and MPLA	Ovalbumin	Pulmonary	Mice	T_{em}	[42]
Solid lipid nanoparticles	DPPC and MPLA	Hepatitis B	Rectal, oral	Rats	IgA, IgG, IFN-γ, IL-2	[65, 66]
Cubosomes	Monoolein and Quil-A	Ovalbumin	Oral	Mice	IgG, CTL	[67, 68]
Emulsions	Nanoemulsion $W_{80}5EC$; isopropyl myristate, Cremophor EL-35 and PEG	H1N1 influenza, respiratory syncytial virus, methicillin-resistant *Staphylococcus aureus*, HIV gp120	Nasal	Mice, rats	IgG and IgA, Th1	[69–72]
ISCOMs	Phosphatidylcholine, cholesterol, antigen, Quil A	*Mycobacterium tuberculosis*, human T-cell lymphotropic virus type 1, diphtheria toxoid	Pulmonary, nasal, oral	Mice	IgG and IgA; Th1	[73, 74]

Table 25.3 Continued

Drug delivery system	Composition	Antigen/pathogen	Mucosal route	Animal model	Immunity type	References
Natural polymer-based nanoparticles						
Chitosan	Mannosylated chitosan, chitosan, trimethyl chitosan	*Mycobacterium tuberculosis* Hsp65, swine influenza A virus, hepatitis B, Group A *Streptococcus*	Nasal	Mice, pigs	IgA, Th1; IgA, IgG, and Tem	[75–79]
Gamma polyglutamic acid	Polyglutamic acid and trimethyl chitosan; polyglutamic acid, chitosan, and cholera toxin subunit A1	Group A *Streptococcus*, influenza A virus	Nasal	Mice	IgA and IgG; Th1 and Th2	[79–81]
Hyaluronic acid	Hyaluronic acid microspheres (HYAFF) and enterotoxin from *Escherichia coli* (LT), DOTAP and hyaluronic acid	Influenza hemagglutinin, ovalbumin	Nasal	Mice, rabbits, micropigs	IgA and IgG; IgG and CD8+ T cells	[82, 83]
Pullulan	Cholesteryl group-bearing pullulan; TNF-α and cholesteryl group-bearing pullulan	*Clostridium botulinum* type-A neurotoxin, *Streptococcus pneumonia*, influenza A virus	Nasal	Mice, macaques	IgA and IgG; Th2 and Th17; IgG1 and IgA	[84–86]
Synthetic polymer-based nanoparticles						
PLGA	PLGA and MPLA; PLGA, DDA, and MPLA; PLGA and MPL; PLGA and hydroxypropyl methylcellulose phthalate; PLGA and Eudragit; PEG and PLGA	Ovalbumin; HspX/ EsxS fusion protein of *Mycobacterium tuberculosis*, H5N1 influenza; *Helicobacter pylori*, HIV envelop protein, Hepatitis B	Oral, nasal, colorectal	Mice	IgG and IgA; IgA, Th1 and Th17; IgA and Th1	[87–91]

PEI	Polyethyleneimine, polyethyleneimine-triethyleneglycol, deacylated PEI	H7N9 Influenza, HIV gag, HIV envelop protein	Nasal	Mice, chickens	IgG, IgA, Th1 and Th2: IgG, Th1, CTL;	[92–94]
PCL	poly-ε-caprolactone, PCL and chitosan, PCL and PEI, or PCL and PEG	Hepatitis B, ovalbumin, *Streptococcus equi*	Nasal	Mice	IgG and IgA	[95–98]
PPS	Polypropylene sulfide; Pluronic-stabilized PPS	Ovalbumin, H1N1 influenza	Nasal	Mice	IgG and IgA; CTL	[99, 100]
Dendrimers	G4-PAMAM-NH$_2$	HIV-1 gp120	Nasal	Mice	IgG and IgA	[101]
Lipid-polymer hybrid nanoparticles	DOTAP and hyaluronic acid; PLGA, CAF01, and chitosan; PLGA, DOPC, DOTAP, and DSPE-PEG	*Yersinia pestis*, *Chlamydia trachomatis*, HIV-1 gag	Nasal	Mice	IgG and CD8 + T cells; IgG and IgA	[83, 102, 103]
VLPs	H1N1 influenza, phosphatidylcholine, and phosphatidylethanolamine; DCPC, respiratory syncytial virus; H5N1 Influenza and Matrix M	HIV-1 gp41, respiratory syncytial virus, H5N1 Influenza	Nasal	Nonhuman primates, mice	IgA, IgG, antibody-dependent cell cytotoxicity; Th1	[104–106]
Gas-filled microbubbles	DSPC and PEG	*Salmonella enterica typhimurium,* ovalbumin	Nasal	Mice	IgA, Th1, and Th17	[107, 108]

CAF01, cationic adjuvant formulation 01; *CpG*, cytosine-phosphodiester-guanine; *CTL*, cytotoxic T lymphocytes; *DCPC*, dicaproylphosphatidylcholine; *DDA*, dimethyldioctadecylammonium; *DOPC*, dioleoylphosphatidylcholine; *DOTAP*, dioleoyl trimethylammonium propane; *DPPC*, dipalmitoylphosphatidylcholine; *DPPS*, dipalmitoylphosphatidylserine; *DSPC*, distearoylphosphatidylcholine; *DSPE*, distearoylphosphatidylethanolamine; *HIV*, human immunodeficiency virus; *HN*, hemagglutinin and neuraminidase; *ICMVs*, interbilayer cross-linked multilamellar vesicles; *IFN*, interferon; *Ig*, immunoglobulin; *ISCOMs*, immunostimulatory complexes; *LT*, heat labile toxin; *MPB*, maleimidophenylbutyramide; *MPL or MPLA*, monophosphoryl lipid A; *PCL*, poly ε-caprolactone; *PEG*; polyethylene glycol; *PEI*, polyethyleneimine; *PLGA*, poly(lactic-*co*-glycolic acid); *PPS*, polyphenylene sulfide; *TDB*, trehalose dibehenate; *Tem*, effector memory T cells; *Th*, helper T cells; *VLPs*, virus-like particles.

administration [111]. Spherical particles display increased propensity for phagocytosis, as compared with more elongated shapes [112]. The size of nanoparticles does also affect their interaction with immune cells and their circulation time. Nanoparticles in the size range between 20 and 100 nm administered systemically display prolonged circulation time, as compared with smaller or larger particles [113]. Recent work shows that 50-nm particles are more efficiently taken up by DCs in the pulmonary mucosa and induce costimulatory signals, as compared with 500-nm particles, which are primarily taken up by alveolar macrophages [114]. In another study, small (approximately 190 nm in diameter) spherical particles were shown to induce Th1-biased immune response, while long (approximately 1.5 μm in length) rod-shaped particles induce Th2-biased response [115]. The surface chemistry, for example, surface charge and hydrophobicity, also plays an important role for the cellular processing of nanoparticles. Particles with a negative surface charge display reduced uptake by APCs and consequently induce weaker adaptive immune responses [116]. With increased surface charge of the nanoparticles, the uptake by APCs is increased [117]. Following interaction with the biological milieu, hydrophobic nanoparticles are coated by adsorption of plasma proteins, which prime the nanoparticles for clearance by the reticuloendothelial system [118]. In contrast, hydrophilic particles display prolonged circulation half-life in vivo [119]. Moreover, lipophilic particles easily cross cellular membranes; hence, nanoparticle carriers with both hydrophilic and lipophilic properties are suitable for mucosal antigen delivery. The colloidal stability also affects the cellular interaction, as nanoparticle suspensions can aggregate over time and become internalized by mononuclear cells [120]. For polymeric nanoparticles, additional factors should be considered during formulation of vaccines, for example, the glass transition temperature, crystallinity, and bioadhesiveness. Above the glass transition temperature, there is an increased molecular mobility and free volume of amorphous polymers, which increases the release of loaded cargo from polymers [121]. The amorphous content of polymers correlates with a high antigen release, whereas the crystalline state results in decreased release rate of the loaded antigen [121, 122]. Inclusion of bioadhesive polymers, for example, chitosan, hyaluronic acid, carboxymethyl cellulose, and sodium alginate, enhances the interaction and prolongs the contact time with the mucosal surfaces, and hence, these drug delivery systems are efficient for targeting drugs to specific cells or intracellular compartments within the mucosa [123]. Thus the physicochemical properties of nanoparticle-based drug delivery systems are important, and customizing these properties represents a unique opportunity for tailoring the interaction with immune cells and the specific type of immune response, which is elicited.

25.7   Nanoparticle-based delivery systems for mucosal vaccines

25.7.1   Lipid-based nanoparticles

Liposomes have been investigated extensively for drug delivery applications, and they show great promise for the delivery of vaccine antigens beyond the mucosal

membranes. Liposomes are usually composed of phospholipids based on natural lipids of biomembranes. Liposome vesicles consist of one or several concentric phospholipid bilayers forming uni- or multilamellar vesicles, respectively, with an inner aqueous core [124]. Both the inner aqueous core and outer bilayer membrane of liposomes are suited for delivering hydrophilic or lipophilic cargoes, respectively [124]. Liposomes are considered as proficient carriers of antigens through several mucosal membranes, and they constitute highly versatile carriers because their composition and physicochemical properties can be tailored according to the desired immune response. For example, cationic liposomes can be customized for complexation and delivery of antigens based on peptides, proteins, plasmid DNA, or mRNA, and pH-sensitive liposomes can be tailored for pH-activated release of bioactive agents [125]. In addition, the liposome surface can be decorated with, for example, ligands for specific cell targeting, bioadhesives, polyethylene glycol (PEGylation), and mannose derivatives, and they can be incorporated with toll-like receptor (TLR) ligands, other PRR ligands, and immunoglobulins for their efficient delivery of encapsulated or conjugated antigens [64, 126, 127].

Liposomes have been investigated for mucosal delivery of vaccines directed against many infectious diseases [128], and a number of liposome-based subunit vaccines are currently being tested in clinical trials [124]. Immunization with liposome-based vaccines via the nasal route has been evaluated extensively and shown to induce robust mucosal immune responses and protection against a variety of pathogens. For example, cationic liposomes based on dioleoyltrimethylammoniumpropane (DOTAP) and dimethylaminoethane-carbamoyl (DC) cholesterol were found to induce strong immune responses against the model protein antigen ovalbumin (OVA) in the nasal mucosa [129–131]. Recently, it was shown that the nasal immunity induced by these liposomes confers significant protection against infection with *Streptococcus pneumonia* [58]. Cationic adjuvant formulation 01 (CAF01), which consists of liposomes composed of dimethyldioctadecylammonium (DDA) and trehalose dibehenate (TDB), is another prominent example of an adjuvant, which induces mucosal immunity and protection. CAF01 has been evaluated in several animal models of infection, for example, influenza [132], *Chlamydia trachomatis* [33, 133], *Leishmania* [59], *Streptococcus* [60], and *Mycobacterium tuberculosis* [61]. In addition to nasal delivery, administration of liposomes via other mucosal routes has been investigated for vaccine delivery [41, 62, 64]. Oral administration of liposomal vaccines has been shown to induce protective mucosal immunity against influenza [62, 127] and *Salmonella* infection [63]. Vaccine stability in the acidic environment of the gut following oral delivery is of concern. However, this challenge can be overcome by incorporating mannose [127], galactose [134], chitosan [126], or PEG [135] for targeted delivery through the mucosa of the gastrointestinal tract. Sublingual [64] and intrapulmonary immunization [41] represent additional potentially beneficial routes for mucosal administration of liposome-based vaccines, which needs further investigation.

Liposomes have been surface-modified with polymers for efficient mucosal delivery of vaccine antigens. For example, liposomes modified with the pH-sensitive fusogenic polymer 3-methyl-glutarylated hyperbranched poly(glycidol) or succinylated poly(glycidol) induced humoral and cellular mucosal immunity [136, 137].

Upon additional incorporation of the TLR-9 ligand cytosine-phosphodiester-guanine (CpG) DNA into these liposomes, they induced even stronger immune responses and antitumor effects [138]. In a separate study, CAF01-modified poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles induced strong antibody and Th1-type immune responses against a *C. trachomatis* antigen delivered mucosally [102].

Another category of lipid-based nanoparticles that is studied for mucosal vaccine delivery is interbilayer cross-linked multilamellar vesicles (ICMVs), which are formed by cross-linking the lipid headgroups of multilayered liposomes [139]. Antigens adjuvanted with ICMVs were found to prime 13-fold more CD8^{+} T cells than soluble antigens and induced long-lived memory T cells in both the lungs and vaginal mucosa following pulmonary administration [42].

Solid lipid nanoparticles represent another class of lipid-based nanoparticles, which can be used to deliver vaccine antigens across the mucosa. Solid lipid nanoparticles have been used for rectal delivery of hepatitis B antigen, and strong systemic and robust mucosal immunity was induced, as compared with parenteral immunization [65]. Recently, these nanoparticles were encapsulated in enteric-coated minicapsules, which induced significant immunity against hepatitis B virus [66]. Cubosomes composed of multiple lipid bilayers with aqueous channels represent another type of lipid-based nanoparticles, which have been explored as vaccine delivery systems [140]. Recently, cubosomes were used to deliver antigen across the oral mucosa [67, 68].

Emulsions, prepared by dispersing two immiscible liquids, either water-in-oil (w/o) or oil-in-water (o/w) emulsions stabilized with an amphiphilic surfactant, are able to deliver vaccine antigens through the mucosal surfaces [141]. Soybean oil [142] and squalene are the most commonly used oils for preparing emulsions [143]. The w/o emulsions integrate and more efficiently deliver hydrophilic drugs than o/w emulsions, which are used to encapsulate and deliver hydrophobic drugs [141]. The controlled release characteristics of emulsions are defined by their physicochemical properties, for example, the viscosity of the oil phase, the ratio of oil-to-water phase, and the droplet size [141]. Freund's vaccine adjuvants are classic examples of emulsion-based adjuvants, which have been used as a standard to develop new vaccine adjuvants. The squalene-based o/w emulsions MF59 and AS03 are widely used adjuvants, which have been approved for commercial use since 1997 and 2009, respectively, for influenza vaccines [144]. Nanoemulsions have been used to induce neutralizing serum antibodies and produce a Th1- and Th17-biased cellular immunity in the mucosa against vaccine antigens of anthrax [145], influenza virus [69], human immunodeficiency virus (HIV) [72], hepatitis B virus [142], and respiratory syncytial virus [70]. In another study a nanoemulsion-adjuvated vaccine was developed to induce systemic and mucosal immunity against methicillin-resistant *Staphylococcus aureus* infection [71]. In addition to single emulsions, double emulsions have been used to deliver antigens through the mucosa, and the double emulsion technology was found to preserve the antigen and stabilize the formulation during the emulsification process more efficiently than the single emulsion method [141]. Recently, MF59-based cationic nanoemulsions were used for nonviral delivery of self-amplifying mRNA vaccines expressing HIV [146] or influenza antigens [147].

Immunostimulatory complexes (ISCOMs) are spherical, micellar, and particulate antigen delivery systems of approximately 40 nm in size, which are composed of antigen, cholesterol, phospholipid, saponin, and the adjuvant Quil A [148]. ISCOMATRIX is a similar particulate adjuvant system but without the antigen. Both delivery systems have been approved for veterinary vaccines and are undergoing clinical testing in humans [149]. ISCOMs have been extensively used for delivering vaccine antigens through mucosal surfaces. Using ISCOMs with a fusion of cholera toxin and the *S. aureus* protein A (CTA1-DD) administered orally or nasally, strong cell-mediated and humoral immune responses, including local mucosal IgA, were induced against OVA peptide epitopes [150]. Using the same formulation, it was shown that CTA1-DD/ISCOMs when combined with major outer membrane protein of *C. trachomatis* stimulated CD4^{+} T-cell immunity and protected against *C. trachomatis* infection in the genital tract [151]. Strong humoral and cellular mucosal immune responses were also observed against hepatitis B antigen [152], plasmid DNA of *Haemophilus influenzae* [153], and palmitoylated diphtheria toxin [74] administered orally or nasally with ISCOMs. In another study, loading of the *M. tuberculosis* antigen 85 complex into ISCOMs significantly improved humoral and cellular immune responses after intrapulmonary immunization in mice [154]. ISCOMATRIX was reported to be an effective adjuvant for inducing mucosal immunity against influenza [155] and human T-cell lymphotropic virus type 1 [73].

25.7.2   *Nanoparticles based on natural polymers*

Chitosan is a natural linear polysaccharide isolated primarily from the exoskeleton of crustaceans, and its properties are well documented, including high biocompatibility and biodegradability. In addition, chitosan has mucoadhesive properties, which are attractive for mucosal vaccine delivery. Chitosan has been extensively investigated for delivery of vaccine antigens across the mucosal surfaces in several preclinical animal models of infection, for example, tuberculosis [75], influenza [76], and chlamydia [102]. However, chitosan displays low solubility under physiological conditions, which limits its use for biomedical applications [156]. To overcome this drawback a number of chitosan derivatives have been developed, including trimethyl chitosan, hydroxyethyl chitosan, chitosan ester, phosphorylated chitosan, and sulfated chitosan [157]. Trimethyl chitosan is the most studied chitosan derivative for mucosal vaccine applications. Trimethylated chitosan nanoparticles have been shown to induce strong mucosal immunity against hepatitis B virus following nasal administration [77]. Recently, trimethyl chitosan-coated liposomes were shown to induce high mucosal and systemic antibody titers upon nasal administration [78] and provided protection against infection with Group A *Streptococcus* [79].

Gamma polyglutamic acid, which is a polypeptide composed of glutamic acid monomers, displays high water solubility and biodegradability, and it has been used for mucosal vaccine delivery. Gamma polyglutamic acid-based nanoparticles have been shown to induce protective immunity against influenza virus infection following nasal immunization [80]. In another study, poly-gamma-glutamate/chitosan nanoparticles induced protective mucosal immunity against influenza virus infection [81].

A recent study reported strong mucosal immune responses in the nasal mucosa against Group A *Streptococcus* infection using polyglutamic acid-trimethyl chitosan-based nanoparticles [79].

Hyaluronic acid is a linear polysaccharide composed of glucuronic acid and acetyl glucosamine monomers. It has high biocompatibility, biodegradability, and mucoadhesiveness, which are attractive properties for mucosal vaccine delivery. Hyaluronic acid represents a multifunctional carbohydrate mediator of immune processes because it modulates leukocyte trafficking, which leads to maturation and migration of DCs and subsequent T-cell activation [158]. Hyaluronic acid-based delivery systems have been tested for mucosal vaccine applications. Hyaluronic acid combined with heat labile toxin-based mucosal adjuvant (LTK63) was shown to induce systemic and mucosal antibody responses following nasal administration with the influenza hemagglutinin antigen [82]. In another study, nasal delivery of F1-V, which is a candidate recombinant antigen from *Yersinia pestis*, using nanoparticles composed of hyaluronic acid and cationic liposomes induced strong humoral and a balanced Th1/Th2 immune responses [83].

Pullulan is another water-soluble linear polysaccharide, which has been used for nanoparticle-based vaccine delivery systems designed to deliver antigens across the mucosa. A nanogel consisting of a cationic type of cholesteryl group-bearing pullulan formulated with *Clostridium botulinum* type-A neurotoxin induced strong tetanus toxoid-specific systemic and mucosal immune responses after nasal immunization [84]. In addition, the pullulan-based nanogels induced Th2 and Th17 cytokine responses in the serum and respiratory tract tissues of macaques after nasal coadministration with pneumococcal surface protein A [85]. In an another study, nasal administration of cholesteryl pullulan-encapsulated tumor necrosis factor-α nanoparticles provided protective immunity against influenza infection [86].

25.7.3   Nanoparticles based on synthetic polymers

The use of nanoparticles based on synthetic polymers has been widely explored for delivery of vaccine antigens across the mucosa, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), PLGA, polyethyleneimine (PEI), poly-ε-caprolactone (PCL), and polyphenylene sulfide (PPS). Among these, PLGA and its derivatives, which are aliphatic polyesters, are the most extensively studied delivery systems for vaccine antigens due to their safety, biodegradability, and biocompatibility [159]. Upon in vivo administration, PLGA undergoes hydrolysis into lactic acid and glycolic acid monomers, which are removed from the body via the citric acid cycle [159]. One of the characteristic features of PLGA nanoparticles is the controlled and sustained release of antigen and adjuvant over several weeks or months, which can generate effector T-cell memory responses [160]. Moreover the type of polymer used, the molecular weight, and the manipulation of physicochemical particle properties allow for the design of PLGA nanoparticles with customized release kinetics [159]. Another important feature of PLGA nanoparticles is their ability to encapsulate not only the vaccine antigen but also various ligands for PRRs, for example, TLR ligands. PLGA nanoparticles incorporating the TLR4 ligand monophosphoryl lipid A (MPLA) and

OVA were shown to induce higher mucosal IgA and IgG titers after oral administration than PLGA without MPLA [87]. In a recent study, PLGA nanoparticles prepared with MPLA induced mucosal and systemic immune responses against the HspX/EsxS fusion antigen of *M. tuberculosis* after nasal administration [88]. Oral administration of PLGA nanoparticles loaded with a TLR7 agonist, MPL, and OVA was shown to target M cells and induce mucosal IgA and serum IgG antibodies [161]. Similarly, PLGA nanoparticles formulated with the TLR9/21 agonist CpG oligodeoxynucleotide [162] or the TLR7 agonist imiquimod [163] induced strong mucosal immune responses against protein antigens. A synergistic increase in antigen-specific neutralizing antibodies against influenza virus infection [164] and protection against simian immunodeficiency virus (SIV) [165] was observed when formulating PLGA nanoparticles with both TLR4 and TLR7 agonists, as compared with nanoparticles containing antigens each of the TLR ligands alone. Moreover, combining TLR2, TLR3, and TLR9 ligands with PLGA nanoparticles has been shown to synergistically induce mucosal antiviral protection after mucosal immunization with HIV envelope peptides [166]. PLGA nanoparticles can also be rationally designed for targeted delivery of vaccine antigens across the mucosa. Oral administration of PLGA nanoparticles modified with hydroxypropyl methylcellulose phthalate HP55 was shown to promote immune protection against *Helicobacter pylori* infection by protecting the antigen from degradation in gastric acid [89]. Similarly, PLGA nanoparticle surface coating with the methacrylate-based polymer Eudragit FS30D was shown to prevent the denaturing effects of the low pH in the stomach and enzymatic destruction and protected against genitorectal HIV infection after oral immunization [90]. Other studies have reported formulating PLGA with lectin [161], claudin 4 [167], and PEGylation [168] for specific targeting of M cells in the mucosa and a corresponding increase in mucosal immune responses. PLA nanoparticles have also been used for mucosal delivery of vaccine antigens. PEG [169] and PEG-derived block copolymers of PLA [91] have been used to develop nanoparticles encapsulating antigens for mucosal delivery. Encapsulating nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and NOD2 ligands in PLA nanoparticles coated with the HIV-1 gag p24 antigen enhanced systemic and mucosal immune responses after oral and nasal administration [170]. Mucosal immunization with PLA nanoparticles specifically target DCs leading to improved mucosal immunity [171].

The cationic polymer PEI has primarily been investigated for gene delivery due to its ability to form polyplexes with nucleic acids via attractive electrostatic interactions [172]. However, there is increased evidence that PEI-based nanoparticles can improve the efficacy of conventional vaccines by (i) enhancing the maturation rates of APCs; (ii) increasing the proliferation of effector cells; and (iii) stimulating the production of antibodies, cytokines, and chemokines [172]. One of the characteristic features of PEI nanoparticles is their buffering capacity, that is, the so-called proton sponge effect, under acidic conditions inside the phagolysosomes, which allows phagocytosed or endocytosed antigens to escape from the vacuole and gain entry to the cytosol and subsequent presentation on major histocompatibility complex class I molecules, which is referred to as cross presentation [173]. This improved antigen cross presentation generates strong immune responses [172, 174]. PEI nanoparticle-formulated protein

vaccines were found to induce strong mucosal immunity against influenza [92], HIV [93], and respiratory syncytial virus [175] following mucosal administration. PEI nanoparticles have been used to deliver plasmid DNA and induced strong mucosal immunity against influenza virus [176], herpes simplex virus [176], and coronavirus infection [177] and protection against HIV infection [94]. Recent studies have shown that PEI conjugated to cyclodextrin serves as an efficient carrier for nasal mRNA vaccine delivery, lymph node trafficking, and improved immune responses [178, 179]. Although PEI is proved as a potential candidate for gene delivery, the high transfection efficiency is also associated with high toxicity [180]. Hence, a number of PEI modifications have been shown to reduce its cytotoxicity while maintaining the transfection efficiency [181] and improving biodegradability [182].

PCL is a synthetic biodegradable, semicrystalline, hydrophobic, aliphatic polyester, which can be used for delivering protein-based vaccine antigens or DNA. It was shown that PCL-based nanoparticles can be used to efficiently deliver antigens through the mucosa due to its hydrophobicity and resistance to acidic pH environments [183]. In another study, PCL/PLGA copolymer-based nanoparticles were used to deliver diphtheria toxoid nasally, and they generated higher IgG antibodies than PLGA nanoparticles alone [184]. Nasal administration of PCL nanoparticles has also been shown to increase immunogenicity and mucosal immune responses against *Streptococcus* antigens [98]. Similarly, chitosan [95, 96] and PEG-modified [97] PCL-based nanoparticles have been used to deliver vaccine antigens through the nasal mucosa, and they elicited strong mucosal and systemic immune responses.

PPS is a synthetic, hydrophobic, and biodegradable polymer, which has been used to prepare nanoparticles for delivery of mucosal vaccines. PPS-based nanoparticles can be prepared as small as 20 nm in diameter, and they have been shown to efficiently present antigens to APCs in the draining lymph nodes [185] and activate the alternate complement pathway [186], thereby stimulating cellular and humoral immune responses. When coadministered nasally with OVA, these nanoparticles promote mucosal and systemic CTL responses, and addition of the TLR5 ligand flagellin further enhances the humoral responses in the lungs and in the vaginal and rectal mucosa [99]. However, the magnitude and quality of the mucosal immune responses after nasal immunization were shown to vary in a nanoparticle size-dependent manner [187]. In another study, PPS-based nanoparticles with CpG were shown to generate 10-fold higher frequencies of effector $CD8^+$ T cells in lungs than CpG and antigen alone, and they induced protective immunity in lungs against influenza virus infection [100].

Dendrimers are synthetic polymers, which are three-dimensional, hyperbranched, and monodisperse structures containing a central core surrounded by branched peripheral groups and numerous functional groups on the surface [188]. Depending on the polymer used, dendrimers can be hydrophilic or hydrophobic and can incorporate the antigen inside the core or on the surface [188]. Dendrimer properties like nanoscale size, a high degree of branching, polyvalency, and water solubility make them interesting systems for delivery of antigens and nucleic acids [188]. In one study, oral administration of a rhesus C–C chemokine receptor type 5-derived cyclopeptide conjugated with tetragalloyl-D-lysine dendrimer in rhesus macaques led to a statistically significant increase in the antigen-specific stool IgA response, which had a

neutralizing activity against SIV infection [189]. Recently, intranasal administration of HIV-1 gp120 peptide complexed with fourth-generation polyamidoamine (G4-PAMAM) dendrimers was found to induce peptide-specific IgG and IgA responses in serum, nasal fluids, and vaginal washes of mice [101].

25.7.4 Lipid-polymer hybrid nanoparticles

Lipid-polymer hybrid nanoparticles (LPNs), which combine the properties of lipids and polymers, have become prominent drug delivery systems during the past few years [190]. Usually, LPNs are made up of three components: (i) an inner polymer core, which encapsulate drugs or vaccine antigens; (ii) a lipid layer covering the polymer core; and (iii) an outer PEG layer, which prolongs the in vivo circulation time and provides steric stabilization to LPNs [191]. LPNs consisting of a poly(β-amino ester) core enveloped by a phospholipid bilayer shell have been tested for mRNA-based vaccine delivery and were found to significantly enhance the expression of the reporter protein luciferase within 6 h after nasal administration [103]. We have previously demonstrated engineering of LPNs comprising PLGA modified with CAF01 using a quality-by-design approach [192]. It was recently shown that these LPNs, when coformulated with chitosan, significantly increase mucosal immune responses against the recombinant *C. trachomatis* fusion antigen CTH522 delivered nasally, as compared with CAF01 alone [102]. In another study, nasal delivery of LPNs consisting of cationic liposomes composed of DOTAP, dioleoyl phosphatidylethanolamine, and hyaluronic acid and coloaded with the recombinant fusion protein F1-V of *Y. pestis* led to strong humoral immune responses with 11-, 23-, and 15-fold increases in F1-V-specific total IgG, IgG1, and IgG2c titers, respectively, and a balanced Th1/Th2 humoral immune responses [83]. Hence, LPNs possess many advantages, for example, controlled delivery, high encapsulation efficiency, adjustable drug release profile, and potential to deliver both hydrophilic and hydrophobic antigens, and they also demonstrate potential for novel therapeutic vaccine strategies [193].

25.7.5 Virus-like particles and virosomes

Virus-like particles (VLP), which are composed of viral structural proteins that self-assemble into particle structures, mimic live viruses but lack the viral genome [194]. VLPs can present viral antigens in their native conformation, and they stimulate high immune responses. Recombivax (hepatitis B virus), Gardasil (human papillomavirus), and Hecolin (hepatitis E) represent examples of VLP-based marketed vaccines [195]. A number of other VLP-based vaccines are under development. However, these vaccines often require coadministration of adjuvants to be effective. Virosomes are vaccine delivery systems, where viral membrane proteins are integrated into unilamellar vesicles, which are composed of viral and other natural or synthetic lipids [128]. Unlike VLPs, virosomes have been shown to possess intrinsic adjuvanticity [196], although addition of an adjuvant may further potentiate vaccine efficacy [197]. A number of reports describe the application of virosomes for mucosal vaccine delivery. In a simian model of HIV infection, nasal administration of HIV-1 gp41 peptide subunit

virosomes conferred full protection against a vaginal simian-HIV challenge, which correlated with mucosal IgA and IgG responses and antibody-dependent cellular cytotoxicity [104]. Immunogenicity and antiviral activity induced by the same vaccine construct were also demonstrated in a randomized phase I clinical trial [198]. Nasal and sublingual administration of virosomes adjuvanted with innate immune receptor ligands, for example, TLR2 (Pam3CsK4) and/or NOD2-ligands (L18-muramyl dipeptide), or adjuvants, for example, c-di- adenosine monophosphate, c-di-guanosine monophosphate, cholera toxin B subunit, or ISCOMs (Matrix M), were shown to protect against influenza virus and respiratory syncytial virus infections in mice by promoting mucosal and systemic humoral and cellular immune responses [105, 106]. When used to deliver plasmid DNA, virosomes induced strong Th1 immune responses and improved vaccine efficacy following nasal administration [199, 200] owing to efficient delivery of DNA to DCs by virosomes [201].

25.7.6 Gas-filled microbubbles

Gas-filled microbubbles are micron-sized spherical structures, which comprise an inert high-molecular weight gas entrapped in a lipid- or polymer-based shell, and were originally developed as ultrasound contrast agents for imaging of the blood compartment [202]. These microbubbles have also been used for delivery of antigens [203], small molecule-based active pharmaceutical ingredients [204], and nucleic acid-based therapeutics [205] due to their characteristic capability of sonoporation upon ultrasound application [206]. However, it has been shown that lipid-based microbubbles can be taken up by APCs and deliver their antigenic cargo without ultrasound application, eventually leading to T-cell activation [207, 208]. Gas-filled microbubbles can be tailored by incorporating cationic lipids or adjuvants for enhanced immunogenicity of the antigenic cargo [204], which allows their application for mucosal vaccine delivery. In a recent study, α-galactosylceramide-adjuvanted microbubbles, which displayed the *Salmonella*-derived SseB antigen on their surface, induced strong antibody responses in the gut following nasal administration and significantly reduced the bacterial load after *Salmonella* infection [107]. In another study, therapeutic nasal administration of allergen-loaded microbubbles was found to suppress experimental allergic asthma in mice and was accompanied by a decrease in eosinophils, neutrophils, and IgE in the bronchoalveolar lavage and reduced frequencies of Th2 cytokine- and IL-17-producing $CD4^+$ T cells in the lungs [108]. The development of cationic microbubbles has further enabled noninvasive delivery of nucleic acid therapeutics in vivo via the formation of strong attractive electrostatic interactions between cationic microbubbles and nucleic acids, which protect nucleic acids from degradation by nucleases [205].

25.8 Nanoparticulate pulmonary mucosal vaccination

Nanoparticulate formulations are being engineered for pulmonary mucosal vaccination to mimic pathogens while still maintaining the safety of vaccine antigens. Although pulmonary mucosal vaccination is a promising approach for inducing protection

against respiratory infections, many technical challenges persist. These include (i) design of appropriate and safe adjuvants and vaccine formulations for pulmonary administration; (ii) identification of the region in the respiratory tract, which is optimal for vaccine delivery and induction of protective immunogenicity; (iii) optimization of particle size and aerosolization properties for pulmonary distribution; (iv) optimization of antigen dose to stimulate protective immunity and avoid induction of tolerance; and (v) engineering of affordable devices, which are optimal for preclinical and clinical pulmonary vaccine administration [209, 210]. Appropriate formulation of antigens into solid dosage forms can result in improved vaccine stability and eliminate the need for costly cold-chain storage associated with traditional liquid vaccine dosage forms. Spray drying is one of the most commonly used scalable methods to manufacture dry powder aerosolizable antigens and nanoparticulate adjuvants [211]. Using spray drying, we have previously engineered a dry powder-based formulation for inhalation of the CAF01 adjuvant, which had preserved adjuvant activity after drying [212]. We also showed that a dry powder-based formulation of the tuberculosis subunit vaccine H56/CAF01 prepared by spray drying displays preserved vaccine-induced humoral and cell-mediated immune responses, as compared with the liquid formulation [213]. Recently, we demonstrated that the H56/CAF01 vaccine induces strong lung mucosal and systemic IgA and polyfunctional Th1 and Th17 responses after parenteral prime and intrapulmonary boost immunization [41]. Similarly, dry powder-based vaccine formulations prepared by spray drying have been evaluated against influenza [214], pertussis [215], diphtheria [216], hepatitis B [217], *S. pneumonia* surface protein A [218], and measles [219] antigens upon pulmonary immunization. Previously a dry powder Bacillus Calmette-Guérin (BCG) vaccine prepared by spray drying displayed improved viability, as compared with a lyophilized vaccine [220], and significantly reduced bacterial burden and lung pathology upon an experimental tuberculosis challenge, as compared with the standard parenteral liquid BCG vaccine [221]. To optimize the critical quality attributes of the dry powder-based subunit vaccines, for example, particle size and aerosolization properties, a systematic quality-by-design (QbD) approach is now frequently used. Using QbD, we optimized the formulation of dry powder CAF01 [211]. Similarly, other studies have demonstrated QbD-based formulation optimization of vaccines against tuberculosis [222, 223], influenza [224], hepatitis B [225], *Neisseria meningitidis* serogroup B [226], and flavivirus [227]. It is known that the particle size affects the deposition site of particles delivered through inhalation, with particles below approximately 3 μm in aerodynamic diameter depositing in the alveolar region and particles above approximately 5 μm in aerodynamic diameter depositing in the conducting airways [228]. Similarly the particle size, surface charge, and surface chemistry are important parameters, which determine the uptake and trafficking of particles by APCs and the fate and dynamics of particle clearance from the lungs [229–231]. However, the regions of lung deposition of particles optimal for safety, immunogenicity, and efficacy of vaccines is not well known. In one study, deep lung deposition of a monovalent influenza vaccine is correlated with superior immune responses [232]. However, in a recent study, the site of antigen deposition was shown to be of minor relevance in inducing protection against influenza virus infection [233]. Noninvasive and high-resolution molecular imaging techniques

that are clinically translatable, for example, positron emission tomography (PET); single-photon emission tomography (SPECT); magnetic resonance imaging (MRI); or combined multiimaging modalities such as PET-CT, SPECT-CT, and PET-MRI, aim to offer the possibility of generating this knowledge [234, 235]. Using SPECT-CT, we have shown the biodistribution and pharmacokinetics of the H56/CAF01 vaccine upon pulmonary immunization in mice [41]. Similarly, SPECT-CT imaging was used for the regional distribution and quantification of inhaled particles in an aerosolized human papillomavirus (HPV) vaccine in human volunteers [236]. PET-CT imaging was used in other studies for evaluating immune responses in human patients against a prostate cancer [237] and HPV vaccine [238]. MRI has been used to track immune cell recruitment and vaccine efficacy against cancer [239, 240]. Using a QbD approach, we have recently formulated a novel gadoteridol-loaded CAF01 formulation and evaluated real-time MRI contrast enhancement of lungs in mice following intrapulmonary administration [241]. Vaccine delivery to the lungs in preclinical rodent models require an efficient device that can aerosolize the formulation and deposit the aerosols evenly in the lungs of the animals. A number of devices have been developed and tested for delivering powders to lungs in preclinical animal studies, including whole-body, intranasal, and intratracheal exposure systems [242]. Pulmonary immunizations were performed with a dry powder insufflator (Penn-Century, Inc., Wyndmoor, PA, USA) to deliver dry powder BCG vaccine [243] and a subunit tuberculosis vaccine combining cutinase-like proteins 1 and 6 and MPT83, a secreted lipoprotein antigen with the adjuvant Lipokel [244] and a whole inactivated influenza virus vaccine [43, 245], which were found to be protective against an experimental challenge. In another study, a novel aerosol generator was developed and was shown to distribute powders homogeneously in mice lungs as compared with the dry powder insufflator [246]. Recently the PreciseInhale platform (Inhalation Sciences AB, Sweden) has enabled the administration of dry powders with high accuracy and reproducibility, with low tracheal deposition [247, 248]. Despite many challenges, considerable progress has been made for developing and testing dry powder vaccines for pulmonary delivery with a growing optimism for commercializing the first aerosolized mucosal vaccine.

25.9   Conclusions and future perspectives

Nanoparticle-based drug delivery systems offer unique opportunities in the development of novel subunit vaccines and immunotherapies, as compared with conventional vaccines based on live or inactivated pathogens. A number of nanoparticle-based delivery systems for mucosal vaccination have been described, which have their own advantages and disadvantages. The new generation of mucosal vaccines, which use components of microbes to elicit an immune response, does require not only the development of novel classes of vaccine delivery systems and adjuvants but also an understanding of the mechanisms of action of existing ones. Although outstanding progress has been made in the development of nanoparticles for delivering antigens through the mucosal surfaces, challenges and unmet needs remain. Presently, targeted delivery in mucosal vaccination aims only at mucosal epithelium and can benefit from

M cell-targeted approaches. Similarly, immune correlates of mucosal adjuvant and vaccine-mediated protection are not well established, the knowledge of which can help in developing novel mucosal vaccines. The development of novel devices for noninvasive delivery and experimental testing of mucosal vaccines will greatly benefit the field. Another area of future research is the evaluation of the impact of physicochemical properties of nanoparticles on specific immune pathways in the mucosa. Finally the overarching goal of most adjuvant research is to develop new adjuvants that can be translated to clinical use. Nanotechnology offers the platform technology for exploring each of these ideas and promises the potential for the successful realization of developing novel nanoparticulate mucosal vaccines against infectious diseases, cancer, and autoimmunity.

Acknowledgments

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