CHAPTER FIFTEEN

Nasal Vaccine Delivery

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15.1 INTRODUCTION

15.1.1 Mucosal infections

Mucosal infections are caused by emerging pathogens such as bacteria and viruses, which are mucosally transmitted and replicate in the mucosal tissues. These pathogens enter the human through single or multiple mucosal sites such as oral, nasal, conjunctival, respiratory, gastrointestinal, and genitourinary tracts, invade the bloodstream, and finally lead to infection. Approximately 68% of deaths in children younger than 5 years are due to mucosal infections.[1] Bacteria such as *Streptococcus pneumoniae*,[2] *Staphylococcus aureus*,[3]

anaerobic bacteria,[4] *Streptococcus viridans*,[5] α-*Streptococci*, Enterobacteriaceae, and viruses such as *Haemophilus influenzae*, Corona virus, and human adenovirus[6] cause mucosal infections. The mucosal infections are treated by antibiotics, and vaccines are administered through oral, parenteral, and nasal routes. A nasal vaccine induces mucosal immune responses and systemic immunity, which provides better protection against infectious agents. Yet developing vaccine delivery systems that induce humoral and cell-mediated response with mucosal immunity has been challenging to date. Different mucosal routes are being explored using different delivery systems, and the nasal route is preferred as compared to other mucosal routes.

15.1.2 Importance of nasal vaccines

The mucosal route of vaccine delivery is one of the better alternatives to conventional multiple injection vaccines, which employ traumatic procedures and may also lead to the spread of infectious agents via contaminated syringes. Mucosal vaccine development has grown extensively, and a multitude of vaccine delivery systems have been developed for application via ocular, nasal, oral, rectal, and vaginal routes. Of all the different routes of mucosal vaccination, the oral and nasal routes are most accepted and easily accessible. For many decades, intranasal applications of tobacco snuff, cocaine, and various hallucinogenic and psychotropic agents have been in practice.[7] Similarly, for many years synthetic drugs have been administered intranasally for their local effect on the mucosa (eg, antihistamines, antibiotics, and nonpeptide drugs).[8] The first nasal influenza vaccine was introduced in 2001, but it was later withdrawn from the market due to potential toxicity problems. Another intranasal vaccine (Flumist) was launched in 2003 and was administered using a syringe sprayer. Success of a vaccine depends not only on the delivery but also on the route of administration; for example, nasal immunization elicits potent immunoglobulin A (IgA) secretion in the respiratory tract.[9] Nasal administrations of vaccines have been shown to achieve a better systemic bioavailability and protection from gastric enzymes compared with parenteral and oral administration. Nasal delivery of vaccines acts as a "first entry block," that is, blocks the pathogen entry, while invading to the mucosal surface by inducing local microbial-specific immune responses, thus increasing the general efficacy of the vaccine. In addition, vaccine uptake into the blood circulatory system by absorption through mucosa can be relatively fast.[10] It is the most appropriate method of immunization because it is rich in T cells, B cells, and plasma cells and stimulates both antigen-specific systemic and mucosal adaptive immune responses. It provides better patient compliance due to the needle-free delivery.

15.1.3 Benefits and challenges of developing nasal vaccines

The nasal route is considered an attractive route for vaccine administration with the following advantages:

- Better patient compliance
- Numerous microvilli present in the nasal epithelium provide a better absorption surface
- Mucosal and systemic immune response can be induced

- Easy immunization of large population groups
- Nasal immunization does not require needles and syringes

Many challenges stand in the way of developing nasal vaccines. When nasal vaccines are administered directly on mucosal surface, the antigens may be diluted by mucosal secretions, seized in mucus gels, attacked by proteases and nucleases, and obstructed by epithelial barriers. Major challenges in the development of nasal vaccines include:

- A relatively large dose of vaccine is required, and it is difficult to administer through the nasal route and also difficult to monitor the actual dose that crosses the mucosa.
- Costly innovative vaccination strategy.
- Efficacy of nasal vaccines may be limited due to the including mucociliary clearance and the inefficient uptake of soluble antigens by nasal epithelial cells in the nasal cavity.[11]
- Nasal delivery may require adjuvants to enhance their immunogenicity and delivery to the mucosal tissues.
- Lack of multiple human-compatible mucosal adjuvants.
- Rapid nasal clearance may not allow sufficient retention for antigen to be taken up by antigen-presenting cells (APCs) in the nasal-associated lymphoid tissue (NALT).
- Several enzymes that are present in the nasal mucosa might affect the stability of drugs. For example, proteins and peptides are subjected to degradation by proteases and amino-peptidase at the mucosal membrane.[12]
- Delivery volume in the nasal cavity is restricted to 25–200 μL.[13]
- High molecular weight compounds cannot be delivered through this route (mass cutoff ~1 kDa).[14]
- Low antigen entrapment efficiency mode.
- Normal defense mechanisms like mucociliary clearance and ciliary beating affect the permeability of the drug.[15]

15.2 THE NASAL ROUTE

The nose is a vital organ in the human body for breathing. The nose has a more complex role as a complete system of defense against inhaled air and air conditioning. The nasal anatomical and physiological structure provides support for nasal immunization against upper respiratory mucosal diseases. Nasal anatomy, nasal morphology and physiology, nasal secretions, nasal mucosa, olfactory region, and blood supply to the nasal cavity are described with respect to their link with the vaccine administration route.

15.2.1 Nasal anatomy

The nasal cavity is protected by the viscerocranium in the human head. The human nose is divided by the median septum into two symmetrical halves; each half opens to the face through the nostrils and extends posteriorly to the nasopharynx.[16] The nasal cavity and the nasal vestibule are the anterior part, which opens to the face through the nostrils. The atrium is placed in an intermediate region between the vestibule and the respiratory

region. The respiratory region, the nasal turbinates, occupies more area of the nasal cavity. It possesses lateral walls that divide it into three sections composed of the superior nasal turbinate (superior region), nasal turbinate (middle region), and inferior turbinate (inferior region). The attachment of the turbinate to the lateral wall is more complex in animals than in humans. These folds provide the nasal cavity with a very high surface area compared to its small volume.

15.2.2 Nasal morphology and physiology

The basic functions of the nose are filtration of particles and heating and humidification of inspired air before it reaches the lungs. The olfactory region situated above the superior nasal turbinate possesses specialized ciliated olfactory nerve cells for smell perception.

The human nasal cavity has a total volume of 15–20 mL and a total surface area of approximately 150 cm^2,[17] of which the respiratory region covers about 85%. Each nasal cavity can be subdivided into different regions such as nasal vestibule, turbinate (inferior, middle, and superior), olfactory region, frontal sinus, sphenoidal sinus, and cribriform plate of ethmoid bone. NALT is present in the nasal cavity, which is situated in the nasopharynx. The central axon of these nerve cells passes through the cribriform plate of the ethmoid bone and into the olfactory bulb. The nasal vestibule has numerous nasal hair (vibrissae) that filter large airborne particles (Fig. 15.1).

The anterior section of the nasal portion is constituted by a stratified squamous epithelium with sebaceous glands and the posterior section of the nasal portion by pseudostratified columnar cells presenting microvilli. The nasal respiratory mucosa is considered the most important area for delivering drugs systemically, as its made of
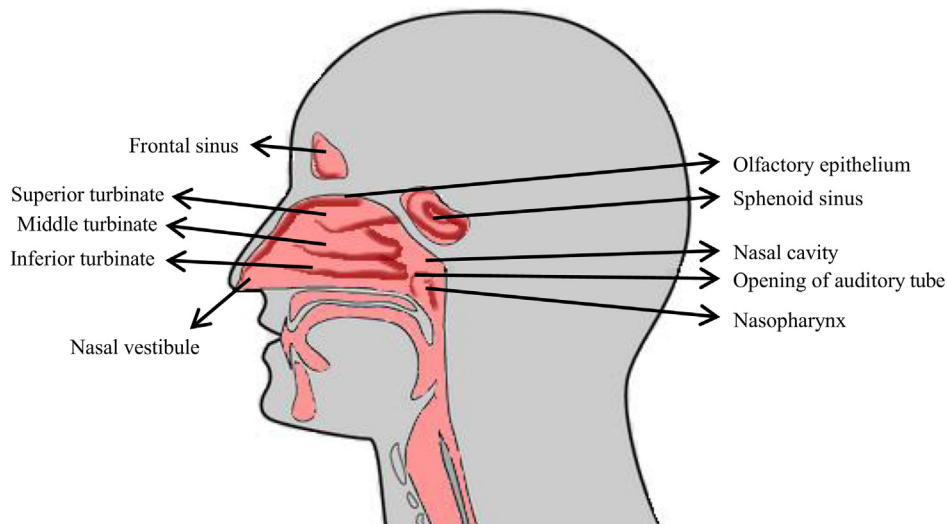

Figure 15.1 *Anatomy and physiology of the nasal passage.*

epithelium, basement membrane, and lamina propria.[18] The nasal respiratory epithelium consists of pseudostratified columnar epithelial cells, goblet cells, basal cells, and mucous and serous glands.[19,20] Most of the epithelial cells on their apical surface with microvilli and the major part have fine projections, called cilia. The nasal secretion, nasal mucosa, olfactory region, and blood supply to the nasal cavity play a major role in nasal physiology.

15.2.2.1 Nasal Secretions

Nasal secretions originate mostly from submucosal glands and goblet cells. Mucus is composed of water (95%); glycoproteins (2%); albumin, immunogobulins, lysozyme, lactoferrin and other proteins (1%); inorganic salts (1%); and lipids (<1%).[11] Glycoproteins are in low proportion, which provides mucus with its characteristic viscoelastic properties. The mucus layer is divided into the low viscosity lower layer of about 5–10 μm thick and the more viscous upper layer of about 0.5–5 μm thick. The human nasal pH is approximately 5–8 with an average baseline of 6.3.

15.2.2.2 Nasal Mucosa

The nasal respiratory mucosa consists of epithelium, basement membrane, and lamina propria. The nasal respiratory epithelium contains pseudostratified columnar epithelial cells, goblet cells, basal cells, mucous, and serous glands. Many of the epithelial cells are covered on their apical surface with fine projections of microvilli (called cilia), which enhance the respiratory surface area. The nasal epithelium is covered with a thin mucus layer, which is produced by secretory glands and goblet cells. Nasal mucus is responsible for several physiological functions, such as humidification and warming of the inhaled air,[21] and offers physical and enzymatic protection.

15.2.2.3 Olfactory Region

The olfactory region is located in the top of the nasal cavity about 2–10 cm^2 and extends down the septum and lateral wall. Neuroepithelium, which is the only part of the central nervous system (CNS), is directly exposed to the external environment. The olfactory area is made of pseudostratified columnar epithelium and is composed of supporting cells, basal cells, microvillar cells, and the typical receptor or olfactory cells.[22] Olfactory regions also contain olfactory receptors (receptors for smell sensations) and small serous glands (which produce secretions that act as a solvent). The total surface area of the olfactory epithelium is 200–400 mm.

15.2.2.4 Blood Supply to Nasal Cavity

The nasal cavity vasculature requires rich blood supply to fulfill the basic physiological functions such as heating, humidification, olfaction, mucociliary clearance, and immunological roles. The nasal vascular bed is intended as such for rapid and easy exchange of fluid and dissolved excipients between blood vessels and nasal tissue. The capillary flow in the nasal mucosa was reported to be 0.5 mL/g/min.[23]

15.3 IMMUNE RESPONSE TO MUCOSAL INFECTION

15.3.1 Antigen uptake in the nose

Antigen uptake in the nose is mostly by two mechanisms: paracellular (aqueous pathway) and transcellular (lipoidal) processes (Fig. 15.2). The most efficient area for drug absorption is the highly vascularized lateral wall of the nasal cavity: the mucosa lined over the turbinates or conchae.

- In the paracellular mechanism, the antigen uptake nasally is through the aqueous route of transport, and this route is slow and passive. The process mainly depends on an inverse log–log correlation between intranasal absorption and the molecular weight of water-soluble compounds/antigens. Poor bioavailability is observed for antigens with a molecular weight greater than 1 kDa in the paracellular process.[24] An example is chitosan widening the tight junctions between epithelial cells.
- In the transcellular mechanism, the antigen uptake nasally is through a lipoidal route and is responsible for the transport of lipophilic antigen based on lipophilicity.

Other than transcellular and paracellular processes, antigens may also cross cell membranes by an active transport route via passive diffusion (depends on pH of environment and pKa of the drug), carrier-mediated means, or transport through the opening of tight junctions in mucosa (ie, through organic cation or amino acid transport), and endocytic process [uptake of antigen mediated by microfold (M) cells].[25]

15.3.2 Factors affecting nasal absorption

There are various factors that affect the antigen uptake in the nasal cavity after nasal immunization, but the bioavailability of a nasal delivery vaccine depends on two major factors: nasal physiological factor and physiochemical properties of nasal vaccine formulation.

15.3.2.1 Nasal Physiological Factor

There are different physiological factors that regulate the absorption of antigen.

- The permeability of a nasal vaccine depends on the type of cells and number of cells in the nasal cavity.
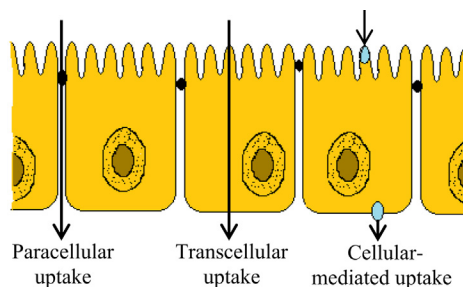

Figure 15.2 *Antigen uptake mechanism.*

- Secretions of nasal mucosa enzymes such as lactate dehydrogenase,[26] oxidative, con-jugative enzymes, peptidases, and proteases also act as a barrier and degrade the nasal vaccine.
- Stimulation of nasal mucosa plays a role in mucosal absorption. Parasympathetic stimulates increase permeability of a nasal vaccine.
- Viscous nasal mucus secretion may retard the uptake of antigen.
- Permeation of antigen is altered at night (chronokinetics) due to clearance rates and mucosal secretions.
- Generally, pH of the nasal cavity for adults is 5.5–6.5 and for infants is 5.0–7.0; for better antigenic absorption, the developed nasal formulation pH should be within 4.5–6.5.[27]
- Mucociliary clearance (MCC) takes about 21 min in the nasal cavity, and increased MCC decreases antigen uptake.[28]
- Mucociliary functioning may affect nasal mucosa due to diseases such as the common cold, rhinitis, etc.
- Environmental conditions (ie, increases in temperature and increases in mucus secretion) also affect the mucociliary clearance.

15.3.2.2 Physiochemical Properties of Nasal Vaccine Formulation

- Antigen concentration, dose, and volume of administration are three interrelated parameters that affect the performance of the nasal antigen delivery.[29]
- pH of nasal formulation should be in the range between 4.5 and 6.5 because lysozymes found in nasal secretions are responsible for destruction of certain bacteria at acidic pH.[30] The lysosomes will be inactivated at pH above 7 and susceptible to microbial infection in nasal tissue.
- Pharmaceutical dosage form is another important factor to consider while developing a nasal formulation. Nasal drops are simpler and more convenient than powder, gel, and suspension sprays.[31]
- Pharmaceutical excipients used in the formulation such as buffer components, antioxidants, preservatives, surfactants, humactants, solubilizers, and gelling/viscosifying agents also play a crucial role in the delivery of the antigen.

15.3.3 Deposition and clearance of antigens

Nasal clearance is one of the major challenges in the development of a nasal vaccine. In nasal mucosa, mucus acts as a sticky fluid and cilia act as a motivator, which prevents foreign substances, pathogens, and particles from being carried by inhaled air into the lungs. In physiological conditions, mucus is transported at a rate of 5 mm/min, and its transit time in the human nasal cavity is 15–20 min.[32] It depends on the length, density, and beat frequency of cilia as well as the amount and viscoelastic properties of the mucus. The absorption of antigen is influenced by the residence (contact) time between the antigen and the epithelial tissue in mucosa.

If the mucociliary clearance increases, the antigen absorption decreases (ie, inversely proportional to each other).

Nasal mucociliary clearance can also be stimulated or inhibited by the active ingredient (ie, antigen and/or inactive ingredients such as preservatives, absorption enhancers, and/or other excipients), thus affecting the delivery of antigen to the absorption site. A prolonged residence time in the nasal cavity may also be achieved by using bioadhesive polymers or a particulate delivery system or by increasing the viscosity of the formulation.

Deposition of antigen in an anterior and posterior region of the nose also affects the antigen absorption of nasal formulation. The anterior portion provides a longer nasal residence time for antigen with low permeability, but antigen permeability is higher in the posterior portion of the nose with shorter nasal residence time. An example is that nasal sprays are deposited anteriorly and cleared slowly into the nasal pharynx by mucociliary clearance. Nasal drops are deposited posteriorly and removed rapidly into the nasal pharynx.[33]

15.3.4 Nasal immune response[34–36]

NALT is the primary target site for nasally administered vaccines, and it consists of lymphoid follicles that occur directly beneath the mucosal follicle-associated epithelial (FAE) cells. NALT is in the Waldeyer's ring, which includes the pharynx and tonsils. The inductive site for nasal immunity is NALT, M cells, B cells, T cells, dendritic cells (DCs), and regional and cervical lymph nodes. Nasally immunized vaccine is transported to NALT through the FAE that contains specialized villous M cells for process and presentation. Villous M cells lack a brush border, which facilitates the binding and delivery of antigens and also does not secrete mucus or any enzymes. NALT is involved in the induction of DC, B cell, and T cell responses following nasal vaccination and also produces local defense against invading pathogens. Part of the soluble antigens may migrate to regional draining lymph nodes through the major histocompatibility complex (MHC) to Th cells. Activation of antigen-specific CD4+ T helper cells (Th cells) interacts with B cells, which develop into IgA committed (IgA+). The IgA+ B cells rapidly migrate from the NALT to the draining cervical lymph nodes in the nasal passage where they differentiate into IgA-producing plasma in the presence of cytokines such as IL-5 and IL-6 that are produced by Th2 cells and secrete IgA in dimers. Dimeric IgA then becomes S-IgA by binding to the polymeric Ig receptor, which transports IgA to effector sites. S-IgA is able to bind toxins, bacteria, or viruses and neutralize their activity, thus preventing entry into the body or reaching the internal organs, and forms a first barrier of defense against invading antigens. Intranasally administered vaccines elicit neutralizing IgA, preventing colonization of the throat and systemic IgG antibodies and facilitating clearance from systemic sites (Fig. 15.3). Immune-competent cells in tonsillar lymphoid tissue and ciliated and nonciliated cells
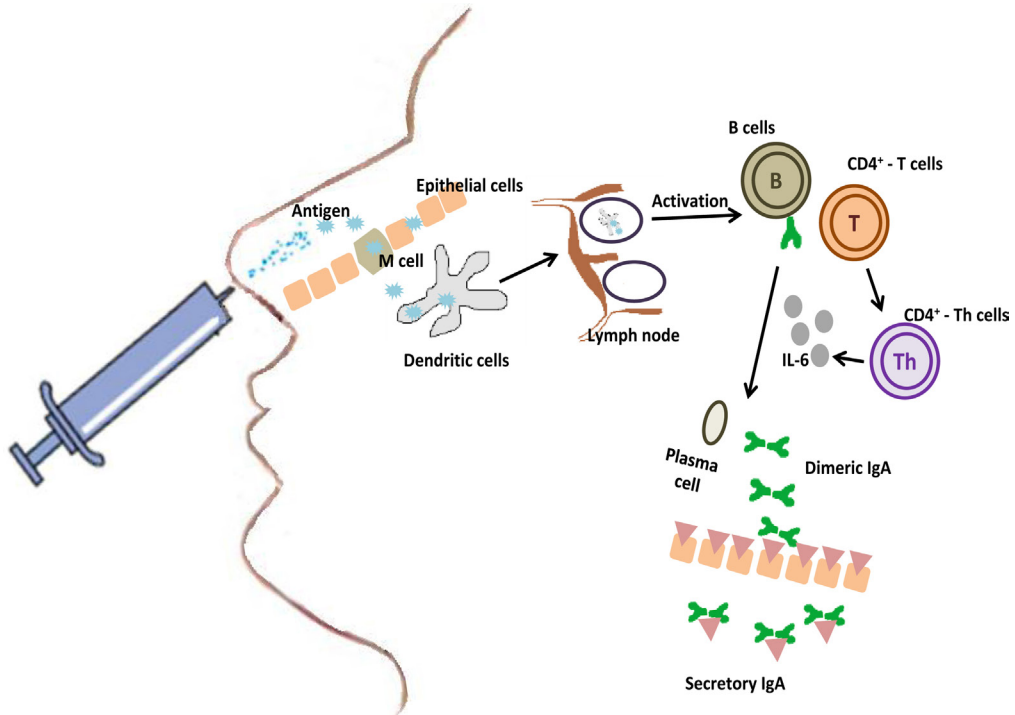
Figure 15.3 *Schematic representation of immune response induction after nasal immunization.*

also play a role in immunity generation. The production of IgA by both the adenoid tissue and the nasal mucosa contributes significantly to immune protection against inhaled bacteria and viruses.[37]

15.3.5 Protective response against mucosal infection

Mucosa defends against invading pathogens through two types of immune systems: the innate immunity system and adaptive immune system. The key role of innate immunity and adaptive immunity at the mucosal surface are discussed in the following sections.

15.3.5.1 Innate Immunity at the Mucosal Surface

Mucosal surfaces of the nasal tract and respiratory tract are adorned with a potential bar-rier known as epithelial cell lines. Epithelial cells in nasal mucosa are active participants in mucosal defense. Epithelia and their associated gland produce innate defenses includ-ing mucins and antimicrobial proteins.[38] Epithelial cells detect the dangerous/foreign microbial components through pattern recognition receptors such as Toll-like receptors (TLRs) and send the cytokine and chemokine signals to mucous membrane–associated APCs, such as DCs and macrophages, to trigger nonspecific/innate defenses and stimu-late adaptive immune responses.

15.3.5.2 Adaptive Immunity at the Mucosal Surface

An important characteristic of mucosa is the production and secretion of dimeric IgA that is resistant to degradation in the protease-rich surroundings of mucosal surfaces. In humans, production of IgA is more comparable to other immunoglobulin isotypes, and high concentrations of IgA antibodies (more than 1 mg/mL) are present in the mucosal surface–associated secretions. IgA facilitates the entrapment of microbes and foreign bodies into the mucus by avoiding direct contact of pathogens with the mucosal surface, which is known as "immune exclusion."

15.4 NASAL VACCINE DELIVERY SYSTEMS

There are numerous mucosal routes of immunization available as an alternative to current parenteral immunization, namely oral, nasal, pulmonary, vaginal, and rectal. The nasal route is more attractive for several reasons. It is a practical site for easy self-administration,[39] with the use of commercially available wide delivery devices. Nasal immunization generally requires much lower doses of antigen compared with the oral or sometimes parenteral route. Nasal immunization does not expose antigens to low pH and digestive enzymes like protease and nuclease, and a protective coating of mucus limits access of antigen into the mucosal epithelium. Administration of vaccines through the nasal route may predominantly induce potent immune responses. Different types of delivery vehicles suitable for the nasal drug delivery systems are being explored; some are being researched, and a few are in clinical phases. They are classified into two major categories: replicating delivery systems and nonreplicating delivery systems.

15.4.1 Replicating delivery system

In a replicating delivery system genetically altered live virus acts as a vector; it proliferates in the host tissues after immunization. It is one of the more prevalent and effective presentations of the antigen and more likely to be effective at stimulating an immune response following oral and nasal delivery. There are different virus vector (Rhabdo virus, polio virus, influenza virus, cowpea mosaic virus)[40–42] and bacterial vector (*Shigella, Salmonella, Listeria*)[43] replicating delivery systems able to induce a CTL response that produces longer-lasting immunity. Introduction of multiple foreign genes can replace the nonessential regions of the viral genome, resulting in an immune response against multiple pathogens. This tactic enables the recombinant virus to be used as a vaccine for two or more infectious agents. Vaccine priming with HIV-env-expressing influenza virus induced systemic cellular response in mice.[44] A single intranasal dose of *Shigella* vector based HIV-1 vaccine produced CD8+ T cell response comparable to systemic immunization.[45] Replicating antigen delivery systems are not preferred due to their complexity of large exploration.

15.4.2 Nonreplicating delivery system

Nonreplicating delivery systems are those which mimic the antigens to the immune system and cause similar uptake by APCs. Different kinds of nonreplicating delivery systems include:

• Liposomes
• Micro- and nanoparticulate systems
• Immune-stimulating complexes (ISCOMs)
• Virus-like particles (VLPs)
• Emulsions
• Bioadhesive delivery systems

15.4.2.1 Liposomes

Liposomes are small artificial vesicles made of bilayer lipid molecules such as phospholipid and cholesterol which encapsulate antigens. Liposomes are a promising delivery system because of their size, ampiphilic nature, and biocompatibility. These vesicles form spontaneously when an aqueous solution is added to a dried film of the lipid components. The hydrophilic (water-soluble or polar head) portion of the lipid molecule is present toward the aqueous phase, and the hydrophobic (water-insoluble) portion is allied inside the membrane. Many of the phospholipids used to make liposomes are from food products (eg, egg yolk or soybeans),[46] so they are nontoxic and safe. The potency of liposomes depends on different factors such as the surface of the lipid layers, electric charge, composition, and method of preparation.[47] Liposomes can also act as an adjuvant,[48] which leads to the induction of potential immune response with low antigen payload. They can also convert nonimmunogenic substances into immunogenic forms (eg, by rendering soluble substances particulate in nature). Liposomes are taken up by macrophages and by M cells for antigen processing and/or presentation to other lymphoid cells for the induction of immune responses. They can also directly present antigens to lymphoid cells for the induction of immune responses. Nasal application of liposomes containing bacterial polysaccharide antigens has been tested in BALB/c mice and revealed that enhanced immune responses in pulmonary secretions was observed in mice after immunization of the liposomal antigen in comparison with antigen alone and as an oral immunization.[49] Apart from this, many investigations carried out on liposomes using different antigens are described in Table 15.1. A nasal influenza vaccine, based on a liposome (virosomal) formulation of influenza virus subunits, has recently been marketed in Europe by the Swiss Serum Institute (Berne, Switzerland). Liposomes could be a promising delivery system for nasal vaccines.

15.4.2.2 Micro- and Nanoparticulate Systems

Particulate carriers have attracted considerable interest as antigen carriers for achieving delivery of antigens at a specific site in the body. Nanoparticles range in size from 1 to

Table 15.1 Delivery system, composition, antigen, and immune response

Delivery system	Composition	Antigen	Immune response	Reference
Liposome	DPPC, Chol, DP	Protein/MPL	Systemic IgG and IgA response	[50]
	DPPC, Chol, DP	*Streptococcus mutans* protein	Systemic and mucosal antibody responses	[51]
	Octyl-beta-D-glucopyranoside	Influenza virus glycoprotein	Local IgA response	[52]
	PC, Chol, DCP	Subunit influenza antigen	Systemic IgG and IgA response	[53]
	Glycol chitosan coated liposomes	Subunit DNA vaccine	Humoral, cellular, and mucosal immunity	[54]
Particulate system	PLGA	Bovine para influenza virus type–3	Systemic IgG responses	[55]
	PLGA	Inactivated PRRS virus	Systemic IgG and MHC–I and MHC–II mediated responses	[56]
	Calcium phosphate	Influenza A virus	Producing CD4+ and CD8+ effector T cells	[57]
	Chitosan	Tumor pGRP DNA	Systemic IgG responses	[58]
	PLGA–coated gelatin	Tetanus toxoid antigen	Humoral, cellular, and mucosal immunity	[59]
	PLGA	Hepatitis B antigen	Humoral, cellular, and mucosal immunity	[60,61]
	Chitosan–tripolyphosphate	Influenza virus	Humoral, cellular, and mucosal immunity	[62]
ISCOM	Quil A	Outer-membrane protein of *Chlamydia trachomatis*	Genital Th1 responses	[63]
	Quil A	Influenza virus glycoprotein	Systemic IgG, IgA, and CTL responses	[64]
	Quil A	Membrane antigens of *Mycoplasma mycoides* subs. mycoides	Systemic and mucosal antibody responses	[65]
	Quil A	Envelope proteins of respiratory syncytial virus (RSV)	IgA antibody and CTL responses	[66]

Table 15.1 Delivery system, composition, antigen, and immune response (*cont.*)

Delivery system	Composition	Antigen	Immune response	Reference
Virus–like particle	Norwalk virus–like particles (NV VLPs antigen)	Norovirus	Systemic IgG1, IgG2, and IgA responses	[67]
	1918 influenza A virus–like particle	Glycoprotein of influenza virus	Systemic IgG responses	[68]
	Adjuvanted Norwalk virus–like particle	Noro virus	Systemic and mucosal antibody responses	[69]
	Simian immunodeficiency virus (SIV) virus–like particles	SIV virus	Specific serum IgG2 or Th1–oriented immune response	[70]
Emulsion	Influenza virus	W805EC (O/W emulsion)	Humoral and cellular immunity	[71]
	Ovalbumin	O/W nanoemulsion	Cellular immunity and other specific responses	[72]
	HIV gp120	O/W nanoemulsion	Th1–polarized cellular immune response	[73]
	Hepatitis B	Nanoemulsion	Th1–associated cellular immunity	[74]
Bioadhesive delivery system	Influenza (H1N1)	Esterified hyaluronic acid microsphere	Serum IgG responses	[75]
	HIV rpg160	Emulsomes (fat, phospholipid, EDTA)	Selected mucosal IgA and IgG responses	[76]
	HIV–1	Capric acid and hydroxyl propyl methylcellulose	Humoral, cellular, and mucosal immunity	[77]
	Tetanus toxoid	Dextran microsphere	IgG and IgA immune responses	[78]

1000 nm (1 μm), while microparticles range from 1 to 1000 μm;[79] the smaller particle size promotes faster adsorption. Other added advantages include a more stable system protecting from the hostile environment of nasal mucosa, prolonged release, bioadhesion properties, and induction of a significant immune response with reduced dose. Depending on the method of preparation, microparticles or nanoparticles can be prepared with different properties and release characteristics. The polymers used for the particulate system are biomolecules such as proteins, peptides, polynucleotides, and polysaccharides or synthetic polymers such as polylactide–polyglycolide copolymers,[80] polyacrylates, poly-ε-caprolacton,[81] and *N*-trimethyl chitosan-poly(γ-glutamic acid).[82] Nano- and micro-sized particulate carriers are prepared with the antigenic molecule and deliver it to the desired site of action to induce potent and long–lasting immune responses.

The mechanisms by which these particulate systems alter the induction of immune responses are size-dependent penetration, depot effect, repetitive antigen display, cross-presentation, and release of soluble mediators such as cytokines that regulate the immune response.[83] Many studies carried out on micro- and nanoparticulate-based nasal vaccine delivery systems using different antigens are shown in Table 15.1. Hence a polymer-based micro-/nanoparticulate system can be exploited as a viable nasal vaccine delivery system that is capable of delivering a multitude of antigens at the targeted sites and inducing desired immune response.

15.4.2.3 Immune Stimulating Complexes

An ISCOM is a highly versatile and effective antigen presentation system, which is a spherical open cage-like structure (typically 30–40 nm in diameter)[84] that forms spontaneously on mixing cholesterol, a lipid such as phosphatidyl choline, and the mixture of saponins that comprise Quillaja saponins A.[85] The antigen-enveloped Quillaja saponin acts as a strong inherent adjuvant as well. In 1984 Morein and colleagues showed the potential induction of immune responses upon immunization with ISCOMs containing viral and bacterial membrane glycoproteins. ISCOMs are the potent inducers of not only humoral (antibody-mediated) immune responses but also cellular (T cell-mediated) immune responses. Presentation of antigens unified with the ISCOM matrix allows their processing via the endogenous and exogenous pathways, resulting in the stimulation of both CD4+ and CD8+ T cells.[86] Moreover, adjuvant activity of the Quil A moiety induces long-lasting immune responses. The antibody levels stimulated by ISCOMs are found to be equivalent to those after immunization with conventional adjuvants such as complete Freund's adjuvant or alum.[87] Some studies revealed that immunization with ISCOMs induces a wide range of immune responses including all subclasses of IgG and cell-mediated immune responses such as delayed-type hypersensitivity (DTH) in vivo, antigen-specific proliferative responses and cytokine production in vitro.[88–90] An additional property of ISCOMs is their capability to enter the endogenous antigen-processing pathway and then MHC class I–restricted cytotoxic T cells (CTL). Intranasal immunization of *Echinococcus granulosus* surface antigen ISCOMs evokes higher serum IgA titer in relation to IgG than the subcutaneous route in mice.[91] Several studies on different antigens using ISCOM-based delivery systems are shown in Table 15.1. Hence ISCOM-based delivery systems may be suitable for nasal vaccine administration.

15.4.2.4 Virus-Like Particles

VLPs are a promising option for vaccine delivery to the nasal site because they are easy to access, highly vascularized, self adjuvanting, and highly immunostimulatory and have a relatively large surface area and low proteolytic activity.[92] VLPs are inert with a 20–100 nm self-assembled empty capsid protein,[93] which contains no DNA/RNA and shows a similar size and shape as viruses. VLPs are efficiently taken up by DCs and

induce potent immune responses after nasal immunization, which induces systemic immunity as well as both local and distal mucosal immunity via the common mucosal immune system (CMIS). It is even more superior to parenteral administration at eliciting IgA at distal mucosal sites. The VLPs can be produced within mammalian cells, insect cells, yeast, bacteria, and even plants through recombinant DNA techniques.[94,95] Due to these properties, VLPs are exploited as the delivery system for different protein/peptide antigens. Despite successful usage and potent immunity, VLPs as a delivery system for protein antigens are still limited by the relatively complicated genetic modification on protein fusion and the subsequently required structural integrity characterization. VLP technology has been used for many years. An examples includes recombinant hepatitis B surface antigen (HBsAg), produced in *Saccharomyces cerevisiae* or *Pichia pastoris* yeasts.[96] The entire recombinant core antigen (HBcAg) of the hepatitis B virus (HBV) promotes Th1 immunomodulation of the immune response to coadministered antigens, including the HBV surface antigen (HBsAg) after nasal administration. This concept also applies to HIV, dengue, and chronic hepatitis C immunotheraphy; details are discussed in Table 15.1. VLPs elicit a potent immune response and increase the immunogenicity of poorly immunogenic antigens, including self-proteins.

15.4.2.5 Emulsions

The potential of micro-/nanoemulsion for various routes of administration has continuously been explored for the past two decades.[97] Due to low globule size and lipophilic nature, emulsions are widely chosen as a delivery system to enhance uptake across nasal mucosa. Emulsions are of various types including oil–in–water (O/W) and water–in–oil (W/O). Most of the novel adjuvants are made up of emulsions such as incomplete Freund's adjuvant, adjuvant 65, montanide, and MF59.[98] Emulsions are isotropic, transparent, thermodynamically stable, and low viscosity colloidal dispersions, which are stabilized by an interfacial film of alternating surfactants and cosurfactant molecules. Ease of administration and scale-up is one of the important advantages over other drug delivery systems such as solid dispersion, liposomes, and particulate delivery systems. Emulsion formulation contains different inactive ingredients such as oil (soybean oil, sesame oil, isopropyl myristate, etc.), surfactant (Tweens, Span80, chromophores, PEG, lecithin, cetrimide, etc.), cosurfactant (ethanol, propanol, PEG, etc.), and aqueous phase. Mucoadhesive polymer such as different grades of carbopol (eg, 974P/971P/980P, sodium alginate, pluronics, etc.) is generally incorporated in the composition of an emulsion to prolong the release of antigens. Micro-/nanoemulsion provides good sprayability for nasal vaccine delivery compared with other particulate delivery systems. Factors to consider during the preparation of a formulation include nature and concentration of oil, surfactant, cosurfactant, and aqueous phase; ratio of oil/surfactant/cosurfactant; and temperature and pH of the environment. Dilutability, particle size, pH, zeta potential, viscosity, and freeze/thaw cycling are critical parameters in

the development of emulsion vaccines. Nanoemulsion nasal vaccines have been studied for several diseases like hepatitis B, HIV, and influenza.[99] Some studies reported that the nasal vaccine immunization produced strong IgG and IgA antibody levels, which is similar to alum adjuvant-based vaccines. The emulsion-based delivery approach might be promising for low-cost nasal vaccine immunization for the populations of developing and underdeveloped countries.

15.4.2.6 *Bioadhesive Delivery Systems*

In a bioadhesive delivery system the antigen carrier system adheres to a biological tissue for an extended period. Nasal mucosa bioadhesion protects the antigen from mucosal enzymes and increases retention time. Bioadhesive-based nasal vaccines are preferred to overcome nasal clearance issue,[100] to facilitate absorption, and for extended antigen delivery. Bioadhesive polymers can be used to increase the nasal residence time by slowing mucocilliary clearance, which allows absorption for a longer time with the nasal mucosa and results in a subsequent increased in absorption. Different bioadhesive polymers such as chitosan, carrageenan, carbopol, hydroxypropyl methylcellulose (HPMC), K15M and E5, sodium alginate, sodium carboxy methylcellulose, polyvinyl pyrrolidone (PVP) 90, and xanthan gum are used in the formulations.[101] Bioadhesion occurs between polymer–mucin chains through van der Waals, hydrogen, hydrophobic, and electrostatic forces.[102] Nasal immunization with different antigens such as tetanus toxoid, influenza, pertussis, and diphtheria following encapsulation and administration using different bioadhesive polymers such as pluronic F127, chitosan and its combination,[103,104] and PEG-coated polylactic acid[80] shows enhanced immune response via induction of IgG and IgA responses. Chitosan-based influenza nasal vaccine produced effective immune response in human clinical trials.[105] Bioadhesive drug delivery systems may be a potential tool for extended antigen delivery through nasal immunization.

15.5 DIFFERENT DOSAGE FORMS OF VACCINE THROUGH THE NASAL ROUTE

Intranasal immunizations are simple, easy, convenient, and safer than other routes of administration. The delivery system selection depends upon the antigen being used for the proposed indication, patient type, and marketing preferences. There are different options available to deliver nasal vaccine such as drops,[106] powder, aerosol sprays, and the application of nasal gel.

15.5.1 Nasal drops

Nasal drops are convenient and the most simple method for delivery of nasal vaccines. The nasal drops are administered using a nasal dropper or syringes. The major disadvantage of this system is the lack of dose precision[107] and difficulty for the pediatric

population. Some studies reported that nasal drops deposit human serum albumin in the nostrils more efficiently than nasal sprays.[108]

15.5.2 Nasal powder

Nasal powder formulations are highly stable compared to liquid formulations. Nasal powders can extend the residence time for powder formulations on the nasal mucosa, potentially increasing the local and systemic immune response.[109] However, the production of nasal dry powders is quite complicated with required particle size, particle distribution, and performance characteristics when compared with other dosage forms.

15.5.3 Aerosol

The aerosol route of delivery of vaccines is one of the most preferred for nasal administration compared with other nasal dosage forms and also less reactogenic than the subcutaneous route of administration.[110] Aerosol vaccination via the lungs targets an epithelium critical to host defense against inhaled pathogens and provides an exciting opportunity in the development of newer and more effective tuberculosis (TB), measles, and influenza vaccines. An aerosol vaccination usually depends on the target pathogen and the sites of the inductive immunity. The aerosols are available in liquid (solution, suspension, and emulsion) and solid forms. In addition to vaccine antigens, carrier/solvent, emulsifier/surfactants, corrosion inhibitors, and propellant selection and compatability play a critical role in achieving required immunogenicity in infants. Aerosol vaccine immunogenecity achievement is based on antigen particle size for prevention of upper respiratory (eg, *Boredetella pertussis*, *Chlamydia pneumonia*) and lower respiratory (eg, *Streptococcus pneumoniae*, *Bacillus anthracis*) bacteria and virus disease. Larger particles (~5 μm) are needed for the aerosol vaccination to prevent upper respiratory tract infection and smaller particles (≤ 3 μm) for lower respiratory tract infection.[111] The dried forms of vaccines with optimum particle size are traditionally prepared by freeze drying or spray drying. The aerosol form of vaccines was administered to many human subjects for a longer period and found to provide excellent protection for diseases such as influenza A and measles.[112,113] Therefore aerosol immunization may be a promising method of vaccination.

15.5.4 Nasal gel

Nasal gels are generally used for colds, allergies, low humidity, or overuse of decongestant. Nasal vaccine gels are a high–viscosity solution or suspension in which antigenic molecules are dispersed. The advantages of a nasal gel include the reduction of nasal clearance and anterior leakage due to highly viscous formulation, reduction of irritation by using soothing/emollient excipients, and target delivery to mucosa for better absorption.[114] In addition, it may potentially enhance the immune response, reduce the antigen and/or adjuvant dose, sustain antigen release, and improve antigen uptake with enhanced

antigen stability. Special application techniques are required for the administration of nasal gel vaccines because of their highly viscous formulation and poor spreading abilities. A wide variety of gelling polymers are available for formulation such as pullulan, deaceylated gellan gum, xantham gum, chitosan, and polyethylene glycol, used to encapsulate vaccine/adjuvant formulation as gel particles. Viscosity, sol–gel transition temperature and gelling time, and gel strength and its texture are critical parameters in the development of nasal gel vaccines. Recently, pneumococcal surface protein-A nasal gel vaccine,[115] *Clostridium botulinum* type-A neurotoxin BoHc/A, and tetanus toxoid[116] were studied in animal models and enhanced both humoral and cellular immunity. Nasal gel is an alternative and promising novel dosage delivery system to achieve the immune response.

SUMMARY

Nasal vaccine administration has been considered as an alternative method and found equivalent or superior to parenteral and other mucosal administration. It avoids the risk of transmitting diseases like hepatitis B, HIV, and other agents through improper injection practices and improves patient compliance. The intranasal vaccine delivery system induces both mucosal and systemic immune response, which avoids entry of pathogen in all mucosal routes. Multiple delivery systems are being explored (both developmental and clinical phases) and can open the door for practical development of nasal vaccine delivery systems.

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