Expert Opinion

Liposomes as delivery systems for nasal vaccination: strategies and outcomes

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Importance of the field: Among the particulate systems that have been envisaged in vaccine delivery, liposomes are very attractive. These phospholipid vesicles can indeed deliver a wide range of molecules. They have been shown to enhance considerably the immunogenicity of weak protein antigens or synthetic peptides. Also, they offer a wide range of pharmaceutical options for the design of vaccines. In the past decade, the nasal mucosa has emerged as an effective route for vaccine delivery, together with the opportunity to develop non-invasive approaches in vaccination.
Areas covered in this review: This review focuses on the recent strategies and outcomes that have been developed around the use of liposomes in nasal vaccination.
What the reader will gain: The various formulation parameters, including lipid composition, size, charge and mucoadhesiveness, that have been investigated in the design of liposomal vaccine candidates dedicated to nasal vaccination are outlined. Also, an overview of the immunological and protective responses obtained with the developed formulations is presented.
Take home message: This review illustrates the high potential of liposomes as nasal vaccine delivery systems.

Keywords: liposome, mucosal immunity, nasal mucosa, nasal vaccination, vaccine delivery

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1. Introduction

Vaccine strategies aim to mimic the process of natural infection supplying antigens to the immune system. So far, most vaccines have been developed using live-attenuated organisms, killed whole organisms or inactivated toxins. However, these 'classical' approaches may have some limits. Live-attenuated vaccines, which have high immunogenicity, may occasionally trigger side effects such as paralytic polio. Also, with live-attenuated pathogens a reversion to virulence is to be feared, particularly in immune-deficient patients. Therefore, recent efforts in the field of vaccinology have focused on developing synthetic vaccines composed of protein, peptide or polysaccharide antigens or of antigen-encoding DNA. However, these vaccines require the use of particulate drug delivery systems (DDS) to transport antigens or DNA, to protect them from the environment, but also to increase their immunogenicity [1,2].

Most pathogens initiate infection by interacting with host mucosal surfaces, including the respiratory one. In spite of this consideration, most of the vaccines in use so far are administered by systemic injection. This route of administration

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Article highlights.

- This article reviews the recent strategies and outcomes that have been developed around the use of liposomes in nasal vaccination.
- Nasal vaccination with liposomes has been used to target extracellular and intracellular pathogens, as well as to prevent tumor metastasis.
- Local and systemic humoral and cellular immune responses, as well as efficient protection, were reported after nasal immunization with liposomal vaccine candidates; liposomes were necessary to achieve these responses. Importantly, liposomal vaccines were able to trigger immune responses at distant mucosa.
- Liposomal-based formulations delivered through the nasal route can lead to protective immunity whatever the nature of the antigen (protein, peptide, DNA) or its mode of association to the vesicles (encapsulation, coupling, membrane embedding).
- Liposomal constructs can be widely improved in terms of efficiency through changes in various formulation parameters, including size, charge or lipid composition. A complete characterization of the liposomal constructs is essential for a comprehensive analysis of the contribution of these parameters and their possible interactions.
- Further research is needed to explore and evaluate fully the potential of liposomal-based constructs in nasal vaccination. Formulation strategies such as incorporation of adjuvants, mucoadhesion and targeting of cells involved in the immune response deserve more investigations. Also, safety issues should be adressed.

This box summarizes key points contained in the article.

leads to a strong systemic immune response, but to little or no mucosal response. In the past decade, the use of the nasal cavity as a route for drug delivery has been an area of considerable interest. Nowadays, this route represents a great alternative for the delivery of drugs, particularly those that are difficult to deliver by routes other than injection [3]. The nasal mucosa has also emerged as an effective route for vaccination, as intranasal (i.n.) administration of vaccines elicits not only a mucosal immune response but also a systemic response [4]. The nasal route may provide the opportunity to comply with one of the challenges for vaccination, which is the development of non-invasive approaches [5]. Several particulate DDS have been envisaged in nasal vaccination, including microemulsions, polymeric microparticles, liposomes and immune-stimulating complexes [4,6,7]. This review focuses on the recent strategies and outcomes that have been developed around the use of liposomes and their derivatives (e.g., proteoliposomes, archaeosomes).

2. The nasal cavity as a route for vaccination

The use of the nasal mucosa for vaccine delivery requires the anatomical, functional and immunological characteristics of the nasal cavity to be taken into consideration.

2.1 Anatomical and functional characteristics of the nasal mucosa

The main functions of the nose are olfaction, regulation of humidity and temperature of inhaled air, and removal of large particles or microorganisms. The nasal cavity, which represents a total surface of ~ 150 cm^2, is divided into 5 anatomical and functional regions: the nasal vestibule, the atrium, the respiratory region, the olfactory region and the nasopharynx [8]. Among these regions, the respiratory region is of great relevance for nasal delivery, as it is the most permeable region owing to its large surface area and its rich vasculature [3,9,10]. This part of the nasal cavity is, however, actively involved in preventing foreign substances and pathogens from entering the organism. The respiratory region is indeed covered by a pseudo-stratified epithelium, composed of columnar cells interspersed with goblet cells, which acts as a protective physical barrier. Tight junctions, called zona occludens, interconnect epithelial cells, providing the epithelium with barrier properties. These junctions have indeed an estimated diameter of 3.9 – 8.4 Å, which restricts the paracellular passage of particles. Although tight junctions are dynamics and highly regulated, absorption enhancers can open them to a limited extent, suggesting that in order to reach the nasal submucosa, large particles must probably cross the epithelium by endocytosis or carrier-mediated transport processes [9].

The respiratory region is also the region where the production of mucus takes place. In normal conditions, nasal mucus is composed of ~ 95% water, 2% mucins, 1% salts, 1% of other proteins including albumin, and < 1% lipids [8,10,11]. It is produced continuously by globlet cells of the respiratory epithelium and by submucosal glands, and forms over the epithelial layer an extra physical barrier. With the exception of specific disease states, the thickness of the mucus layer in the nasal tract is limited (5 µm) compared with other mucosal surfaces. The nasal mucosa is therefore considered to be highly accessible. Mucus also acts as an adhesive; it traps inhaled soluble substances or particles. As residence time at mucosal surfaces is a critical factor for the efficacy of mucosal vaccines, strategies have been developed to increase adhesiveness of antigen delivery systems to the nasal mucus [10]. However, the mucus is continuously secreted and cleared from the nasal cavity owing to the beating of the cilia that are present on columnar cells. This is the so-called mucociliary clearance that drives the mucus towards the nasopharynx. The mucus flow rate is ~ 5 mm/min, and the mucus layer is renewed approximately every 20 min, leading to the efficient clearance of inhaled particles. Therefore, mucociliary clearance, rather than mucus alone, may represent a critical barrier for the delivery of vaccines at the nasal mucosa. To overcome this barrier, particulate vaccine candidates should cross the mucus layer rapidly rather than adhere to it [11]. Transport of particles through the mucus is a complex process. It depends on the properties of the mucus, as well as on the particles [10,11]. Mucus is a viscoelastic and sticky gel that

entraps particles by adhesive interactions resulting from the negative charges of the carboxyl or sulfate groups and the periodic hydrophobic regions present along the mucin strands. In addition, mucus acts as a net. Indeed, crosslinked and entangled mucin fibers form a network with mesh sizes of the order of 10 – 100 nm. Therefore, surface charge and size of delivery systems are certainly pivotal pharmaceutical factors when developing nasal vaccine candidates.

2.2 Immunological characteristics of the nasal mucosa

Mucosal surfaces are the major site of entry of pathogens. Therefore, these surfaces possess highly specialized immune systems that protect them from infections, the so-called mucosa-associated lymphoid tissues (MALT).

Nasal mucosa is equipped with a MALT, called the nasopharynx-associated lymphoid tissue (NALT), which shares significant structural and functional similarities with the Peyer's patches of the gastrointestinal mucosa [4,6,7,12,13]. NALT is a well-organized subepithelial lymphoid structure composed of follicles of B cells and $CD4^+$ and $CD8^+$ T lymphocytes that are associated to an antigen-presenting cell (APC) network made of macrophages, B cells and dendritic cells (DC). This structure is covered by an epithelial layer that contains microfold M cells that act as antigen-sampling and delivering cells. Indeed, M cells possess the capacity to capture and transport transcellularly a wide range of materials. Therefore, NALT possess all the cells needed for the induction of a local humoral or cellular immune response. In regions where lymphoid structures and M cells are absent, DC can traffic into the epithelial layer to sample antigens that are present at the surface or within the mucosa lumen. Following i.n. administration, antigens may elicit an immune response through two hypothetical pathways [4,6,7,13]. They may be taken up by M cells and transported to the subepithelial lymphoid follicles where they will be presented by APC to the adjacent T cells. Alternatively, antigens may be taken up by intraepithelial DC that will then migrate to local lymphoid tissues or exit the mucosa through lymphatics to present antigens to naive T cells in draining lymph nodes. Data suggest that particulate antigens would be preferentially taken up by M cells, whereas small soluble antigens would get access to the nasal epithelium where they would be taken up by intraepithelial DC [4,7,9].

3. The adaptative immune response after nasal vaccination

Ideally, vaccination at a given mucosa should provide both humoral and cellular immune responses against the pathogen at the relevant mucosal surface and throughout the body [12,13]. Nasal immunization is effective in inducing a local humoral immune response [4,6,7,13]. This response is mainly mediated by the production and secretion of immunoglobulins A (IgA) by mucosal B cells differentiated into plasma cells. In mucosal secretions, IgA neutralize microorganisms or block their attachment to the epithelium thus preventing mucosal colonization and invasion by pathogens. Nasal immunization is also effective at inducing a systemic humoral response characterized by the production of IgA and IgG [4,6,7,13]. Indeed, as mentioned above, DC of the nasal cavity can readily traffic and carry antigens to systemic inductive sites such as the lymph nodes and the spleen, resulting in the induction of a systemic immune response. This response is important, as it provides the organism with a defence mechanism against pathogens that infect the host through both mucosal and systemic routes. Interestingly, the systemic antibody response induced by nasal vaccination appears to be greater than the one triggered by other mucosal immunization routes [4,6,7]. Also, sometimes, this response can be higher than the one induced by the parenteral route. By contrast, systemic immunization is generally ineffective at inducing a mucosal IgA response.

Cellular immunity, mediated by $CD4^+$ T helper (T_H) cells and $CD8^+$ cytotoxic T lymphocytes (CTL), also plays a major role in the protection against infectious agents. Whereas CTL are in charge of the destruction of tumoral cells or cells infected by intracellular pathogens, activated T_H cells differentiated into T_H1 or T_H2 subtype help antibody production by B cells and cellular immunity, respectively, by producing specific cytokines. Although less investigated than the humoral response, there is evidence that vaccination by the nasal route can trigger cellular immunity [4,6,7].

MALT act as local immune systems, but are also able to communicate with distant mucosal surfaces through an integrated network, called the 'common mucosal system', which connects inductive sites with effector sites. Indeed, B and T lymphocytes that have been primed in a mucosal lymphoid tissue are able to recirculate through other mucosa to trigger an immune response at distant sites. There is evidence that nasal administration of vaccines can induce specific humoral and cytotoxic responses in the upper and lower airway mucosa, the salivary glands and/or the genital tract [4,7,13].

Thus, the broad characteristics of the immune response induced through the nasal mucosa confer to nasal vaccination several advantages over parenteral vaccination.

4. Liposomes as vaccine delivery systems

Liposomes are vesicles composed of one (unilamellar vesicles) or more (multilamellar vesicles) phospholipid membranes surrounding an aqueous core. Developed in the 1960s, they were first used as a model of biological membranes [14]. However, in the past decade, they created much interest in the pharmaceutical industry as DDS [15].

Owing to their structure, liposomes offer a wide range of options for the design of vaccine candidates. Proteins, peptides or DNA can be encapsulated free inside the aqueous core of the liposomes, embedded within the lipid bilayer or

associated at the surface by simple adsorption, hydrophobic anchor insertion or covalent binding (**Figure 1** and **Tables 1 – 3**). Encapsulation is usually carried out during the formulation step [16] or by post-encapsulation using the pH- or ion-gradient principle [17]. An example of antigen insertion inside the membrane of liposomes is given by proteoliposomes and cochleates prepared from microorganisms by detergent extraction. In these structures, protein antigens of the microorganisms are entrapped inside a phospholipid membrane containing lipopolysaccharides (LPS) and other microorganism components [18-20]. Surface adsorption of negatively charged antigens and DNA is achieved by using cationic liposomes [21,22]. Surface covalent coupling is the mode of choice for association of peptides. Several approaches have been used to achieve this coupling [23]. One approach consists of the covalent coupling of the peptides to preformed liposomes that contain hydrophobic anchors (e.g., derivatives of phosphatidylethanolamine) (**Figure 1**). These anchors are functionalized with reactive end groups (maleimide, bromoacetyl, carboxylic acid, etc.) that are able to react with free SH, NH_2 or CHO groups that are present or have been introduced on the peptide. The conjugation reaction can be performed in aqueous media under mild conditions with high yield and is generally very chemoselective [24]. Moreover, it gives access to well-controlled ligand-to-epitope ratio at the surface of the liposomes. Alternatively, peptides can be conjugated in a first step to lipophilic anchors such as long chain fatty acids before the insertion of the resulting lipopeptide into the liposomes. Then, the lipopeptide can be either introduced into the liposomes during the same formulation step as the constitutive phospholipids or inserted in preformed liposomes by a post-insertion technique. This last method allows the conjugation of the peptide only on the outer surface of the vesicles [23]. The same kind of reaction has also been developed for the bioconjugation of polysaccharide epitopes and the design of synthetic carbohydrate-based vaccine candidates [25]. Liposomes are able to present multiple copies of an antigen [2]. Final constructs can also contain multiple copies of structurally independent epitopes that can fulfil different immunological roles. As reported by the authors' group [26] and others [27], the combination of T-cytotoxic and T-helper epitopes in the same liposomal construct leads to improved T-cytotoxic activity compared with constructs containing the T-cytotoxic epitope only, probably through the recruitment of $CD4^+$ T lymphocytes. In the same way, the simultaneous presence of B and T-helper epitopes on the same vesicle is of prime importance in the design of a highly immunogenic synthetic peptide-based vaccine candidate leading to a humoral response [28].

As vaccine delivery systems, liposomes can also enhance considerably the immune response of weak protein antigens or of synthetic peptides, which are generally poorly immunogenic [29]. Liposomes are indeed efficiently endocytosed by APC, the key cells in the initiation of the adaptative immune response. To enhance the immune response triggered by weak antigens or to drive the immune response through a desired pathway, the adjuvant activity of liposomes can be potentiated or modulated by the incorporation of immunopotentiators such as Toll-like receptor (TLR) ligands, including lipopolysaccharide, Pam_2 or Pam_3 derivatives and CpG (**Figure 2**) [26,29-32]. The use of formulations associating liposomes and adjuvants allows the simultaneous presentation of the antigen and the adjuvant to the same APC. In these formulations, the adjuvant can be encapsulated, adsorbed at the surface or incorporated into the membrane of the liposome. Lipopeptide adjuvants inserted in the membrane of liposomes can be modified by addition of a reactive function to serve as an anchor for peptide antigens [33]. However, the antigen and the adjuvant do not necessarily need to be conjugated together for immune efficiency [26,32,34]. On the contrary, the independent addition of the adjuvant and the peptide in the same liposome brings higher design versatility (e.g., adjuvant types, adjuvant/antigen ratio) (unpublished data). If the addition of an adjuvant increases the immunoactivity of liposomes, it may conversely bring benefit for the adjuvant. For example, incorporation of lipophilic adjuvants into liposomes can increase their aqueous solubility.

To increase their delivery efficacy, the surface of liposomes can be modified [31]. As an example, APC targeting has been achieved by the coupling of mannosylated derivatives that recognize APC mannose receptors (**Figure 2**) [35,36]. Mucus, which acts as a physical barrier and is rapidly cleared, may dramatically reduce the access of particulate vaccine candidates to APC. Likewise, to counteract this fact, mucins that are present in the mucus have been exploited to promote adhesion of particles at mucosal surfaces [31]. Numerous polymers, such as alginate, chitosan, hyaluronan and hydroxyethyl cellulose, which present mucoadhesive properties, have been successfully adsorbed at the surface of particles (**Figure 2**) [10].

Finally, numerous physicochemical parameters can be modified to optimize the role of liposomes as vaccine candidates [31], including the structure/size and the lipid composition that determines the charge and the fluidity of the liposomal membrane bilayer (**Figure 2**). After oral administration, larger antigens (~ 5 μm) held within the Peyer's patch stimulate a local mucosal immune response while smaller antigens escaping to the peripheral lymphatics induce a systemic immune response [37,38]. Also, after nasal administration, polymer particles in the size range 20 – 200 nm are usually taken up by receptor-mediated endocytosis and elicit a cellular biased response, whereas particles with a size between 0.5 and 5.0 μm are predominantly taken up by phagocytosis and/or macropinocytosis, eliciting a humoral response [7]. The size of liposomes is therefore a parameter to explore when used at mucosal surfaces. The surface charge of nanoparticles including liposomes, reflected by the zeta potential, has been correlated to their stability [39]. As described above, surface charge is also used to associate proteins or antigen-encoding DNA to liposomes.

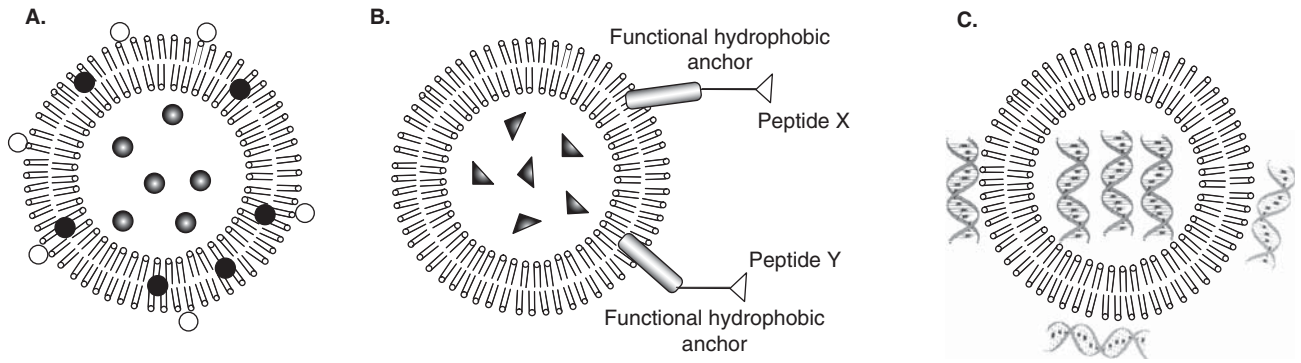

Figure 1. Schematic illustrations of the modes of association of proteins, peptides or antigen-encoding DNA to liposomes. A. Encapsulation in the core (grey circles), insertion inside the phospholipid bilayer (black circles) or surface adsorption (open circles) of protein antigens. **B.** Encapsulation (grey triangles) or surface coupling through functional hydrophobic anchors (open triangles) of peptide antigens. **C.** Encapsulation or adsorption of antigen-encoding DNA.

Table 1. Liposome-based constructions for nasal delivery of DNA.

Composition	Structure/size	Zeta potential	Plasmid/DNA	Mode of DNA association	Targeted disease	Immune response	Ref.
Glycol chitosan PC/DOPE/Chol	DRV < 1 μm	+10 mV	pRc/CMV-HBs(S)	Encapsulation	Hepatitis B	Mucosal humoral (including vagina) Systemic humoral Cellular	[48]
DMRIE/DOPE	NA	Cationic	Reporter gene firefly luciferase	Adsorption	None	Vaginal humoral Systemic humoral Proliferative and cytotoxic T-lymphocyte response in the spleen and iliac lymph nodes	[56]
EPC/DOPE/DOTAP	240 – 1000 nm	+30 mV	pVAX-hsp65	Encapsulation Adsorption	Tuberculosis	Systemic humoral Cellular	[53]
NA	100 nm	Cationic	pCI-HA10	Encapsulation	Influenza	Mucosal humoral Systemic humoral T-cell proliferation Protection	[45]
DOTAP or DOTMA/Chol	NA	Cationic	CpG	Adsorption	Pulmonary metastasis	Cellular Prevention of tumor cell proliferation	[22]

Chol: Cholesterol; DMRIE: 1,2-dimyristyloxypropyl-3-dimethyl-hydroxylethyl ammonium; DOAP: Dioleoyl trimethylammonium-propane; DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane; DOTMA: *N*-[1-(2,3-dioleyloxy)propy]-*N,N,N*-trimethylammonium chloride; DRV: Dehydration–rehydratation vesicle; EPC: Egg phosphatidylcholine; NA: Not available; PC: Phosphatidylcholine; SUV: Small unilamellar vesicle.

Furthermore, considering that cell membranes have a negative charge, cationic liposomes should easily bind to cells and deliver or retain the antigen in the vicinity of the treated tissues and therefore show an increased efficacy [40]. Therefore, the surface charge of liposomes provides possibilities to formulate vaccine candidates as well as to modulate their activity. Finally, lipid composition can either increase or decrease the stability of liposomes, as well as their adjuvant activity, which both contribute to their efficacy as vaccine delivery systems [31].

Thus, the high and broad versatile structure of liposomes confers to these DDS a large potential in vaccination, including one at mucosal surfaces (**Figure 2**).

5. Liposomes as delivery systems for nasal vaccination

So far, a significant number of studies have investigated the potential of liposomes as a delivery system for nasal vaccination. Vaccine targets have been mainly pathogens

Table 2. Liposome-based constructions for nasal delivery of peptides.

Lipid composition	Structure/size	Zeta potential (mV)	Peptide	Adjuvants	Mode of peptide association	Immune response	Ref.
PC/PG/Chol	SUV < 150 nm	NA	Peptide derived from pili protein from *P. aeruginosa*	$Pam_{2\ or\ 3}$ derivatives	Covalent coupling	Mucosal humoral Systemic humoral	[34]
PS/PC	MLV	NA	Nucleoprotein 366 – 374 peptide from *Influenza* (CTL epitope)	Anti-CD40 mAb	Encapsulation	Mucosal humoral Mucosal cytotoxic Protection	[44]
DOPC/DOPE/ DOPG/Chol	NA	NA	LCMV peptides: GP33 – 41, GP283 – 291, GP61 – 80, NP396 – 404, NP309 – 328	CpG	Covalent coupling	Mucosal humoral Cytotoxic Protection	[47]

Chol: Cholesterol; DOPC: Dioleylphosphatidylcholine; DOPE: Dioleylphosphatidylethanolamine; DOPG: Dioleylphosphatidylglycerol; LCMV: Lymphocytic choriomeningitidis virus; MLV: Multilamellar vesicle; NA: Not available; PC: Phosphatidylcholine; PG: Phosphatidylglycerol; PS: Phosphatidylserine; SUV: Small unilamellar vesicle.

such as viruses (e.g., influenza [41-45], human immunodeficiency virus (HIV) [46], *Vibrio cholerae* [18,20], lymphocytic choriomeningitis virus [47], hepatitis B virus [48,49], respiratory syncytial virus [50], Newcastle disease virus [40,51,52]) and bacteria (e.g., *Mycobacterium tuberculosis* [53], *Pseudomonas aeruginosa* [34], *Actinobacillus pleuropneumoniae* [54,55]). These studies are presented in the **Tables 1 – 3** according to the nature of the antigen (protein, peptide or antigen-encoding DNA). The vaccinal responses that were obtained with the developed liposomal formulations as well as the main pharmaceutical characteristics that influenced these responses are described in the following paragraphs.

5.1 Delivery and immunogenic efficacy

To be efficient, a particulate system dedicated to mucosal vaccination must at first allow the delivery of the antigen at the inductive site. In their work [56], Klavinskis and co-workers addressed the efficiency of cationic liposomes in delivering DNA to the nasal mucosa after i.n. administration in mice. By using the reporter gene firefly luciferase as a model, these authors found that incorporating DNA into cationic liposomes increased the quantity of DNA delivered to the nasal tissue 30-fold. The capacity of liposomes to modulate the deposition, absorption and retention of protein antigen at the nasal mucosa was evaluated by Christensen and co-workers [42] using ovalbumin (OVA) as an antigen and the mucus-secreting Calu-3 cells as an *in vitro* model of airway epithelial barrier. In this set up, addition of liposomes significantly increased the quantity of OVA present in the cell layer, as well as its transport across the mucus layer.

The production of specific IgA at mucosal surfaces constitutes a critical first line of defence against pathogens that infect the host through mucosa. When investigating the mucosal immune response triggered by the i.n. administration of liposomal vaccine candidates in laboratory animals, all studies [18,19,34,40,41,44-49,51,52,54,55,57-66] evidenced a local production of specific IgA in bronchoalveolar lavages or nasal secretions, whatever the nature of the transported molecule (DNA, peptide or protein) or the targeted pathogen (**Tables 1 – 3**). In the studies where mucosal IgA were observed, specific IgG were concomitantly detected in serum (**Tables 1 – 3**). The possibility of inducing a genital/rectal humoral immune response is attractive for vaccines targeting pathogens that disseminate during sexual contacts, such as HIV. Klavinskis *et al.* [56] reported the presence of specific IgA in vaginal fluids of mice that received a single i.n. injection of DNA–liposome complexes. Specific IgA were also measured in vaginal washes and/or feces from mice immunized i.n. with protein-based liposomal vaccines [46,59,62,64]. In some studies, the presence of specific antibodies was also reported in saliva or bile [18,46,59,64].

The association of a humoral response to a cell-mediated immune response increases the efficiency of a vaccine candidate. Intranasal administration of DNA- or protein-based liposomes was reported to trigger T-cell

Table 3. Liposome-based constructions for nasal delivery of proteins.

Composition	Structure/size	Zeta potential	Protein	Mode of protein association	Immune response	Ref.
PC/Chol/dicetyl phosphate	LMV 1 – 4 μm	Anionic	HBsAg for the boost (i.n.) after a HBsAg-DNA vaccine prime (i.m.)	Encapsulation	Mucosal humoral (including vagina) and mucosal cellular (lung) Systemic humoral Cellular Protection	[49]
Egg PC or DPPC + Chol + DPPS/Chol + SA/Chol	MLV 1.0 – 1.2 μm	Anionic Neutral Cationic	Inactivated Newcastle disease virus ± LPS	Encapsulation	PC/DPPS: systemic and mucosal humoral SA: poor humoral Protection	[40,51,52]
PC/Chol + Tremella or xanthan gum	MLV 1.9 μm for plain liposomes	-17 mV for plain liposomes	Inactivated influenza H5N3 virus	Encapsulation	Mucosal humoral Systemic humoral	[41]
CCS/Chol DOTAP/Chol DDAB DMTAP DMPC/DMPG DOPE/Chol	CCS < 20 nm Others 50 nm – 10 μm	Anionic Neutral Cationic	Mono and trivalent vaccines from influenza	Encapsulation Adsorption	Mucosal humoral (including lung) Systemic humoral Cellular Persistant protective immunity	[63]
DDAB/TDB	450 nm	+60 mV	OVA or influenza	Adsorption	Systemic humoral Cellular	[42]
DOPC	NA	NA	RSV protein fused with bacterial thioredoxin	Encapsulation	Pivotal role of alveolar macrophages	[50]
Sphingomyelin/DOPE/PC/PS	NA	NA	HIVgp160	Encapsulation	Mucosal humoral (including rectal and vaginal mucosa) Systemic humoral Cellular	[46]
Olive oil, castor oil, SPC, Chol	Biphasic lipid vesicles 20 – 790 nm	-6 mV	OmlA from *A. pleuropneumoniae* ± CpG	Binding/association	Mucosal humoral Systemic humoral	[54,55]
PC/DDAB/Chol	MLV	Cationic	*Yersinia pestis* killed whole cell	Encapsulation/ entrapment	Mucosal humoral (including lung) Cellular T-cell proliferation Protection	[58]

AMVAD: Archeal lipid mucosal vaccine adjuvant and delivery vaccine; CCS: Ceramide carbamoyl-spermine; Chol: Cholesterol; DCP: Dicethylphosphate; DDAB: Dimethyl-dioctadecylammonium bromide; DMPC: Dimyristoyl phosphatidylcholine; DMPG: Dimyristoyl phosphatidylglycerol; DMTAP: Dimyristoyl-3-trimethylammonium-propane; DOPC: Dioleoyl phosphatidylcholine; DOPE: Dioleoyl phosphatidylethanolamine; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane; DPPC: 1,1-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine; DPPS: 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine; DRV: Dehydratation-rehydratation vesicle; EPC: Egg phosphatidylcholine; i.m.: Intramuscular; i.n.: Intranasal; LMV: Large multilamellar vesicle; LPS: Lipopolysaccharide; MPL: Monophosphoryl lipid A; MSHA: Mannose-sensitive hemagglutinin; NA: Not available; OME: Outer membrane extract; OmlA: Outer membrane lipoproteins A; OMP: Outer membrane proteins; OVA: Ovalbumin; PC: Phosphatidylcholine; PS: Phosphatidylserine; RSV: Respiratory syncytial virus; SA: Stearylamine; SPC: Soja phosphatidylcholine; TDB: Trehalose 6,6′-dibehenate.

Table 3. Liposome-based constructions for nasal delivery of proteins (continued).

Composition	Structure/size	Zeta potential	Protein	Mode of protein association	Immune response	Ref.
Phospholipids of OME from *V. cholerae*	Cochleates Tubular shape 16 μm	NA	OMP from *V. cholerae* + LPS	Membrane insertion Encapsulation	Mucosal humoral (including rectal mucosa) Systemic humoral Vibriocidal antibodies	[18]
DPPC/Chol/dicetylphosphate	NA	NA	*S. mutans* antigen ± MPL	Encapsulation/ association	Mucosal humoral (including vagina) Systemic humoral	[59-61]
DOTAP/Chol/protamine	200 nm	NA	Plasmid DNA: CpG Anthrax protective antigen protein	Mixture	Mucosal humoral (including lung) Systemic humoral T-cell proliferation Neutralizing antibodies	[65]
Phospholipids of OME from *V. cholerae*	Proteoliposomes 170 nm	-23 mV	OMP from *V. cholerae*/LPS/ MSHA	Membrane insertion	Systemic humoral Vibriocidal antibodies	[20]
Phospholipids of OME from *N. meningitidis*	Cochleates and proteoliposomes	NA	OVA + OME from *N. meningitis*/LPS	Membrane insertion	Mucosal humoral Systemic humoral Cellular	[19]
Archeal polar lipids	Archaeosomes 100 nm AMVAD > 5 μm	Anionic	OVA	Encapsulation	Systemic humoral Mucosal humoral for AMVAD (including feces, vagina) Cytotoxic Sustained over several months Subject to memory boost	[64]
DOTAP/Chol	NA	Cationic	CD52 peptide conjugated to diphtheria toxoid	Mixture	Mucosal humoral (including vagina) Systemic humoral	[62]
PC/DCP/Chol Chitosan	DRV 2.4 μm	Anionic (without chitosan)	Tetanus toxoid ± CpG	Encapsulation	Mucosal humoral Systemic humoral	[57]
PC/Chol	2.3 μm	NA	Tetanus toxoid ± CpG	Encapsulation	Mucosal humoral Systemic humoral	[66]

AMVAD: Archeal lipid mucosal vaccine adjuvant and delivery vaccine; CCS: Ceramide carbamoyl-spermine; Chol: Cholesterol; DCP: Dicethylphosphate; DDAB: Dimethyl-dioctadecylammonium bromide; DMPC: Dimyristoyl phosphatidylcholine; DMPG: Dimyristoyl phosphatidylglycerol; DMTAP: Dimyristoyl-3-trimethylammonium-propane; DOPC: Dioleoyl phosphatidylcholine; DOPE: Dioleoyl phosphatidylethanolamine; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane; DPPC: 1,1-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine; DPPS: 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine; DRV: Dehydratation-rehydratation vesicle; EPC: Egg phosphatidylcholine; i.m.: Intramuscular; i.n.: Intranasal; LMV: Large multilamellar vesicle; LPS: Lipopolysaccharide; MPL: Monophosphoryl lipid A; MSHA: Mannose-sensitive hemagglutinin; NA: Not available; OME: Outer membrane extract; OmlA: Outer membrane lipoproteins A; OMP: Outer membrane proteins; OVA: Ovalbumin; PC: Phosphatidylcholine; PS: Phosphatidylserine; RSV: Respiratory syncytial virus; SA: Stearylamine; SPC: Soja phosphatidylcholine; TDB: Trehalose 6,6′-dibehenate.

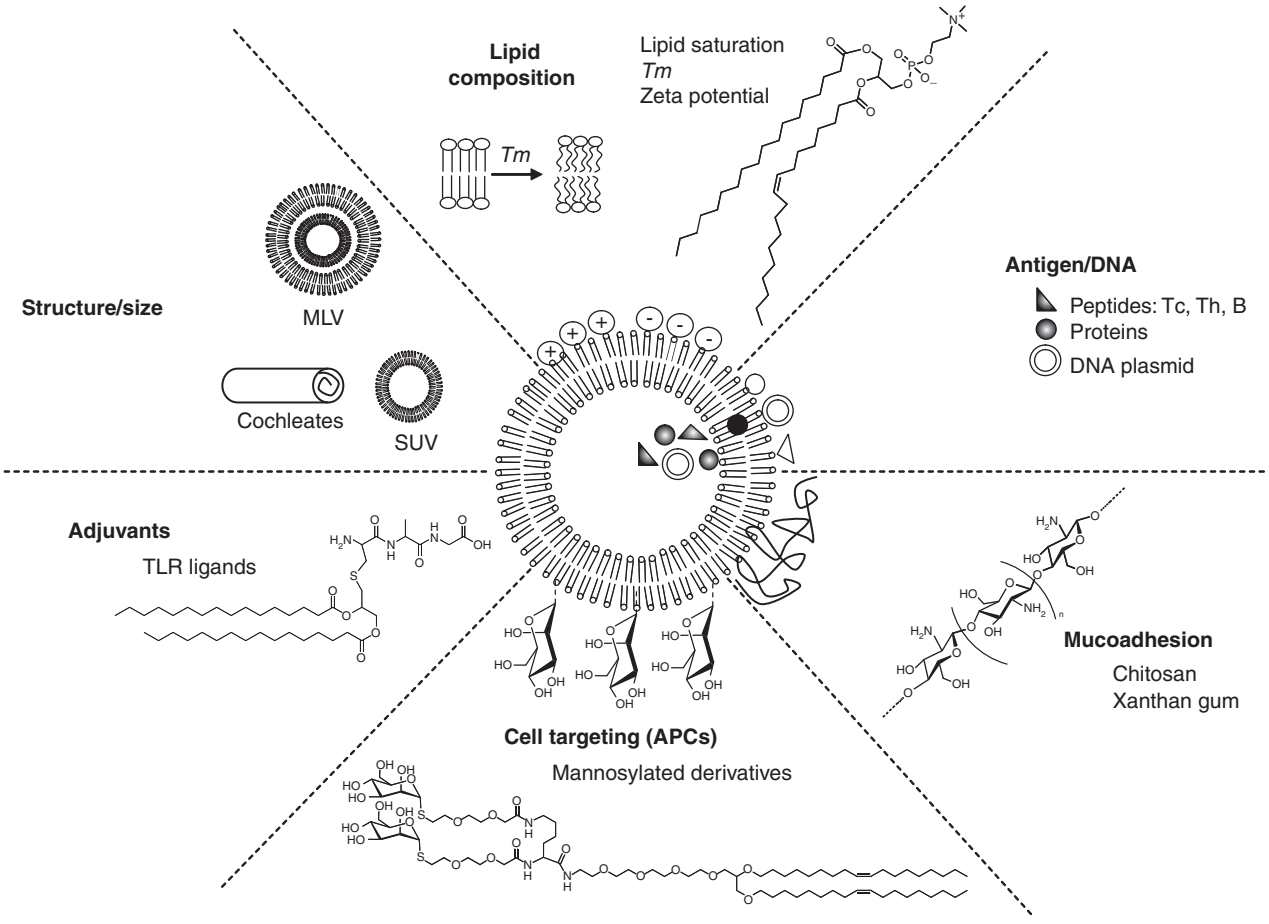

Figure 2. Design of liposomal vaccine candidates.

APCs: Antigen-presenting cells; MLV: Multilamellar vesicles; SUV: Small unilamellar vesicles; Tc: T-cytotoxic peptide; Th: T-helper peptide; TLR: Toll-like receptor; *Tm:* Phase transition temperature.

proliferation [45,56,58,65,67], IFN-γ producing T cells [22,45,48,53,58,67] or both T_H1- and T_H2-type $CD4^+$ T cells [46] in the mucosal and systemic compartments of immunized animals (**Tables 1** and **3**). The induction of antigen-specific effector and memory CTL responses was also reported after i.n. administration of DNA-, peptide- or protein-based liposomal constructs (**Tables 1 – 3**) [21,44,46,47,64].

One of the major challenges when designing vaccine candidates is the induction of long-term immunity. Only a few groups aimed to determine the persistence of the immunity triggered by their constructs. Serum antibodies and protective immunity were shown to persist for at least 9 months in response to an influenza protein-based vaccine [63]. An archeal lipid vaccine candidate also elicited sustained systemic immune responses over several months and was subject to memory boost responses, although IgA in fecal and vaginal wash samples declined [64].

Numerous authors evaluated the potential of their liposomal vaccine candidates to confer protection by characterizing the capacity of the elicited antibodies to neutralize the antigen [18,20,43,46,65] or by assessing survival or pathogen titers in immunized animals after i.n. challenge [19,22,40,44,45,47,51,58,67]. In all cases, nasal vaccination confered protection.

In summary, both humoral and cellular immune responses were obtained after nasal immunization of laboratory animals with liposomal vaccine candidates and these responses led to an efficient protection. Importantly, in numerous studies evidence was provided that liposomes are necessary to achieve efficient responses [22,40,41,45,48,49,51,52,58,59,61,65-67].

5.2 Pharmaceutical design

The immune efficiency highlighted in the previous paragraph appears to be intimately related to the composition and the physicochemical characteristics of the liposomal vaccine constructions that were used in the different studies. The most important of these parameters are outlined and exemplified below.

5.2.1 Mode of association of antigens or DNA to liposomes

Encapsulation, surface coupling or adsorption, as well as membrane embedding have been used to design nasal liposomal vaccine candidates (**Tables 1 – 3**). The encapsulation has been used for the formulation of protein-, peptide- or DNA-based vaccine candidates (**Tables 1 – 3**). In general, all have been encapsulated inside the core of the liposomes during the formulation step. When using mucosal routes for immunization, encapsulation in a reservoir structure can be preferred in order to protect antigen or antigen-encoding DNA from enzyme degradation [7,68]. Khatri *et al.* [48] evaluated the potential of liposomes in protecting DNA from DNase I and found that encapsulated DNA was indeed protected compared with naked DNA when tested *in vitro.* The surface covalent coupling of peptides was used to formulate vaccine candidates directed against bacteria (*P. aeruginosa*) [34] or virus (*Lymphocytic choriomeningitis virus*) [47]. The coupling was achieved by means of thiol reactive [34] or disuccinimidyl suberate functions [47]. For protein-based constructs, two microorganisms have been used to prepared proteoliposomes or cochleates (*Neisseria meningitis* or *V. cholerae*) in which protein antigens are inserted in the phospholipid membrane [18-20]. These preparations have been exploited to generate an immune response directed against the microorganism itself [18] or a heterologous antigen, namely OVA [19]. Proteins and antigen-encoding DNA can be encapsulated into the core of liposomes, but may also be adsorbed at the surface of cationic vesicles by means of electrostatic interactions [22,42,53-56,63,65]. Adsorption at the surface of liposomes could result, however, in a greater exposure of antigens or DNA to the enzymatic environment when compared with encapsulation. Interestingly, Rosada and co-workers [53] compared the protection triggered by the i.n. administration of a plasmid encoding for *Mycobacterium leprae* heat-shock protein 65 (DNA-hsp65) that was either encapsulated or adsorbed at the surface of cationic liposomes. Surprisingly, only adsorbed DNA achieved effective protection against tuberculosis. Exposure of DNA at the surface of liposomes may indeed favor the interaction of CpG motifs present in the plasmid DNA, with receptors such as TLR leading to a greater immunostimulatory effect [53].

5.2.2 Size

Although liposomes of a wide range of size (from 150 nm to > 5 µm) have been evaluated, only one study has addressed the impact of the size of the liposomes on the immune response evoked after i.n. administration [64]. In this study, unilamellar liposomes (average diameter of 100 nm) made from archaeal polar lipids (archaeosomes) and encapsulating OVA were compared with aggregated structures (> 5 µm) obtained after addition of $CaCl_2$ to archaeosomes. The results showed that i.n. administration of unilamellar liposomes, although inducing an anti-OVA IgG antibody response in serum, failed to evoke a specific IgA response at the nasal mucosa [64]. By contrast, aggregated archaeosomes elicited a mucosal IgA antibody response together with systemic humoral and CTL responses [64]. In agreement with the observations made for polymer particles [69,70], these data suggest that the size may indeed play an important role in the efficacy of liposomes as nasal vaccine delivery systems. However, it must be pointed out that in the study of Patel and co-workers [64], not only the size but also the structure of the constructs was changed. Although liposomes of various structural characteristics (e.g., unilamellar vesicles, multilamellar vesicles, liposome aggregates) have been evaluated through the literature (**Tables 1 – 3**), no study clearly assessed the influence of the structure of liposomes on the immune responses evoked on nasal administration.

5.2.3 Surface charge

As hypothesized above, the use of cationic liposomes may favor binding to cell membranes and consequently increase vaccine efficacy [40]. Joseph and co-workers [63] used a new polycationic lipid, the ceramide carbamoyl-spermine (CSS), as well as dimyristoyl-3-trimethylammonium-propane and dioleoyl-3-trimethylammonium-propane, to formulate positive influenza vaccines, and compared the efficacy of these preparations with the one triggered by vaccine candidates formulated with neutral or anionic lipids on i.n. administration in mice [63]. Whereas the neutral and anionic vaccine candidates were poorly immunogenic, the vaccines formulated with cationic lipids, especially CSS, elicited strong humoral and cellular responses and provided protective immunity [63]. Interestingly, in this study, high antibody titers and protective immunity were induced for at least 9 months. One group reported [40,51,52], however, that positive liposomes containing dipalmitoylphosphatidylcholine (DPPC) and stearylamine (SA) led to a lower humoral immune response after i.n. vaccination of chickens against the Newcastle disease (despite their high affinity for cells) than negative liposomes composed of DPPC and/or phosphatidylserine (PS). The same team confirmed that negative liposomes made of egg phosphatidylcholine (EPC) and dipalmitoylphosphatidylserine elicited the highest antibody titers against the Newcastle virus compared with neutral or positive ones (EPC or EPC/SA, respectively) [40,51,52]. These last results suggest that physicochemical characteristics other than electrostatic interactions have to be taken into account when formulating positive liposomes. In the present case [40,51,52], the change in surface charge of the liposomal vaccine was achieved by changes in the lipid composition, which may also have an impact on the membrane fluidity of liposomes.

5.2.4 Membrane fluidity

Bilayers of liposomes are mainly composed of phospholipids that confer either rigidity or fluidity to the membrane. This property may influence adsorption of liposomes at the cell surface and therefore antigen delivery. The assembly of the lipids in a vesicle membrane undergoes a membrane phase

transition around a critical temperature (*Tm*). Above *Tm,* lipids are in a fluid state, whereas below *Tm* the membrane is in a gel phase where the lipids are packed orderly, conferring rigidity to the membrane. The transition temperature of the membrane depends on the nature of the polar head, as well as on the length and saturation of the carbon chain of the phospholipids. Tseng and co-workers [40] evaluated the adjuvant effect of liposomal formulations composed of PS or SA with high *Tm* (62 and 58°C, respectively) and of phosphatidylcholine (PC) with a *Tm* of 43°C on i.n. vaccination of chickens against Newcastle disease [40]. Chickens immunized with the liposomal vaccine candidate formulated with PC showed the highest mucosal IgA and serum IgG antibody responses, as well as the best protection on viral challenge. To confirm these data, the authors showed that liposomes made of PC were taken up more efficiently by chicken macrophages *in vitro* than liposomes composed of PS or SA. Also, the authors assumed that PC-based liposomes, owing to their low *Tm,* would become more flexible and fluid at body temperature, facilitating their attachment to the cell surface and allowing a better antigen delivery to the nasal cavity. On the contrary, the high *Tm* of PS and SA liposomes would render them resistant to adsorption at the nasal surface owing to their rigid state at the chicken's body temperature [40].

5.2.5 Incorporation of adjuvants

A key step in the design of a liposome-based mucosal vaccine candidate is the rational choice of the adjuvant. De Magistris [71] summarized the characteristics of the ideal mucosal adjuvant, which in particular has to be effective with low doses of antigen, any type of antigens and few injections, and has to induce a persistent response. Only a few immunopotentiators show these properties. For example, LPS (TLR4 ligand from Gram-negative bacteria) [18-20], CpG (TLR9 ligand) [22,47,65,66] and MALP-2 or Braun protein derivatives (TLR2 ligands) [34] have shown their potential in nasal vaccination against bacteria or virus. Interaction between CD40 and CD40 ligand is believed to play an important role in activation of APCs and in immunoglobulin production [72]. A liposomal vaccine candidate encapsulating a CTL epitope of influenza A virus nucleoprotein together with an anti-CD40 antibody has been evaluated after i.n. administration in mice and found to induce successfully a protective CTL response [44]. Likewise, the use of liposomes containing the strong immunostimulator trehalose 6,6′-dibehenate (TDB) increased cell-mediated immunity as well as the humoral immune response induced by a commercial influenza vaccine after nasal application in mice [42]. Therefore, several successful incorporations of adjuvants to liposomal formulations dedicated to nasal vaccination have been reported in the literature [18-20,22,34,41,44,47,51,54,55,57,59,60,65]. It should be pointed out, however, that in the example of TDB [42], the liposomes used as carriers were also strongly cationic and had a phase transition temperature > 37°C, two characteristics that may play a significant role in the adjuvant activity of the candidate vaccine.

5.2.6 Bioadhesive properties

Several mucoadhesive liposomal preparations have been evaluated as vaccine delivery systems through the nasal route. In these studies, chitosan and other polysaccharides were used as bioadhesives. Chitosan is a biocompatible, biodegradable and non-toxic cationic polysaccharide [73]. Its adsorption at the surface of negative liposomes results in an increase in the zeta potential of the vesicles up to a relatively constant value as chitosan concentration increases [74]. The resulting positive surface charge facilitates the electrostatic interaction of liposomes with the physiological mucus. Owing to its cationic properties, chitosan also has a great potential to complex negatively charged DNA plasmids. Also, chitosan is known for its ability to open transiently the tight junctions of mucosal membranes, improving access of the antigen to the submucosa [75]. Khatri *et al.* [48] prepared glycol chitosan-coated liposomes encapsulating plasmid DNA encoding surface protein of hepatitis B virus, and evaluated the ability of these preparations to adhere to mucins *in vitro,* as well as to interact with the nasal tissue and to induce humoral mucosal and cellular immune responses after i.n. administration in rats. Chitosan-coated liposomes were found to adsorb quantitatively more mucins than plain liposomes and to adhere to rat nasal tissue to a greater extent than uncoated vesicles. Also, following i.n. administration chitosan-coated liposomes elicited humoral mucosal and cellular immune responses that were significant compared with naked DNA. In the same way, dipalmitoylphosphatidylcholine/dicetyl phosphate negative liposomes encapsulating tetanus toxoid were coated with chitosan and evaluated for their capacity to induce a systemic humoral response after i.n. administration in mice [76]. Following a priming dose, chitosan-coated liposomes engendered high antitetanus IgG titers. Furthermore, after boosting, animals that received the chitosan formulation showed the highest IgG or IgG1 titers compared with mice administered with the free antigen or with liposomes coated with other bioadhesive molecules (hyaluronan and carbopol) [76]. Chiou and co-workers [41] prepared bioadhesive liposomal vaccine candidates against the avian influenza virus by mixing liposomes encapsulating the inactivated virus with two polysaccharides showing viscous properties, xanthan gum (XG) or tremella (T). By mixing different ratios of XG or T with liposomes, vaccines of low and high viscosity were formulated. The two types of bioadhesive vaccine candidate (XG or T) showed increased immunogenicity compared with non-bioadhesive formulations when administered i.n. to chickens, with the low-viscosity vaccine resulting in greater antibody responses compared with the high-viscosity one. It should be pointed out nonetheless that XG and T are known also for their immunoadjuvant properties. All together, these studies suggest that bioadhesives are promising tools for increasing the efficiency of liposomes as nasal vaccine delivery systems.

Nevertheless, in the work of Amin *et al.* [57], tetanus toxoid-loaded liposomes coated with chitosan were not as efficient as uncoated liposomes at inducing a mucosal IgA response and led to an equivalent humoral systemic response after nasal administration in rabbits. The free amine form of chitosan used, which led to deprotonation at pH > 6.5, and its low concentration (0.025%) compared with other studies could explain these conflicting results [57].

5.2.7 APC targeting

Recognition and uptake of antigens by immature DC involve receptor-mediated endocytosis/phagocytosis by means of nonspecific pattern recognition receptors such as C-type lectins that recognize specific pathogen-associated carbohydrate structures including mannose [77-80]. Accordingly, mannose receptor-targeting presents a promising approach in the vaccine field [81]. To increase further the effectiveness of a liposomal peptide-based vaccine against *P. aeruginosa,* the authors' group successfully combined B- and T-helper peptides in the same structure and completed this construct with mannosylated derivatives able to target DC [34]. This strategy, first developed for the systemic administration of peptide-based vaccine candidates [35,36], allowed a more rapid production of specific IgG in serum after i.n. vaccination in mice [34].

These studies highlight that formulation aspects such as lipid composition, size, charge and bioadhesiveness are important parameters for the efficacy of liposomal vaccine candidates at the nasal mucosa.

6. Conclusion

The nasal route has been clearly identified as a promising route for vaccine delivery owing to its privileged localization at one of the primary sites of infection and its ability to initiate an efficient immune response, including at distant mucosa. In recent years, numerous groups have worked on the development of liposome-based formulations for nasal vaccination. Protein-, peptide- or DNA-antigens mainly derived from virus or bacteria have been successfully encapsulated, coupled, adsorbed or embedded into liposomes. To optimize the activity of these vaccine candidates, various particulate physicochemical parameters have been modulated. Also, strong adjuvants, mucoadhesive components and/or -APC-targeting molecules have been incorporated in the developed formulations. On evaluation of these vaccine candidates in laboratory animals, mucosal and systemic humoral responses have been constantly observed. Cellular responses have also been reported, although less frequently investigated. More importantly, when assessed, the evoked immune responses led to an efficient protection. On a pharmaceutical point of view, these studies showed that formulation aspects such as lipid composition, size, charge and bioadhesiveness influence the efficacy of liposomal delivery systems dedicated to nasal vaccination.

7. Expert opinion

Liposomes are very attractive delivery systems for vaccination. They can indeed deliver a wide range of molecules. They considerably enhance the immunogenicity of weak protein antigens or synthetic peptides. Also, they offer a wide range of pharmaceutical options for the design of vaccine candidates. As illustrated in this review, a significant number of studies have illustrated so far the high potential of liposomes as delivery systems for nasal vaccination. The key findings in this field of research have been that liposomal formulations can: i) lead to protective immunity whatever the nature of the antigen (protein, peptide, DNA) or its mode of association to the vesicle (encapsulation, coupling, membrane embedding); ii) be used to target extracellular and intracellular pathogens, as well as to prevent tumor metastasis; iii) trigger immune responses at distant mucosa, which is an essential goal in the field of mucosal vaccination; and iv) be widely improved in terms of efficiency through changes in various formulation parameters.

Liposomes are highly versatile DDS. A wide range of physicochemical factors (e.g., size, charge, lipid composition) have been thus exploited to design liposome-based nasal vaccines. However, modifying a given physicochemical parameter may impact on another one. As a consequence, a complete characterization of liposomal formulations is essential for a comprehensive analysis of the data; however, this characterization has not been systematically provided in the literature. Likewise, only a few studies have evaluated a given formulation parameter in a given model. As a striking exemple, both unilamellar and multilamellar liposomes have been used to formulate nasal vaccines, but their evaluation was carried out in distinct studies using different models. Also, there is a lack of comparative studies between different formulation factors, which makes it difficult to understand what the critical physichochemical factors to take into account are when designing liposomal delivery systems dedicated to nasal vaccination, and, more importantly, how these different factors interact with each other. Therefore, although there is evidence that liposome-based nasal vaccines can be widely improved in terms of efficiency through changes in various formulation parameters, data in this field are still needed. A systematic evaluation in a given model could reveal the contribution of each component and highlight the possible synergies between components. In this respect, as suggested by Pattani *et al.* [82], the development of microarrays associated to a common database would be of great interest to determine the immunological profile of liposomal vaccines with specific physicochemical characteristics.

Beside physicochemical factors, three very attractive approaches have been investigated to increase further the immune efficiency of liposome-based vaccines after nasal administration: incorporation of adjuvants, mucoadhesion, and targeting of cells involved in the immune response, such as APC. However, these different strategies deserve more

investigation in the future. As highlighted above, liposome coating with mucoadhesive molecules gave conflicting results. Although promising, APC targeting has been only poorly investigated. Likewise, the incorporation of adjuvants has been reported to enhance successfully the immune response triggered by liposomal formulations dedicated to nasal vaccination. However, its potential in driving the immune response through a desired pathway has not been explored so far. Depending on the targeted disease, specific immune profiles focusing on humoral, cellular and/or mucosal responses may indeed be required, and the choice of the adjuvant is determinant in this respect.

Safety is a key issue for the development of vaccine candidates. Among their many advantages, liposomes, with the exception of cationic vesicles, are characterized by a marked absence of intrinsic toxicity. However, in the nasal cavity the olfactory region involved in smell perception may be sensitive to any component (antigen or adjuvant) of the vaccine formulation. The local toxicity of liposomal formulations dedicated to nasal vaccination has been addressed by several groups. Only a mild local inflammatory response was observed in mice and rabbits immunized i.n. with a liposomal vaccine candidate based on polycationic lipids [63]. No nasal irritation or sneezing or burning symptoms were reported in human volunteers after i.n. administration of blank neutral liposomes [66]. Also, multilamellar cationic liposomes did not alter the viability of airway epithelial cell monolayers in culture, as a model of nasal mucosa [42]. Although limited, these data suggest that liposome-based vaccine candidates are well tolerated by the nasal mucosa. The use of liposomes may, on the contrary, contribute to decreasing or abrogating the risk of local toxicity of nasal vaccines. Indeed, owing to their efficiency, liposomes may provide an opportunity to administer smaller quantities of antigen and adjuvant [47,53]. However, the nasal cavity contains olfactory neurons that are in direct contact with the olfactory bulb in the brain. This anatomical characteristic, which is looked at by the pharmaceutical industry as a chance to bypass the blood–brain barrier and to deliver drugs to the central nervous system, raises some safety issues. One of these issues is the possibility of antigen or particle deposition in the brain. This question may deserve consideration, including for liposomal vaccine candidates.

As illustrated in this review, the high and broadly versatile structure of liposomes confers to these DDS great potential in nasal vaccination and further research is needed to explore and evaluate this potential fully. Beyond this, liposomes could be also a powerful tool to study the mechanism of the innate and adaptative immune responses at the nasal mucosa.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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