Current prospects and future challenges for nasal vaccine delivery

Current Prospects And Future Challenges For Nasal Vaccine Delivery

Abstract

Nasal delivery offers many benefits over traditional approaches to vaccine administration. These include ease of administration without needles that reduces issues associated with needlestick injuries and disposal. Additionally, this route offers easy access to a key part of the immune system that can stimulate other mucosal sites throughout the body. Increased acceptance of nasal vaccine products in both adults and children has led to a burgeoning pipeline of nasal delivery technology. Key challenges and opportunities for the future will include translating in vivo data to clinical outcomes. Particular focus should be brought to designing delivery strategies that take into account the broad range of diseases, populations and healthcare delivery settings that stand to benefit from this unique mucosal route.

Key-words nasal, vaccine, needle-free, influenza, mucosal

In this review the current state of the art in nasal vaccine delivery will be described along with future prospects. A brief introduction to the anatomy and physiology of the nasal cavity will highlight the advantages and disadvantages of the route. Encapsulation and presentation methods along with particular formulation considerations for the nasal route will also be discussed.

There are many mucosal routes which have been regarded as potential sites for vaccine delivery such as oral, nasal, pulmonary, conjunctival, rectal and vaginal mucosa. However, for practical and cultural reasons researchers have tended to focus only on oral, nasal, and pulmonary administration.[1] Needle-free vaccines offer many advantages over traditional vaccination approaches including convenience, cost, ease of administration and disposal.
There are several needle free methods of vaccination such as transdermal delivery and mucosal delivery.[2,3] Mucosal immunization has been successfully used in human vaccination. The human mucosal immune system is large and specialized in performing inspection for foreign antigens to protect the surfaces themselves and of course human body interior. Since most infections affect or start from mucosal surfaces, using a mucosal route of vaccination is of great interest and provides a rational reason to induce a protective immune response.[3] Nasal delivery of vaccine offers an easily accessible route to the immune system.
The nose has the function of olfactory detection (sense of smell) and also filtration, humidification and temperature control of air as it enters the respiratory system. Moving from front to back the areas of the nasal cavity are the nasal vestibule, the respiratory region, and the olfactory region. The nasal cavity is divided by the septum to form the left and right nares, which lead into the left and right choana before opening onto the nasopharynx at the top of the throat. The turbinates bound the nasal walls and are responsible for air conditioning and the large mucosal surface area of the nasal cavity. The nose is also the main port of entry for many pathogens. The first barrier to foreign bodies is hair at the entrance to the nares, the nostrils, which successfully keeps out larger particles. The entire surface of the nasal cavity is covered in a mucus layer, which traps smaller particles. Mucus is an aqueous, viscoelastic and adhesive gel [4] that contains several types of mucins (abbreviated to MUC) MUC1, MUC4, MUC5A and MUC5B, MUC16, that are produced by either goblet cells or mucus subglands.[5,6] Cilia perform a mechanical clearing role termed mucociliary clearance by beating and thus transporting the mucus blanket with entrapped pathogens to the back of the throat at a rate of 5-6 mm per minute, either to be destroyed in the stomach or expectorated via sneezing and/or coughing. This function

minimises the amount of particles able to enter the body through the mucosal surface.[7] The nasal route has been used to deliver vaccines for respiratory infections and sexually transmitted infections.[8] The rationale for targeting mucosal tissue in the genital tracts can be attributed to the mucosal immune system.

The Mucosal Immune System

The mucosal immune system provides local protection against pathogens that enter the body through the mucosal membranes. The mucosal immune activities are associated with lymphoid tissues, i.e. mucosa-associated lymphoid tissue (MALT), which is present in mucosal tissue in the nose, lungs, gastrointestinal tract and vaginal/rectal surfaces.[9] The MALT is classified into specific subcompartments, depending on the location, including the gut-associated lymphoid tissue (GALT), nasopharynx-associated lymphoid tissue (NALT),[10] bronchus-associated lymphoid tissue (BALT). The mucosal routes commonly used for vaccination strategies are depicted in Figure 1. The mucosal immune systems are protected by immune cells that populate the region along the mucosal surfaces, and also epithelial cells and mucus that acts as physical barrier before the pathogen gain access to the underlying tissues.

[Figure 1 near here]

Respiratory Epithelial Cells

The epithelial cell layers cover the mucosal surfaces including the respiratory, gastrointestinal and urogenital tracts exposed to the outer environments. The epithelial cell layer acts as a barrier that is equipped with some supporting elements such as the mucus and cilia in preventing penetration of pathogens (Figure 2).

Furthermore, the epithelial cells can detect and uptake pathogenic organisms and/or antigenic components by performing nonspecific endocytosis or interacting with pattern recognition receptors such as Toll-like receptors (TLRs).[11-14] The epithelial cells together with lymphocytes and underlying antigen presenting cells (e.g. dendritic cells (DCs) and macrophages), cytokines and chemokines perform an innate, non-specific and adaptive immune response to encounter the invasion of pathogenic organisms or immunogenic substances.[14,15]

[Figure 2 near here]

Nasopharynx-Associated Lymphoid Tissue (NALT)

The NALT can be simply defined as organized mucosal immune system in the nasal mucosa that consist of lymphoid tissue, B cells, T cells and antigen presenting cells (APCs) and are covered by an epithelial layer containing memory (M) cells.[16] M cells are present in the epithelial cell layers and have specialization in transporting antigen across the epithelium.[17,18]

Whenever the nasal mucosa is exposed to pathogens or antigenic substances, the intruder will interact with the mucosal immune system. The type of interaction is highly dependent on the characteristics of the antigen. The pathogen or immunogenic substances may be able to pass through the nasal epithelium and interact with the APCs such as macrophages and DCs. These APCs will process the antigen and migrate to the lymph node where the immunogenic portion will be presented to the T cells. This marks the activation of the immune response cascade. A soluble antigen might be recognized by the APCs,[19] while particulate antigen is generally taken up by the M cells and transported to the NALT.[20] The NALT is also drained to the lymph node where further antigen processing will occur. A schematic representation of this process in more detail mechanisms is presented in Figure 3[21].

[Figure 3 near here]

Immunoglobulin A (IgA)

In addition to the MALT, the mucosal immune system also produces the antibody immunoglobulin A (IgA), that plays an important role in mucosal immunity at mucosal surfaces.[22] IgA constitutes up to 15 % of the total immunoglobulin, which is predominantly present in external secretions including the mucus in the bronchial, urogenital and digestive tracts, saliva and tears.[23] It was found that the production of IgA in humans could be over 1 mg/ml in secretions associated with the mucosal surfaces.[18] A small amount of IgA can be found in the serum while most of the IgA is located in external secretions known as secretory IgA (sIgA).[24] IgA consist of a dimer or tetramer, a joining J-chain polypeptide and a polypeptide chain called the secretory component. [24, 25] IgA has several functions in mucosal defense including the entrapment of antigens or pathogens in mucus to prevent them from direct contact with the mucosal surface.[15, 26] In addition, sIgA may also block or provide steric hindrance to surfaces of pathogenic molecules that may inhibit their attachment to the epithelium.[27]

The predominance of IgA in mucosal areas is a result of mutual collaboration between plasma cells and epithelial cells. The activated plasma cells in the lamina propria, adjacent to mucosal surfaces produce polymeric IgA (pIgA), while the epithelial cells in the mucosal surfaces express an Ig receptor called the polymeric Ig receptor (pIgR). The released pIgA from activated plasma cells binds to pIgR, and is then taken up into the cell via endocytosis. IgA is transported across mucosal epithelial cells before being released onto the luminal surface of the epithelial cells. Proteolysis cleavage of the pIgR allows IgA to be secreted into mucosal secretions. [15, 25, 28]

Mucosal Vaccines

New vaccine formulations should be able to induce innate and adaptive immune response; involving antigen-specific memory T and B cells that will respond effectively to the invading pathogens.[29, 30] Interaction with pathogens or antigens can produce the IgA secretion as an antibody response.[31] Intracellular antigens, can be produced by invading viruses that replicate within the host cell, or derive from cytoplasmic bacteria, while the extracellular antigens include bacteria, parasites, and toxins in the tissues. Intracellular antigens are generally processed in the host cells, coupled to a major histocompatibility complex-I (MHC-I), a cell surface molecule, and transported to the cell surface.[32,32] The presence of MHC-I on the cell surface will lead to activation of CD8+ T-cells to become cytotoxic T-lymphocytes (CTLs). Extracellular antigens are endocytosed and presented on MHC-II molecules for activation of CD4+ T-helper (Th) cells.[32-34]

The activation of Th cells will release a specific set of cytokines that modulate the B cell and CD8+ CTL immune response, depending on the nature of the stimulant.[35] Th cell types Th-1, Th-2 or Th-17 will be induced accordingly. A Th-1 response develops in the presence of interleukin 12 (IL-12), which is in turn synthesized primarily by DCs and/or natural killer (NK) cells in the presence of bacteria or virus. The Th-1 response is marked by the production of the Th-1 cytokines e.g. interferon-gamma (IFN-γ) and tumour necrosis factor-beta (TNF-β). A Th-2 response is driven by the presence of IL-4 and results in the production of specific cytokines IL-4, IL-5, IL-9 and IL-13.[36] It can be seen that the production of IL-4 generates a feedback loop that results in increased generation of a Th-2 response at the local site.

Nasal vaccination can also result in stimulation of Th-17 CD4+ cells. Th-17 cells are responsible for the secretion of the proinflammatory interleukins IL-17A and IL-22, as well as

IL-17F and IL-21. It Is known that the Th-17 family of cytokines respond to extracellular bacterial and fungal pathogens, and Th-17 cells enhance generation of Th-1 cells through an increased IFN-γ activation giving rise to a Th-1/Th-17 immune response that activates macrophages and other innate responses.[36-38] Stimulation of epithelial cells by the Th-17 family of cytokines can aid tissue repair and secretion of antimicrobial peptides, which can exert a protective effect in pulmonary infection.[39] There is contradictory evidence, however, regarding the role of Th-17 response in nasal immunization. Early work on the role of Th polarization in nasal immunization indicated that this route always promotes a Th-17 response. [40] Later research has indicated that the response is more nuanced, with some contradictory evidence regarding advantages and disadvantages of IL-17A induction.[41,42, 43] Predominance of one set of cytokines over the other is generally indicative of polarization of Th responses, for example the presence of IL-4 and absence of IFN-γ indicate a classical Th-2 polarized immune reaction[44] although these cytokines can also be released at the same time.[45,46, 47] The varying cytokine profiles related to CTL and antibody production are fundamental in affording protection against a specific pathogen. Specific macrophage activation was found to play a crucial role in the eradication of Mycobacterium tuberculosis bacterial infections,[48] showing that the induction of specific immune responses may play a key role in determining whether a given vaccine product is effective.

The recently discovered innate lymphoid cells (ILCs) act as an early source of cytokines to regulate and direct mucosal immune responses. [49] Unlike B or T cells, however, they do not exhibit antigen specificity. Group 1 ILCs (ILC1s) include NK cells and produce Th-1 type cytokines IFN-γ and tumor necrosis factor-α (TNF-α); group 2 ILCs (ILC2s) produce Th-2 type cytokines IL4, IL-5 and/or IL-13, while group 3 ILCs (ILC3s) include lymphoid tissue inducer cells that produce Th-17 type cytokines IL-17 and/or IL-22. Both ILC1s and ILC3s have been implicated in type 1 and Th17 cell-mediated immunity and disease.[50] Because they are involved in early release of cytokines at mucosal sites, ILCs have been implicated in directing immune response at the mucosal surface, as shown by a number of recent studies. [51, 52] NK cells and ILC1-like cells damped the immune response after vaginal administration of ovalbumin and cholera toxin to mice.[53] NK cells have been shown to enhance Th proliferation through IFN-γ production, [54] while ILC2s play a role in directing Th-2 response.[55] There is also evidence that ILCs can act as APCs, although this may be specific to the lymphoid tissue site involved and is thought to occur to a lesser extent than through the professional APCs.[55] Finally the regulatory T-cells (Tregs) play a role in ILC and Th communication,[54] as well as helping to directly control Th response, which is particularly important in autoimmune dysfunction discussed later.[56]

Advantages of nasal vaccine delivery

The nasal route has great potential for vaccination due to the organized immune systems of the nasal mucosa. The nasal epithelium encloses follicle-associated lymphoid tissues that are important in inducing mucosal immune response. The immune cells such as nearby B-cells can produce IgA at the mucosal sites where the respiratory pathogens invade.[57] Many published studies have shown that nasally administered vaccines induce serum IgG and mucosal IgA that are important for deliberating enhanced efficacy of vaccine.[57, 58] The enhanced induction of mucosal IgA antibodies has been shown to play a significant role in neutralizing pathogens such as *Streptococcus pneumonia*[59] and measles viruses[60] and preventing further infection. Moreover, intranasal immunization has also been reported to induce cross-reactive antibodies that might be indicative of cross-protection.[61, 62] This effect can make vaccines more efficient by reducing the number of vaccinations required since cross-protective vaccines may produce cross-reactive antibodies that recognize more than one antigen. Given the high cost of many antigen production systems this offers a distinct advantage over other routes.

Therapeutic vaccines

While much of the work on nasal vaccine delivery is currently focused on prophylactic vaccines, the access that the nasal route provides to the mucosal immune system also has relevance for therapeutic vaccines used to treat rather than prevent disease. Nasal immunotherapy for treatment of various cancers and Alzheimer's are currently generating much interest. [63,64] A particular focus is the use of therapeutic vaccines for the treatment of autoimmune diseases such as type I diabetes, atherosclerosis, multiple sclerosis, rheumatoid arthritis, lupus and Crohn's disease. These are caused by unchecked immune response to molecules, termed self-antigens, that are capable of inducing an immune response in a host but should not induce an immune response in a healthy individual that produces them, whereas undesirable response to innocuous environmental antigens gives rise to allergy. The autoimmune and inflammatory response is governed by regulatory T-cells (Tregs), with poor function or reduced numbers of Tregs being associated with autoimmune disease. Treatments for this family of diseases are often non-specific, or use immune suppressants that increase susceptibility to infection. Development of effective therapeutic vaccine would correct the inappropriate immune response through generation of tolerance to the self-antigen(s). [65] Treg cells that express the forkhead box P3 transcription factor are known as FoxP3+T-cells, with dysfunction of this subset of Tregs being implicated in a range of chronic inflammatory disorders.[66] It has long been known that oral delivery is effective in generating antigen tolerance, through deliberate introduction of the antigen to food.[67] More recently it has been shown that a similar tolerance induction can be achieved via nasal delivery through activation of the DCs in the draining lymph nodes to enhance induction of FoxP3+T-cells. [68] Examples of successful nasal delivery include immunization to suppress atherosclerosis[69,70] and arthritis. [71] The effect of adjuvant on tolerance is discussed in a later section.

Formulation approaches

Current nasal formulations include, solutions (drops or sprays), powders, gels and solid inserts. [72] Solutions are often described in the literature as they are both the easiest way of formulating a vaccine for an in vivo study or clinical trial, and are the easiest to administer for example in mice where the liquid is often pipetted directly into the nostril. In humans this often means that the subject either has to remain laying down or with their head held back for a period of time after administration, which is not realistic in a mass vaccination setting. Sprays are easier to administer and deliver vaccine further into the nasal cavity, but may still leak out of the nostril or drip into the oral cavity. Including a gelling agent in the formulation that is either mucoadhesive or able to penetrate through mucus offers increased residence time, while advantages of solid formats such as powders or solid inserts include ease of manufacture and stability, while liquids are more prone to degradation. Taste may also be a factor as formulations may travel into the oral cavity, although given that vaccines tend to be administered once or twice only, this is less of an issue than for medicines that are taken on a regular basis.

A range of naturally-occurring, synthetic and semi-synthetic polymers have been investigated as gelling agents in nasal delivery of vaccine. Administering as a gel should improve retention, although there is ongoing debate as to whether positively charged or anionic polymers offer better uptake. Those that have the ability to adhere to mucosal surfaces and selectively target M cells or APCs, should be the most effective. [18, 26] Chitosan has been much investigated, and is a polysaccharide manufactured from chitin found in crustacean shells or fungi by a deacetylation process. Because of the range of sources this polymer is available in a range of molecular weights, but all are made up of repeating units of glucosamine and N-acetylglucosamine and bear a positive charge making it mucoadhesive. Varying the degree of deacetylation affects the charge, as does methylation. Methylating chitosan offers some advantages for mucosal delivery.

Powder formats have the advantage of increased stability over their liquid counterparts and ability to target further into the nasal cavity. An example of this is the Anthrax spray-dried powder formulation suitable for mass vaccination in developed and developing world settings.[73] Possible disadvantages of powders include the ease and cost of administration if specialist applicators are required. Solid inserts are tablets designed to dissolve when in contact with mucus and have been investigated for vaginal delivery in humans and nasal delivery in livestock animals, [74,75] and have many similarities with sublingual formulations.

Soluble antigens tend to be less immunogenic than particulate formulations, additionally encapsulating antigen into particles may improve the transport of the antigens across the nasal mucosa. For this reason there has been a great interest in developing particulate systems as carriers for vaccine products.[76-78] Aspects such as vaccine formulations and delivery strategies are important in designing new vaccines so that efficient induction of the innate and adaptive immune response can be obtained according to the target pathogen.[18,]

[26] Particulate delivery systems that can imitate pathogens such as polymeric nanoparticles and liposomes are considered a promising approach for nasal vaccine delivery.

Nanoparticles are particles in the nanometer $1x10^{-9}$ m size range and can be made of polymers such as chitosan, alginate or synthetic co-polymers such as poly(lactic-co-glycolic acid (PLGA). Varying the molecular weight and/or ratio of lactic to glycolic acid affects the rate of degradation enabling rate of release to be controlled. But PLGA nanoparticles bear a negative charge, which is not compatible with mucosal delivery, hence the plethora of papers investigating various coatings or modifications to adjust this. Those with positive charge and enhanced residence have tended to give the best immunological responses with high serum antibody titers and sIgA levels.[79] Poly(lactic acid) (PLA) and polyethylene glycol (PEG) can also be combined to form co-block polymers able to incorporate antigen [80], varying the molecular weight of the PEG and/or ratio of PEG to PLA alters physicochemical characteristics, release and hence efficacy.[81]

Other polymers investigated include pullulan, a naturally occurring polysaccharide copolymer made up of maltotriose subunits from fungus;[82] pectin, a naturally occurring polysaccharide found in fruits; and the biodegradable synthetic polymer polycaprolactone. [83]

Liposomes are nano- or micrometre sized particles made up of one or more lipid bilayers, which have the ability to incorporate antigen at their surface or inside the aqueous core. There are numerous examples of coated and un-coated liposomal formulations used to deliver vaccine intranasally in a range of formats.[84-90] Chen showed that trimethylchitosan-coated liposome powders offered improved uptake in ex vivo nasal penetration studies when compared with the same liposomes coated in chitosan.[91] Liposomes that also comprise lipid or other material derived from virus are known as virosomes, with material from influenza virus such as hemagglutinin (HA) and neuraminidase being commonly used. [92-102]

Currently there is more evidence to support the hypothesis that particles smaller than 300nm are the most effective at crossing mucus, [103] but there is also evidence to suggest that larger particles are also able to penetrate. Results from intranasal administration of mucoadhesive microparticles suggest that penetration of the entire particle may not be necessary to induce an immune response.[104] It is likely that the overall combination of size and charge are key to achieving maximum immunological effect. Some examples of particulate delivery systems investigated for nasal delivery of vaccine are shown in Table 1.

[Table 1 near here]

Adjuvants

Some materials added to form gels or particles may act as adjuvants as well as delivery vehicles. Alternatively, adjuvants may be added as a separate component to a vaccine product. Adjuvants are materials added to a vaccine to boost the immune response and may also reduce the amount of antigen required to elicit an immune response. Alum is often used in traditional vaccines but is not effective when administered mucosally. Judicious choice of adjuvant can direct the arm of the immune system, as described previously. Often particulate delivery systems are believed to confer both the benefits of optimised delivery across mucus/mucosal tissue and inherent adjuvanting effects. Many studies have investigated these abilities and ascribed immune boosting response to one, other or both qualities.[26]

Mucosal adjuvants that have been tested for intranasal vaccine delivery including: MF59 emulsion (containing squalene oil, the surfactants Span 85 and Tween 80 and citrate buffer) [105, 106], lipopolysaccharide, [84, 107] TLR agonists,[41,108,109] chitosan, [110] trimethylchitosan,[91 110] bacterial outer membrane protein[111] and cholera toxin[112] or heat-labile enterotoxin (LT) from *E.coli*.[113] Some side effects have been found with the use of bacterial toxin when given intranasally, including Bell's palsy (Facial paralysis) and other adverse events related to disorders of the facial nerves.[114-116] It has been suggested that the central nervous system was involved in the palsy as the bacterial toxin was re-directed into the brain. [115, 117] Thus, the use of LT as vaccine adjuvant is no longer recommended. Mast cell activators such as compound 48/80 (C48/80) have shown promise in Anthrax vaccine.[73] As described previously, adjuvants can help to polarize immune response and this effect should be taken into account when considering adjuvant for a particular vaccine type. Mice immunized with an influenza vaccine adjuvanted with a synthetic TLR-4 agonist via the nasal route, exhibited a transient, enhanced IL-17A pathology, characterised by weight loss and morbidity, which was significantly greater than observed in mice given no-adjuvanted antigen.[41] The effect of adjuvants on induction of tolerance has also been noted; an intranasal co-administration of hen egg lysozyme with a TLR2 ligand enhanced Th1-type antibodies in one case, [118] while another TLG2 ligand, Pam3Cys, was shown to increase the risk of developing autoimmune disease [119] PLGA nanoparticles have been shown to boost tolerance in suppression of arthritis [120] and further research by the same group has shown that they are responsible for generation of enhanced Treg cell induction.[68]

Current nasal vaccine products

Licensed intranasal vaccines for humans include the influenza vaccines FluMist/Fluenz™ (MedImmune, MD, USA)[121] and the Nasovac™ live attenuated influenza nasal spray manufactured by the Serum Institute of India, which was developed alongside its live attenuated A(H1N1), more commonly known as swine flu.[122] No serious side effects have been reported associated with the administration of Nasovac indicating its safety,[123] although its efficacy data are not sufficiently available yet.[124] Until recently FluMist was considered one of the most successful intranasal vaccines, it is well tolerated and had exhibited good efficacy.[125] A runny nose/nasal congestion has been reported as the most common adverse events of Flumist, with mild to moderate in severity.[121] However The US CDC (Centre for Disease Control) Advisory Committee on Immunization Practices (ACIP) recently voted that the Flumist nasal spray live attenuated influenza vaccine (LAIV) (sic), should not be used during the 2016-2017 flu season, based on "data showing poor or relatively lower effectiveness of LAIV from 2013 through 2016".[126] At the time of writing no further detail was available. It should be noted that a nasal Live Attenuated Influenza Virus (LAIV) influenza vaccine has been used for over 50 years in Russia and previously the USSR. Data published from a study using the Russian intranasal vaccine showed better herd immunity for intranasal LAIV than inactivated vaccine.[127] Herd immunity is a crucial impact of mas vaccination programs; it is the immunity given to the whole population, even those who have not received a vaccine, because enough of the population (the herd) have received the vaccine that the infection cannot effectively spread. However, it should be noted that the Russian LAIV is administered in 2 doses 3 weeks apart, which increases cost and has the possibility of reducing compliance.

Targeting school age children for influenza has two benefits, first this age group tend to have the highest rates of influenza infection. Secondly targeting children reduces infection rates in through transmission from this group, although transmission rates can vary.[128] In the European Union an intranasal influenza vaccine was licensed in 2011. Damm et al explored the possible effect of introducing this product in Germany and concluded that introducing the vaccine to German schoolchildren would lead to a "substantial reduction in influenza-associated disease at a reasonable cost to the German statutory health insurance system".[129] Researchers looking into the same question for Thailand reached similar conclusions with provisos based on willingness to pay and contact between age groups. [130] This study raised the issue of effectiveness across countries where healthcare systems are either new or emerging and differences in rates and timing of seasonal outbreaks. These findings highlight the differences between high and low- to middle-income countries and demonstrate the need to carefully evaluate the target population and seasonal factors before designing or selecting a vaccine product.

[Table 2 near here]

A recent review describes most of the commonly encountered nasal delivery devices currently on the market. [72] Additionally, there is a range of nasal delivery strategies at various stages along the pre-clinical-clinical pipe-line, some of these may be suitable for vaccine delivery either in their current formats or with some adaptation. A selection of these is shown in Table 2 and will be described briefly. Criticalsorb is a penetration enhancing formulation based on PLGA and PLA, developed by a spin-out from University of Nottingham, UK, currently there are no details for vaccine application. The web-site of µco™ System (Muco System) shows data for a nasal flu vaccine in a non-human primate immunogenicity study, stating that more sIgA was produced in the mucosal membrane compared to injection and nasal liquid spray. and 4-times greater sIgA than a nasal liquid spray.[131] Optinose is a breath-actuated device for delivering powder or liquid, a schematic of the device has been published in the literature,[132] as has data on the use of sumitriptan delivered via the Optinose device[133, 134]. Kurve is a device for delivering liquid formulations "via a controlled, turbulent flow", [135] the makers have published results of a pilot clinical trial detailing their intranasal insulin therapy for Alzheimer's disease and amnestic mild cognitive impairment A,[136] while Archimedes Pharma developed a chitosan-based formulation, ChiSys® , that achieved good success in a clinical trial for a Norovirus vaccine.[137] Because of the proprietary and often pre-approval nature of the devices described (with the exception of Flumist/Fluenz and MAD Nasal), there is a paucity of information regarding design of some of the devices described in this section. The interested reader is referred to the relevant company web-sites (Table 2), which will offer more current information than is possible in this review.

Conclusion

Safety profiles are yet to be established in humans for many of the formulation approaches described in this review. However, the ever-increasing range of clinical trials indicates the accepted need for nasal vaccines that are easy to administer and offer improved benefits over other mucosal routes in terms of cost of formulation and need for skilled personnel to administer. The obvious benefits of directly stimulating the mucosal immune response are clear, but as yet have not been fully realized with the exception of those for influenza, which demonstrate the efficiency of this route. The recent US CDC press release will no doubt impact on the pharmaceutical industry view of riskiness of nasal formats. But with increased need to immunize large populations, potentially in swift response to pandemics such as avian, swine flu and Ebola there is a clear need to have strategies in place. The interplay between formulation or carrier and adjuvant in directing immune response should be investigated. Unfortunately, the high cost of clinical trials and issues with correlating immune responses in animal models with humans have created a bottleneck. There is a growing body of evidence to suggest that genetic material can be successfully delivered via this route, while recent studies have also demonstrated the advantages associated with combining the nasal with other routes of delivery or even combining vaccine with microbicide.[138] This review has focused primarily on prophylactic vaccines but there is encouraging evidence that nasal delivery will have a role to play in the design of therapeutic vaccines for e.g. cancers Alzheimer's and autoimmune diseases. The role of presentation is also important when designing pre-clinical studies – instillation of drops is relatively facile even in mice, while more advanced formulations require more careful consideration than those administered via pipette. The design of ex vivo, cell culture or tissue models that provide better prediction of response in humans is extremely desirable. A "one size fits all" approach is not appropriate for vaccine design where factors relating to target population, disease type and mode of infection, will all impact on both formulation and antigen optimization.

Table 1 Examples of particulate formulations with published in vivo data.

Particle type	Vaccine	Study type	Key findings	Literature source
Chitosan and HSA (human serum albumin)	Hepatitis B Plasmid DNA	Female C57/BL mice compared with plasmid DNA alone and protein antigen	humoral and mucosal immune response	Lebre et al 2016[139]
polycaprolactone /chitosan	Hepatitis B surface antigen (HBsAg)	C57BL/6 mice IN only. Varying doses of HBsAg no comparator formulations	Dose-independent serum IgG and nasal IgA	Jesus et al 2016[83]
TMC	ovalbumin compared with PLGA and TMC-coated PLGA	Female Balb/c compared with PLGA and TMC-coated PLGA (IM and IN)	Serum IgG superior to other IN but inferior to all IM	Slutter et al 2010 [79]
chitosan and glycol chitosan coated PLGA	HBsAg	Female BALB/c mice compared with chitosan coated PLGA and PLGA, HBsAg-Alum sub-cut.	GC-PLGA NPs could induce significantly higher systemic and mucosal immune response than other IN nanoparticles.	Pawar et al 2013[140]
PEG-PLA	HBsAg	BALB/c mice compared with PLA nanoparticles and conventional alum-HBsAg based vaccine	Higher systemic and mucosal response than PLA	Jain et al 2009[80]
Liposomes	Influenza plasmid DNA (H1N1) hemagglutinin (HA)	BALB/c mice challenge study IN compared with IM DNA alone (IN and IM)	Protective effect against challenge	Wang et al 2004[85]
Esterified hyaluronic acid microparticles	Commercial Influenza H1N1 HA and LTK63 or LTR72 adjuvants	mice, rabbits and micro-pigs IN compared with soluble HA + LTK63, or IM with HA	Significantly enhanced serum IgG responses and higher hemagglutination inhibition (HI) titers than other groups	Singh et al 2001[104]
Glycol chitosan coated liposomes	Hepatitis B Plasmid DNA	BALB/c mice prime boost	Humoral mucosal and cellular	Khatri et al 2008 [141]

		compared with DNA alone (IN) and HBsAg protein (IM)	response higher than DNA alone. Cellular response better than IM protein antigen	
Liposomes/ hyaluronic acid	Yersinia pestis (plague)	C57BL/6 mice No IM comparison	Th1/Th2 humoral immune response	Fan et al 2015[90]
Chitosan-coated PLGA	foot-and-mouth disease plasmid DNA	Challenge study in cattle	Higher mucosal, systemic, and cell-mediated immunity than Chitosan - Inactivated antigen nanoparticles	Pan et al 2014[142]
Cationic cholesteryl-group-bearing pullulan	*Clostridium botulinum* type-A neurotoxin subunit antigen	BALB/c mice	Strong tetanus-toxoid-specific systemic and mucosal immune responses	Nochi et al 2010[82]

Table 2 Currently Marketed Technology for Nasal Delivery

Name	Company	Presentation	Drug type	Regulatory status	Marketed products	Company web-site
Criticalsorb	Critical Pharmaceuticals	Powder or aerosol	Small molecule – peptide, HGH,insulin	GRAS status?	None	www.criticalpharmaceuticals.com
µco™	Nasal Delivery System Business	Powder-based mucoadhesive drug carrier plus device	Anti-emetic Migraine, flu vaccine	Phase II, Phase I, pre-clinical	None	www.snbl-nds.co.jp/en/
Optinose	Optinose	Powder or liquid plus device	Small molecule	Clinical trials (various)	None	optinose.com/
Kurve	Kurve	Liquid plus device	Includes Alzheimer's vaccine	Phase II	None	www.kurvetech.com
MAD nasal	Teleflex	Liquid plus device	Attachment for syringe to atomize liquids	Device only/ not vaccines	Marketed as stand-alone device	www.teleflex.com
None	Drug Delivery International	Solid insert	Small molecules & insulin	None found	None found	www.bdpharma
Flumist Fluenz	MedImmune (AstraZeneca)	Nasal gel	Flu vaccine	FDA & EMA	Flumist Fluenz	www.flumistquadrivalent.com/
Bacterial S antigen pores	Tufts University - US	Oral/nasal format not stated	Tetanus toxin and rotavirus VP6 antigen	None	None	www.tufts.edu/
Vaccinetab	Queen's University Belfast, UK	Liposomal liquid, powder or nasal insert	Small molecules and antigen	GRAS	None	www.vaccinetab.com
ChiSys	Archimedes Pharma	Nasal gel	Small molecules and antigen	Phase I, pre-clinical	Small molecule	

Figure Captions

Figure 1. Routes of mucosal vaccination within the mucosa-associated lymphoid tissue (MALT), with several subcompartments including: the nasopharynx-associated lymphoid tissue (NALT), bronchus-associated lymphoid tissue (BALT), gut-associated lymphoid tissue (GALT) and genital tract-associated lymphoid tissue, reproduced from Lycke et al, 2012.[125]

Figure 2. Structure and function of respiratory epithelial cells; equipped with mucus layer (not shown) and ciliated cells, reproduced from Grassin-Delyle (2012)[143].

Figure 3. Pathways demonstrating how particulate antigen triggers local immune response in the nasal mucosa and systemic immune response via the NALT, adapted from Csaba (2009)[21].

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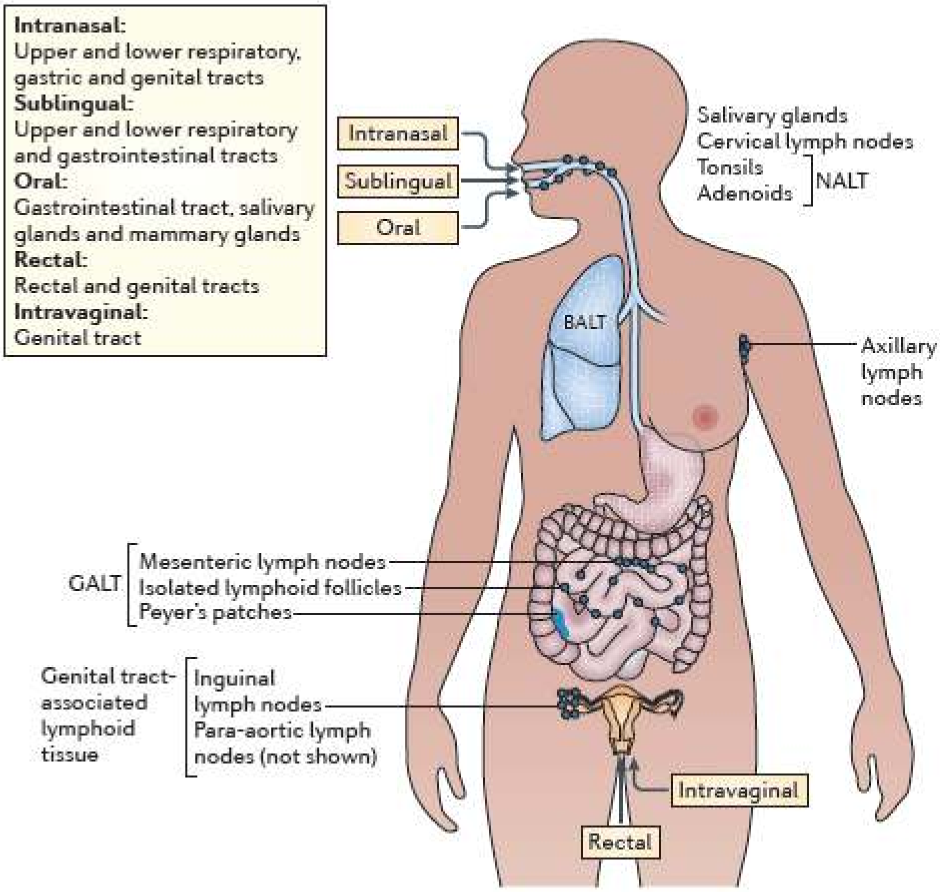
Figure 1



Intranasal:
Upper and lower respiratory, gastric and genital tracts
Sublingual:
Upper and lower respiratory and gastrointestinal tracts
Oral:
Gastrointestinal tract, salivary glands and mammary glands
Rectal:
Rectal and genital tracts
Intravaginal:
Genital tract
Intranasal
Sublingual
Oral
Salivary glands
Cervical lymph nodes
Tonsils
Adenoids
NALT
BALT
Axillary lymph nodes
GALT
Mesenteric lymph nodes
Isolated lymphoid follicles
Peyer's patches
Genital tract-associated lymphoid tissue
Inguinal lymph nodes
Para-aortic lymph nodes (not shown)
Intravaginal
Rectal

Figure 2

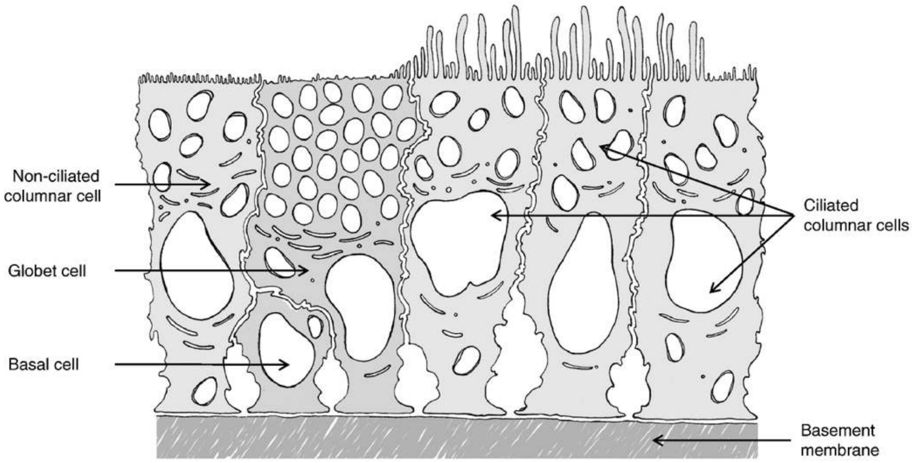
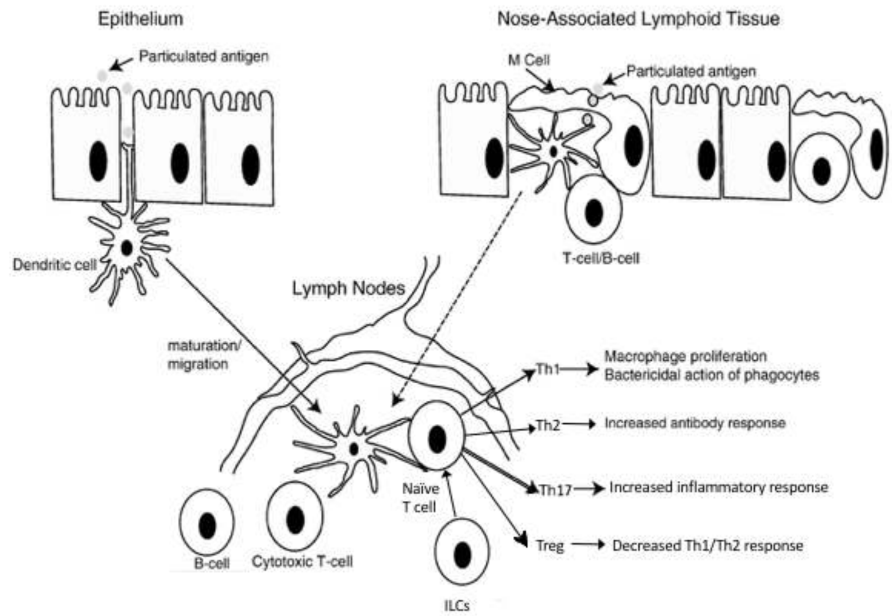

Non-ciliated
columnar cell
Globet cell
Basal cell
Ciliated
columnar cells
Basement
membrane

Figure 3


Epithelium
Particulated antigen
Dendritic cell
Nose-Associated Lymphoid Tissue
M Cell
Particulated antigen
T-cell/B-cell
Lymph Nodes
maturation/
migration
Th1
Macrophage proliferation
Bactericidal action of phagocytes
Th2
Increased antibody response
Th17
Increased inflammatory response
Treg
Decreased Th1/Th2 response
Naïve
T cell
B-cell
Cytotoxic T-cell
ILCs