Review

Nasal vaccines for respiratory infections

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Beginning with Edward Jenner's discovery of the smallpox vaccine, the ever-expanding repertoire of vaccines against pathogens has saved many lives. During the COVID-19 pandemic, a revolutionary mRNA injectable vaccine emerged that effectively controlled the severity of disease caused by SARS-CoV-2. This vaccine induced potent antigen-specific neutralizing serum IgG antibodies, but was limited in its ability to prevent viral invasion at the respiratory surfaces. Nasal vaccines have attracted attention as a potential strategy to combat respiratory infections and prepare for future pandemics. Input from disciplines such as microbiology, biomaterials, bioengineering and chemistry have complemented the immunology to create innovative delivery systems. This approach to vaccine delivery has yielded nasal vaccines that induce secretory IgA as well as serum IgG antibodies, which are expected to prevent pathogen invasion, thereby diminishing transmission and disease severity. For a nasal vaccine to be successful, the complexity of the relevant anatomical, physiological and immunological properties, including the proximity of the central nervous system to the nasal cavity, must be considered. In this Review, we discuss past and current efforts as well as future directions for developing safe and effective nasal vaccines for the prevention of respiratory infections.

Mucosal surfaces covering the airway and digestive tracts are exposed constantly to many inhaled or ingested microbial challenges[1–3]. These surfaces therefore require a highly adapted mucosal immune system that is induced and orchestrated independently from systemic immune responses. Attempts to decrease the toll of mucosal infections have been ongoing for many years. Nasal administration of pulverized scabs of smallpox lesions from recovered individuals was practiced in China during the tenth century[4]. More recently, evidence has shown that nasal vaccines containing live influenza virus were more effective than injected vaccines, based on a significant reduction in absenteeism in around 10,000 Japanese students[5,6]. Although the nasal vaccine was proven effective by epidemiological evidence, it has not yet become a universal approach to controlling respiratory infections.

The COVID-19 pandemic returned mucosal immunization to the forefront of vaccine development strategies. Widely used injectable vaccines—including the SARS-CoV-2 mRNA vaccine—induce pathogen-specific serum IgG neutralizing antibodies that prevent most severe disease (for example, in the lung)[7]. However, injectable vaccines are less effective at inducing mucosal IgA responses and thus cannot protect at the site of pathogen invasion[8–11]. By contrast, nasal vaccines not only induce systemic immune responses (for example, IgG), they also induce pathogen-specific mucosal immunity (for example, secretory IgA (SIgA)), thereby providing comprehensive immunity[12,13] (Fig. 1). Although less studied, nasal vaccines also induce $CD4^+$ and $CD8^+$ T resident memory (T_{RM}) cells in respiratory tissues. Pulmonary T_{RM} cells confer long-term immunity and protect mice against lethal virus infection[14,15]. In addition, respiratory $CD8^+$ T_{RM} cells reduce both infection and transmission of the virus, even in the absence of virus-specific antibodies[16]. Because respiratory infections are initiated at mucosal surfaces of the airways, nasal vaccination is considered a rational means of inducing protection against pathogens such as SARS-CoV-2, influenza virus, respiratory syncytial virus (RSV), *Streptococcus pneumoniae* and others[17–20].

Considerations for nasal vaccine development

The respiratory mucosa provides a physiological and immunological barrier that protects the host[1,6,19,21,22]. These surfaces are covered with ciliated epithelial cells that facilitate the exclusion of inhaled substances[22]. Epithelial goblet cells secrete mucus, which traps inhaled particles that are then cleared from the cavity by ciliary movement[23,24]. Thus, the barrier function of the nasal cavity helps to minimize the entry of pathogens into the body through the respiratory mucosal surface. Similarly, these physiological barriers impair the effective delivery of nasal vaccine antigens.

The airway mucosa also includes nasopharyngeal-associated lymphoid tissue (NALT), the site where antigen-specific immune responses to inhaled antigens are induced[1,6,25] (Fig. 2). Human unpaired nasopharyngeal tonsils (adenoids) and paired palatine tonsils (Waldeyer's ring) constitute the NALT structures. Unlike human nasopharyngeal tissue, rodent NALT develops on the basolateral sides of the cavity[6,25], which may be an important consideration when interpreting results from murine nasal vaccination studies. NALT contains all of the cells that are necessary to initiate and regulate antigen-specific immunity, including antigen-presenting cells (APCs) such as dendritic cells and macrophages, as well as B cells and T cells[6,19,20]. For example, human NALT include resident memory $CD8^+$ T cells and $CD4^+$ T helper (T_H) cells, regulatory T cells, T follicular helper (T_{FH}) cells as

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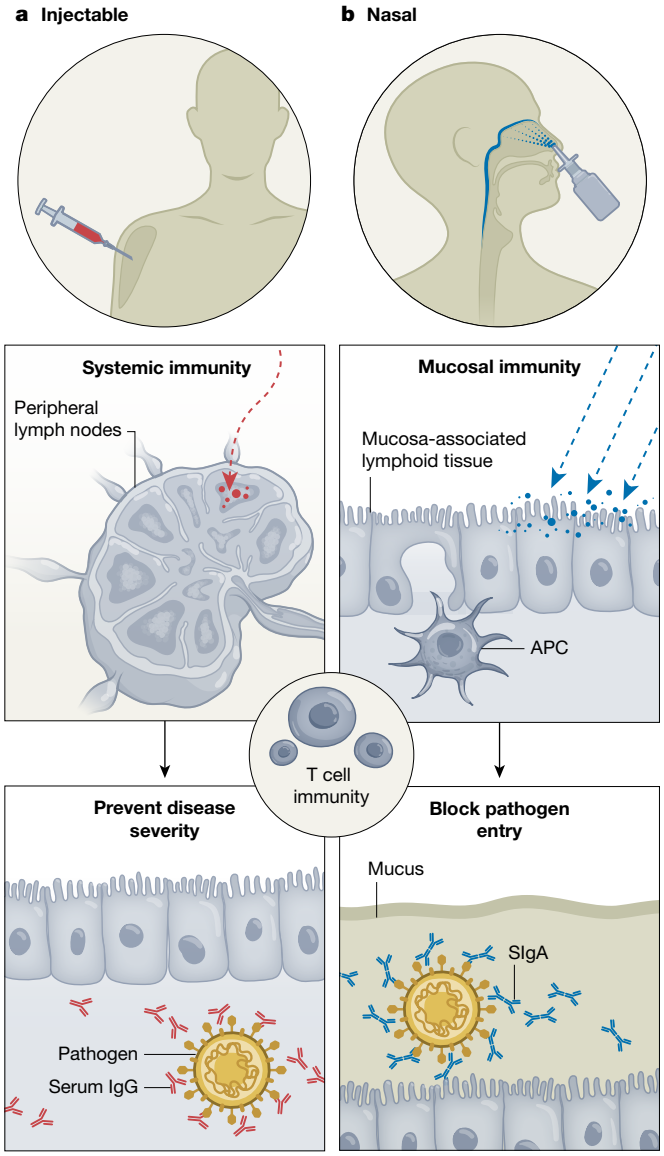

Fig. 1 | Comparison of injectable and mucosal vaccines. a, Currently, most vaccinations are administered by injection, which effectively stimulates systemic immunity and leads to the induction of pathogen-specific serum IgG neutralizing antibodies and T cell immunity, resulting in control of the severity of infectious diseases. However, injectable vaccines are generally less effective in inducing antigen-specific immune responses on mucosal surfaces and thus may not provide adequate protective immunity at the site of pathogen invasion. **b**, By contrast, the preferred formulation of nasal vaccines effectively stimulate the mucosal immune system to produce pathogen-specific SIgA antibodies and systemic (serum IgG) immunity, which together, provide broad protection to the host. Because most infections are initiated at the mucosal surfaces, mucosal vaccination is considered a rational means to induce broad protection against infection.

well as resident memory B cells, which are stable over time in healthy adults[26]. Active germinal centre formation is observed in the adenoid, which contains B and T cells, providing the necessary elements for IgA induction.

The NALT is covered by an epithelial layer containing luminal antigen-scavenging microfold cells (M cells)[6,27]. Inhaled antigens are captured by M cells and transferred immediately to underlying APCs to activate local T_H cells that produce IgA-switching and enhancing cytokines (for example, TGFβ1 and IL-5)[6,19,28–30]. This facilitates the generation of antigen-specific IgA^+ B cells (Fig. 2). NALT-derived IgA^+ B cells predominantly express CCR10 and accumulate in the nasal turbinate and glandular acinar tissues, where the CCR10 ligand CCL28 is expressed[31]. The acquisition of chemokine receptors—such as CCR9 and CCR10—imprints their ability to home to mucosal effector sites expressing the corresponding ligands—CCL25 and CCL28 (refs. 20,32–34). This targeted migration constitutes the common mucosal immune system[20,35]. The effect of this homing was shown when the CCR3–CXCL9/CXCL10-mediated migration of IgA plasma cells into the olfactory mucosa blocked transmission of pathogens to the olfactory bulb and the central nervous system (CNS)[36].

After migration to the nasal mucosa, antigen-specific IgA^+ B cells differentiate into plasma cells. Once they become plasma cells, they produce polymeric IgA[37,38] (Fig. 2). Polymeric IgA binds to the polymeric immunoglobulin receptor after which the complex is transported across the epithelium and into the lumen as SIgA[38,39] (Fig. 2). SIgA uses the multiple Fab antigen-binding sites to capture pathogens and block their adhesion to mucosal surfaces[40]. The importance of SIgA was demonstrated by examining virus shedding from individuals infected with SARS-CoV-2 (ref. 41). SARS-CoV-2 spike (S) protein-specific SIgA antibodies reduced viral RNA load and infectivity. When recombinant monomeric, dimeric and SIgA1 antibodies were engineered from four neutralizing monoclonal IgG antibodies specific for the receptor-binding domain (RBD) of the S protein and their activities were compared with their parental antibodies, the engineered dimeric and SIgA1 antibodies showed higher neutralizing activity against several variants, including the Omicron lineages BA.1, BA.2 and BA.4/5 (ref. 42). Therefore, developing nasal vaccines that effectively induce local antigen-specific SIgA to block the initial invasion by pathogens is a compelling strategy.

In addition to NALT-initiated antigen-specific immunity, the nasal turbinates are lined with a monolayer of epithelium containing respiratory M cells[32,43]. This provides an alternative antigen uptake site for nasally administered antigens. Furthermore, nasally administered antigens can be engulfed directly by dendritic cells that have extended their dendrites between epithelial cells and into the nasal cavity[44]. Following engulfment of the antigens, these cells migrate to regional lymph nodes (for example, cervical lymph nodes) to initiate antigen-specific immunity[6,19,20] (Fig. 2). The nasal cavity is thus an efficient antigen-sampling site that enhances the induction of mucosal antigen-specific immune responses, making it a logical target for vaccine delivery.

The need for a nasal vaccine

Nasal vaccines have practical advantages over injectable formulations[19,20]: (1) by being less intrusive, they avoid the pain and anxiety that patients associate with needles; (2) they can be self-administered, thereby facilitating mass vaccination; (3) nasal vaccination effectively induces mucosal and systemic immunity, thus preventing pathogen invasion of the airway and the severity of disease elsewhere; and (4) the nasal route induces cross-reactive IgA antibodies[45,46]. The latter point was demonstrated using a mouse model of influenza in which nasal, but not systemic, immunization with a recombinant influenza neuraminidase protein induced cross-reactive IgA antibodies from tissue-resident IgA-producing cells. This provided protection against both heterologous and homologous virus infections[46].

A 2003 human clinical trial of FluMist demonstrated broader protection against infection by three different subtypes—influenza A/Texas/36/91 (H1N1), influenza A/Shangdong/9/93 (H3N2) and influenza B/Panama/45/90—than the injectable trivalent inactivated vaccine[47]. FluMist is the only intranasal vaccine approved by the US Food and Drug Administration (FDA). FluMist is now a quadrivalent, attenuated influenza nasal vaccine with efficacy comparable to the flu shot. Approval excludes individuals younger than 2 or older than 50 years of age, or those who are immunocompromised or pregnant. Although it

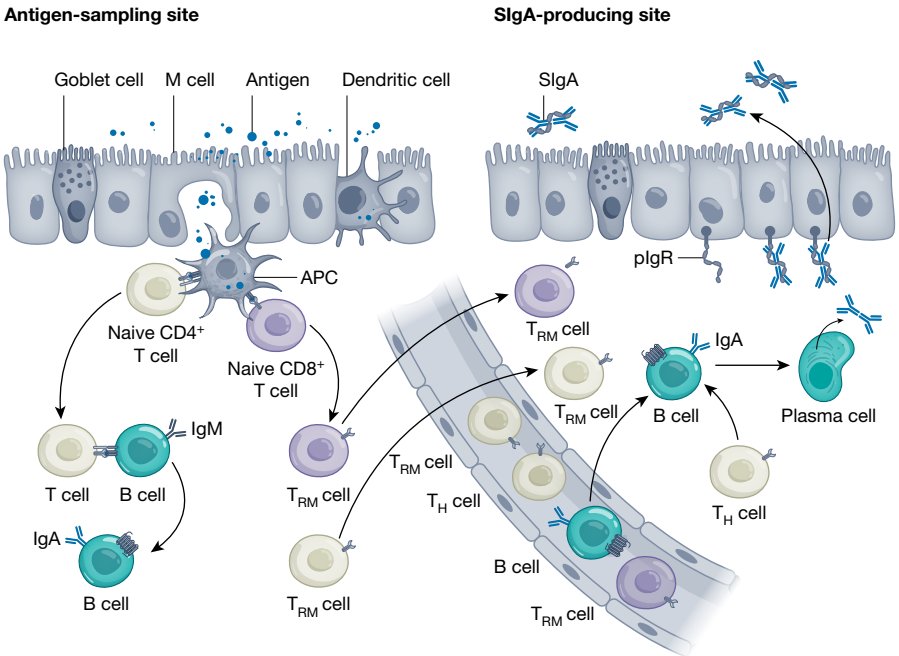

Fig. 2 | A strategy for the induction of antigen-specific mucosal immunity by nasal vaccines. The common mucosal immune system consists of inductive (antigen-sampling) and effector (SIgA-producing) sites for the induction of antigen-specific mucosal immunity (antigen-specific SIgA and T cells). Classical antigen-sampling M cells are located in the epithelium of the nasopharyngeal-associated lymphoid tissue (NALT), whereas respiratory M cells are located in the nasal turbinate epithelium for the initiation of antigen-specific immunity. Nasally administered antigens taken up by classical M cells are captured by underlying dendritic cells where they are phagocytosed, processed and presented as immunogenic peptides to local T_H1, T_H2 and T_{FH} cells as well as antigen-specific IgA B cells in NALT. Nasally administered antigens are also taken up by respiratory M cells and processed by underlying dendritic cells for the induction of antigen-specific immune responses in the cervical lymph nodes. Some of the dendritic cells underlying the nasal epithelium can extend their dendrites into the lumen to directly capture nasally administered antigens and then migrate to the regional lymph nodes (cervical lymph nodes) to initiate antigen-specific immunity. Thus, the nasal cavity is enriched with a diversified antigen-sampling system for the initiation of antigen-specific mucosal antibody and T cell-mediated immunity. Antigen-specific lymphocytes, including IgA B cells and T cells in the organized lymphoid structures (NALT and cervical lymph nodes), also acquire mucosal imprinting molecules, including homing and chemokine receptors, and migrate to mucosal effector sites for the generation of SIgA antibodies. It is thought that after T_{RM} cells are induced at the antigen-sampling site, some of them remain while others migrate to the SIgA-producing site. Following migration of antigen-specific lymphocytes into the lamina propria region of the respiratory tract, including the nasal cavity, IgA^+ B cells differentiate into IgA plasma cells by cytokines produced by antigen-specific T_H2 cells. These IgA plasma cells produce polymeric forms of IgA, which then bind to the polymeric immunoglobulin receptor (pIgR) found on the basal membrane of epithelial cells and get transported to the lumen as SIgA antibodies.

requires a prescription, a key advantage of FluMist is that parents and caregivers can administer this vaccine outside of a healthcare setting (https://www.fda.gov/vaccines-blood-biologics/vaccines/flumist).

Another intranasal live-attenuated influenza vaccine (AstraZeneca, 2018/19 formulation) has demonstrated the induction of distinct IgA and IgG responses in human mucosa and blood, respectively[48]. Mucosal IL-33 associated with T_H2-type immunity and divergent $CD8^+$ T cell and circulating T_{FH} cell responses in the first 8 h and 7 days postnasal immunization, respectively. Furthermore, it was suggested that these responses are correlates of protection induced by nasal vaccination in humans. The current status of influenza nasal vaccine development is summarized in Supplementary Fig. 1.

The secret to success for nasal vaccines

For a nasal vaccine to be successful, it must address the unique physiological environment within the nasal cavity[19,20]. Nasal vaccine antigen is readily diluted by nasal secretions and eliminated by the ciliated epithelium, potentially hindering antigen binding, sampling and processing[23,24,49]. Nasal secretions also contain proteases and aminopeptidases[50] that could degrade vaccine antigens. In humans, the volume of a nasally administered liquid vaccine formulation[51] is limited to approximately 200 µl. These physical and physiological characteristics must be considered for the successful development of nasal vaccines. To this end, the integrated efforts of experts in immunology and other disciplines have created novel delivery systems (for example, replicative and non-replicative forms) to advance the development of nasal vaccines[19,52] (Fig. 3).

Replicating delivery systems for nasal vaccines

Replicating delivery systems include recombinant adenoviruses, influenza viruses, parainfluenza viruses and herpesviruses that replicate within the host to continuously deliver vaccine antigens[53,54]. These offer stable expression of antigenic proteins and adjuvant-free induction of local cytotoxic T cell responses[55]. A clinical trial in seronegative adults using a live, virus-vectored, parainfluenza virus 5 (PIV5) nasal vaccine encoding the RSV F antigen (BLB201) showed no side effects and good immunogenicity[56]. Both nasal and serum RSV F-specific antibody responses and antigen-specific $CD4^+$ and $CD8^+$ T cells were detected. In addition to these systems, bacterial vectors including *Lactobacillus*, *Salmonella* and *Listeria* have been investigated[57]. *Lactobacillus*, which is non-pathogenic and a relatively safe vector, is considered a promising candidate after testing in nasal vaccines targeting *Yersinia pseudotuberculosis*, *Mycobacterium tuberculosis* and SARS-CoV-2 (refs. 58,59).

Non-replicating and biocompatible delivery systems

Non-replicating viral vectors address the safety issues associated with replicating delivery systems. The inherent infectivity of adenoviral vectors resulted in rapid expression and increased production of the vaccine antigen as it simultaneously stimulates innate and adaptive

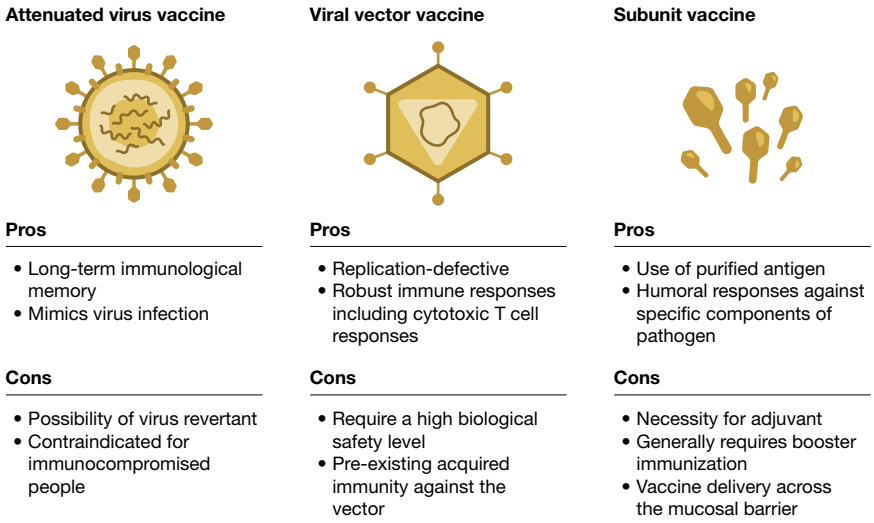

Fig. 3 | A comparison of nasal vaccine formulations. To develop a successful nasal vaccine against respiratory virus infections, the formulation of the vaccine, including the delivery system, must cope with the unique anatomical properties of the nasal cavity. Generally, three forms of nasal vaccines have been considered: attenuated live virus, viral vector and subunit-based formulations. A classical form of virus vaccine, which can be adopted for nasal vaccination, is whole killed virus or reverse genetically modified virus. Live viral vectors including adenoviruses, influenza viruses, parainfluenza and herpesviruses have been shown to be effective replicating delivery systems. To overcome safety concerns of the replicating viral vector, non-replicating forms of viral vectors have been developed that show rapid expression and increased production of the desired vaccine antigen for the induction of antigen-specific immunity. Regarding safety of a vaccine, there is general agreement that subunit-type antigen preparations (or purified antigens) are suitable candidates. A key issue in applying a subunit formulation to nasal vaccines is the development of antigen-delivery vehicles that are robust to the harsh environment of the nasal cavity and thus can effectively deliver the vaccine antigen to the mucosal immune system in the airway tract. To this end, integration of biomaterial and nanomaterial science has led to the development of vaccine-delivery polymers and compounds that can be controlled with respect to size, biological acceptability and safety.

immunity[60]. These vectors are a promising vaccine platform because they do not require co-administered adjuvants.

Other non-replicating vaccine-delivery systems have integrated biomaterials and nanomaterials consisting of natural and synthetic polymers or various synthetic compounds that can be controlled with respect to size, biological acceptability and safety[61–63] (Fig. 3). Thus, nanomaterials with varying covalency can carry antigens, thereby equipping them for the unique environment of a target tissue[19,64]. For example, cationic cholesteryl group pullulan (cCHP)-bearing nanogels are a promising material that effectively delivers a vaccine antigen to the mucosal surfaces of the nasal cavity[32,65,66] (Fig. 4). Initially, a noncationic, cholesteryl pullulan (CHP) nanogel was developed for human use as an injectable vaccine containing cancer peptide antigen[67]. The CHP nanogel is composed of a polysaccharide pullulan and a cholesterol group that forms a hydrophobic aggregate spherical structure[19,32,66]. Protein antigens are easily incorporated into the internal space of the nanomaterials, which act as a chaperone, releasing the antigenic cargo in a refolded native form[68].

Since the nasal epithelium exists in a negatively charged state, cationic amino groups have been introduced into the CHP nanogels[69]. This adapts the nanogels to efficiently attach to the mucosal surface, thus improving their application as a vaccine-delivery system[69–71] (Fig. 4).

Given their potential as a nasal vaccine-delivery system, several cCHP-based vaccines are under development that target various respiratory infections[70–73]. In particular, pneumococcal surface protein A (PspA), expressed by all serotypes of *S. pneumoniae*, is an attractive candidate vaccine antigen, as it induces cross-reactive immune responses among different serotypes[74–76]. To develop a vaccine that targets all *S. pneumonia* serotypes, cCHP nanogels containing PspA (cCHP-PspA) have been formulated and tested in mice and non-human primates[70–72]. When administered intranasally to mice, the cCHP-PspA induced PspA-specific SIgA and IgG antibodies in the nasal lavage fluids and the serum, respectively, which resulted in diminished numbers of bacteria in the nasal cavity and lung. This led to protection against lethal respiratory challenge with pneumococci[70]. The cCHP-PspA nasal vaccine has also been tested in Rhesus macaques[71], where nasal immunization induced high titres of PspA-specific serum IgG and mucosal IgA antibodies[71] and provided protective immunity against intratracheal challenge with different pneumococcal serotypes[72]. These preclinical studies identified the cCHP-PspA nasal vaccine as a promising candidate for future use in human clinical trials. The cCHP system has been adopted to target otitis media caused by non-typeable *Haemophilus influenzae* (NTHi). Because the P6 protein is conserved among 90% of NTHi strains[77], it was incorporated into a cCHP nanogel (cCHP-P6)[73]. cCHP-P6 effectively induced P6-specific IgA antibodies in nasal or middle ear fluids of mice and inhibited NTHi biofilm formation[73]. Thus, the cCHP-P6 nanogel nasal vaccine is a promising candidate to prevent NTHi-derived infections related to otitis media, sinusitis and pneumonia.

The cCHP-based nasal delivery strategy has also been applied to respiratory viral infections. RSV is a leading cause of respiratory infections, particularly in children[78–80] and the elderly[81]. An injectable RSV vaccine using the prefusion F protein antigen has been licensed and used for the older and pregnant women[82]. Although the vaccine is effective in inducing systemic immunity, considering the route of RSV infection, it would make sense to develop a nasal vaccine that blocks viral invasion at the actual site of infection. One study has used an alternative antigen, the ectodomain of the small hydrophobic protein (SHe), a transmembrane surface protein of RSV, for the induction of protective immunity[83]. SHe was linked to the carrier protein PspA (SHe–PspA) in a vaccine antigen and formulated using the cCHP nanogel[84]. Nasal immunization of mice and cotton rats with cCHP containing SHe–PspA (cCHP-[SHe–PspA]) induced both SHe-specific mucosal IgA and serum IgG antibodies. In cotton rats, a preferred model of human RSV infection, these nasal vaccine-induced SHe-specific antibodies controlled viral infection in the airway mucosa and in the lung[84]. This study demonstrated that cCHP is also applicable to subunit-based nasal vaccines against other respiratory viral infections. An obvious strategy for this nasal vaccine development would be to determine whether the bivalent form

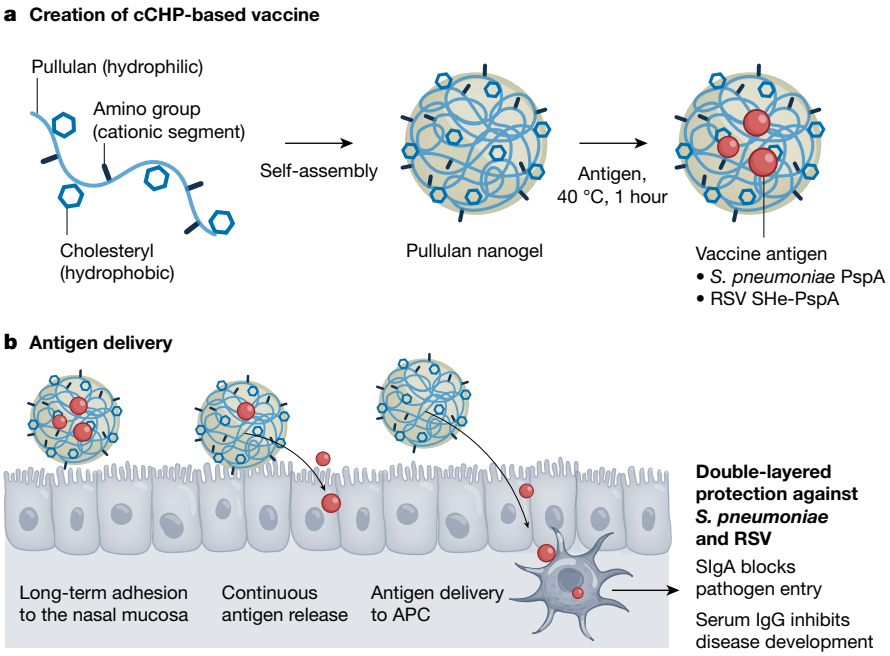

Fig. 4 | cCHP nanogel, a promising nasal vaccine-delivery system. a, The original CHP nanogel consisted of pullulan, a glucose-based polysaccharide and cholesteryl groups that form spherical structures with an average size of 40 nm. Protein-based vaccine antigens are naturally fused inside the CHP nanogel and then released as a refolded native form of the antigen. This unique characteristic is referred to as 'artificial chaperone activity'. Thus, the CHP nanogel effectively delivers the protein vaccine antigen to the target site in its native and active form. To enhance the effective delivery of the vaccine antigen to the nasal epithelium, positively charged amino groups have been introduced into the CHP nanogels to create cCHP. **b**, cCHP binds efficiently to the nasal mucosal surface for a prolonged period of time and continuously releases antigens, enabling vaccine-delivery to APC, such as dendritic cells, in the underlying nasal epithelium. cCHP nanogel-containing candidate vaccine antigens (such as PspA from *S. pneumoniae* and SHe from RSV) effectively deliver the vaccine antigens to APCs for the initiation of antigen-specific immune responses. cCHP nanogel-containing vaccine antigens can thus induce both systemic (IgG) and mucosal (SIgA) responses, resulting in a double layer of protective immunity against respiratory pathogens. Nasal vaccines against several respiratory infections, including RSV, *S. pneumoniae*, NTHi, SARS-CoV-2 and seasonal influenza are under development.

of the cCHP-[SHe–PspA] nasal vaccine can simultaneously induce immunity against both viral (RSV) and bacterial (*S. pneumoniae*) infections.

Other promising biocompatible materials that deliver subunit vaccine antigens to mucosal surfaces of the nasal cavity include a lipid-based delivery vehicle. Liposomes are small artificial vesicles that consist of lipid bilayers containing phospholipids and cholesterol. They have been shown to be effective carriers for inducing antigen-specific immunity[62]. The amphiphilic nature of liposomes allows for the entrapment of antigens within liposomes[63]. Various physicochemical properties of the liposome, including lipid composition, structure and size, can be tailored to the characteristics of the candidate vaccine antigen in order to maximize the immunogenicity[63]. Because the phospholipids used for liposome preparations are derived from food sources (such as egg yolk and soybean), this delivery system is considered to be safe except in individuals allergic to those dietary antigens[85]. Cationic liposomes and surface-modified liposomes with mucoadhesive polymers have been developed to enhance mucosal adhesion and persistence in the nasal cavity after immunization[61]. For example, the nasal delivery of recombinant influenza haemagglutinin trimers using cobalt-porphyrin-phospholipid (CoPoP) liposomes effectively induced haemagglutinin-specific immune responses[86]. CoPoP liposomes are being evaluated in clinical trials for a COVID-19 vaccine (Clinical Trials.gov Identifier: NCT04783311).

Chitosan has favourable biological properties for mucosal delivery, including good degradability and low toxicity[87]. Additional advantages are its mucoadhesive properties and its ability to loosen epithelial tight junctions, thereby facilitating antigen uptake[88]. This delivery system induces balanced antigen-specific T_H1 and T_H2-mediated antibody responses in mice following nasal immunization using chitosan loaded with a protein-based antigen[89]. However, its poor solubility necessitates solubilization under acidic conditions followed by hydrolysis. This might become a drawback for chitosan as a practical mucosal vaccine[90]. Chemical modifications to increase the hydrophilicity of chitosan and ligand modifications to target specific cells and improve cellular uptake (such as galactose, mannose and peptide modifications) are being investigated to improve the applicability of chitosan-based vaccine-delivery systems[90]. Chitosan-based nasal candidate vaccines using diphtheria antigen and Norwalk virus-like particles have been shown to be well tolerated and immunogenic, with the induction of antigen-specific IgG and IgA responses[91–93].

SpyCage, a self-assembling 60-subunit I3-01 protein scaffold that is covalently decorated with recombinant protein antigen, has provided additional proof-of-principle support for the induction of mucosal immunity by nasal immunization[94]. SpyCage coated with the RBD of the SARS-CoV-2 S protein co-administered with the mucosal adjuvant LTA1 from heat-labile *Escherichia coli* enterotoxin (lacking cranial nerve toxicity) was highly immunogenic and enhanced clearance from the nose and lungs of Syrian hamsters.

A nasal vaccine against COVID-19

The current intramuscular COVID-19 vaccines are not very effective at inducing mucosal immunity that prevents subsequent infections or limits transmission to others[10,11]. As SARS-CoV-2 initiates infection in the airway, it is important to target the respiratory immune system directly with a second-generation COVID-19 vaccine. A human SARS-CoV-2 challenge study in seronegative adults given an intranasal vaccine demonstrated that the immune response associated with early mucosal IgA and $CD8^+$ T cell responses is strongly correlated with the

control of virus burden[95]. This study supports a role for nasal vaccines that lead to a reduction in infection and transmission.

Promising results emerged following the nasal administration of viral vector-based vaccine formulations. When the avian paramyxovirus type 3 (APMV3) vaccine vector expressing the SARS-CoV-2 S protein was administered nasally once to hamsters, it induced S-specific IgG and IgA antibody responses with neutralizing activities not only against SARS-CoV-2 isolate WA1/2020, but also against alpha and beta variants[96]. These nasally immunized hamsters exhibited no lung inflammation or viral replication throughout the respiratory tract. In addition, a live, attenuated parainfluenza type 3 virus (B/HPIV3) expressing the prefusion-stabilized SARS-CoV-2 S protein (B/HPIV3/S-6P) was evaluated for immunogenicity and efficacy in non-human primates[97]. This study showed that S-specific airway mucosal IgA and IgG were induced along with S-specific serum antibodies, and these antibodies had neutralizing activities against SARS-CoV-2 variant Alpha, Beta and Delta lineages, but less activity against the Omicron sublineages. After challenge, SARS-CoV-2 was undetectable in the respiratory tract and lung tissues of macaques that were nasally immunized with B/HPIV3 expressing prefusion S[97]. As a similar strategy, a B/HPIV3 candidate vaccine expressing SARS-CoV-2 antigens was developed and tested. A single intranasal immunization of hamsters with a live, replication-competent chimeric B/HPIV3 expressing prefusion-stabilized S proteins induced virus-specific IgG and IgA with neutralizing activity against SARS-CoV-2 A, B.1.1.7 and B.1.351 (ref. 98). After viral challenge, virus was undetectable in the nasal cavity and lungs. Another study examined adenovector-based ChAdOx1 nCoV-19/AZD1222, an approved injectable SARS-CoV-2 vaccine (known as Covishield). Previous studies demonstrated that intramuscular vaccination of rhesus macaques with ChAdOx1 nCoV-19/AZD1222 protected against pneumonia but did not reduce viral shedding from the upper respiratory tract. However, subsequent nasal immunization of macaques and hamsters with ChAdOx1 nCoV-19 reduced detection of the virus in the respiratory tract after challenge infection[99].

It should be noted that the nasally administered ChAd-based vaccines induce not only broadly reactive IgG and IgA responses, but also virus-specific memory $CD8^+$ T cells in the respiratory tract[100]. A single-centre, open-label phase 1 clinical trial of nasal vaccination with ChAdOx1 nCoV-19 was conducted in healthy adults[101], in which nasal ChAdOx1 nCoV-19 was administered as a single booster to two groups of small numbers of participants who had previously received two injections of ChAdOx1 nCoV-19 or BNT162b2. This phase 1 study demonstrated an acceptable tolerability profile of the nasal ChAdOx1 nCoV-19 vaccine. However, detectable mucosal antibodies were observed in a minority of participants with minimal systemic antibody responses. Because the ChAdOx1 vector used for this nasal vaccine is derived from a simian adenovirus serotype, it may have exhibit poor infectivity towards human respiratory epithelium, resulting in low expression of the SARS-CoV-2 antigen. To further examine applicability of the adenovirus vector-based COVID-19 nasal vaccine, a separate study compared replication-deficient adenovirus and single-cycle replicating adenovirus vaccines expressing SARS-CoV-2 S protein[102]. The single-cycle replicating formulation resulted in the generation of higher levels of S-specific antibodies than the replication-deficient formulation. A single nasal or intramuscular vaccination with the single-cycle replicating adenovirus reduced SARS-CoV-2 viral load and lung injury compared with vaccination with the replication-defective adenovirus. It remains to be determined whether repeated vector-based vaccine administration will lead to an anti-vector immune response that reduces future viral vector infectivity and vaccine antigen delivery[103]. Together, these studies support the applicability of a virus vector-based nasal vaccine against SARS-CoV-2 by demonstrating high immunogenicity and protective immunity. However, the safety and immunogenicity of this virus vector-based nasal delivery for human use needs to be investigated further.

Another study[104] further emphasized the importance of the nasal vaccine strategy by developing a fully attenuated replication-competent SARS-CoV-2 vaccine candidate, sCPD9-ΔFCS, as a nasal vaccine and comparing it to the injectable monovalent mRNA vaccine BNT162b2 in prevention of transmission of SARS-CoV-2 variants B.1 and Omicron BA.5. The results showed that sCPD9-ΔFCS outperformed the injectable mRNA vaccine in preventing viral transmission, further highlighting the advantages of a nasal vaccine over the injectable vaccines.

Many people have already had the injectable mRNA vaccine, which provides systemic protective immunity against severe COVID-19 disease. A 'prime-and-spike' (P&S) strategy has been proposed, building on the existing systemic protective immunity generated by injection vaccination (prime) followed by eliciting mucosal immunity using a nasal booster (spike) with prefusion-stabilized (Hexapro), trimeric, recombinant SARS-CoV-2 S protein[105]. The P&S resulted in robust mucosal cellular and humoral immune responses, including tissue-resident memory $CD8^+$ T cells and IgA and IgG antibodies, in experimental rodents. The vaccine-induced immunity protected mice from a viral challenge several months after vaccination, demonstrating the vaccine's durability. The effectiveness of the P&S vaccination strategy was further demonstrated by the protection of immunized hamsters, including the blocking of viral transmission. A primate study further confirmed the efficacy of intratracheal boosting with a bivalent Ad26-based SARS-CoV-2 vaccine candidate to induce mucosal humoral responses with neutralizing activity and cellular immunity that provided near-complete protection against challenge with SARS-CoV-2 BQ.1.1. in injectable mRNA vaccine-primed rhesus macaques[106]. This study suggests that protective efficacy was correlated with mucosal humoral and cellular immune responses and supports the feasibility of developing mucosal vaccines that can block respiratory virus invasion at the airway mucosa.

As evidence for the broad relevance and durability of the P&S strategy, the induction of protective immunity against heterologous XBB.1.16 challenge was examined in nonhuman primates five months after receiving either a nasal booster with a WA1-BA.5 bivalent chimpanzee adenovirus-vectored vaccine, or an injection booster with bivalent mRNA encoding WA1 and BA.5 or XBB.1.16 S proteins[107]. Nasal vaccination elicited durable airway IgG and IgA responses, with minimal viral replication in both the upper and lower respiratory tracts. By contrast, the injected mRNA vaccine was limited to systemic B cell-mediated protection in the lower respiratory tract. This study further supports the broad efficacy of the nasal vaccine in preventing SARS-CoV-2 infection.

In line with the expectation that a nasal vaccine can induce a robust mucosal immunity capable of preventing the invasion and transmission of SARS-CoV-2, a large number of candidate nasal vaccines are undergoing investigation. As of the 30 March 2023 issue of the World Health Organization's *COVID-19 Vaccine Tracker* (https://www.who.int/teams/blueprint/covid-19/covid-19-vaccine-tracker-and-landscape), 19 airway-targeted vaccine candidates including 16 nasal, 1 aerosol and 2 inhaler types are under various stages of clinical evaluations (Supplementary Fig. 1). Most of these airway-administered SARS-CoV-2 vaccines are either replication-competent or attenuated viral vectors. For example, the replication-competent influenza virus vector-based nasal vaccine targeting the RBD of the SARS-CoV-2 S protein[108], and Newcastle disease virus and adenovirus vector-based nasal vaccines expressing the S protein[109,110] are being evaluated in phase 2/3 trials. Non-replicating vector-based vaccines against COVID-19 include a PIV5-based adenovirus vector vaccine expressing the S protein of Wuhan (ancestral strain WA1) that is in phase 1 clinical trials to assess safety, reactogenicity and immunogenicity in healthy adult and young volunteers[111,112]. An attenuated live vaccine with RSV vector expressing S protein—MV-014-212 (ref. 113)—is currently undergoing a phase 1 trial to evaluate its safety and immunogenicity in healthy adults. Furthermore, an attenuated SARS-CoV-2 nasal vaccine, COVI-VAC, is undergoing clinical investigation to evaluate its safety and efficacy in healthy adults[114].

Along with these live-attenuated virus- or viral vector-based vaccine candidates, efforts are also aimed at developing recombinant protein (or subunit)-based nasal vaccines. A recent study showed that nasal administration of SARS-CoV-2 RBD subunit vaccines with mastoparan-7 (mast cell-activating oligopeptide) adjuvant resulted in the preferential induction of polyfunctional central memory T cells (T_{CM} cells)[115]. Nasally induced T_{CM} cell responses are T cell-intrinsic and are maintained after transfer to naive hosts to promote enhanced memory retrieval upon lung antigen challenge in both draining brachial lymph nodes and the lungs. The T_{CM} cell response resulted in antibodies with broad neutralizing activity against multiple variants compared to injection vaccination with the same antigen and adjuvant. Thus, nasal vaccination resulted in protection from clinical disease and less lung histopathology in the hamster challenge model. The study provides supportive evidence for the efficacy of the nasal vaccine and demonstrates the critical importance of T cell immunity in addition to antibody responses. Whereas the vaccine field has historically focused on humoral immunity (for example, antibodies) as an informative parameter for protective immunity, the experience with SARS-CoV-2 infection has re-emphasized the critical importance of T cell-mediated immunity for the induction of memory and protective immunity against pathogens[116,117]. T cell immunity has been shown to control viral infection even in the absence of neutralizing antibodies[16,118]. Clearly, the contributions of T cells remain an important response to be assessed in vaccine development.

Many countries have contributed novel approaches to address nasal immunization for COVID. In Cuba, CIGB-669, a protein subunit candidate vaccine containing the SARS-CoV-2 S protein RBD and the nucleocapsid antigen of hepatitis B virus, has advanced to clinical trials in which it is being evaluated in nasal administration of CIGB-669 alone or in combination with intramuscular vaccination[119]. Another COVID-19 subunit nasal vaccine candidate under evaluation is ACM-001, which consists of SARS-CoV-2 S protein encapsulated with a CpG adjuvant in self-assembled nanoscale vesicles formed by an artificial cell membrane via polymersome technology[120,121].

As a result of these global efforts, at least four countries China, India, Iran and Russia have granted emergency licenses for nasal vaccines. Two forms of mucosal vaccines, an inhaled vaccine with adenovirus vector type 5 expressing S protein[122] and live-attenuated, influenza-expressing, RBD-based nasal spray vaccine[123] have been approved in China. In India, a replication-deficient chimpanzee adenovirus vaccine encoding a prefusion-stabilized S protein[124,125] has been approved as a nasal vaccine for adults. The recombinant S protein-based nasal vaccine combined with the oil-in-water adjuvant system RAS-0 has been shown to be safe and effective for the induction of protective immunity[126] and approved for emergency use in Iran. Russia has also reportedly approved the use of nasal vaccination with a heterologous recombinant adenovirus rAd26 and rAd5-based vaccine that was originally developed as an injectable vaccine[127].

The reality of nasal mRNA vaccines

Given the success of the mRNA-based vaccine during the COVID-19 pandemic, the mucosal delivery of mRNA vaccines needs to be considered in preparation for future pandemics. Innovative mRNA nasal delivery systems are being developed to protect mRNA from degradation by enzymes in nasal secretions and to prevent its clearance across mucosal barriers[128]. A cationic polyethyleneimine-based nasal vaccine-delivery vehicle facilitates mRNA delivery across the nasal epithelium owing to its biocompatible cyclodextrin, which minimizes mucosal damage and transiently opens tight junctions[129]. Thus, a cationic cyclodextrin-polyethyleneimine 2k conjugate complexed with anionic mRNA encoding HIV gp120 has been tested for nasal mRNA vaccination. This resulted in the induction of potent mucosal and systemic T_H1, T_H2 and T_H17 immune responses[130]. A similar strategy, but using a cationic liposomal formulation, has been considered for nasal delivery of nucleic acid-based therapies for the prevention and treatment of neurologic diseases[131,132]. Despite the hurdles that exist for maintaining mRNA stability in the harsh environment of the nasal cavity, the merging of expertise in bioengineering, mucosal immunology and vaccinology is leading to novel delivery vehicles for nasal immunization targeting respiratory pathogens. For example, an inhalable, biodegradable polyamine-co-ester polyplex has been studied for the delivery of mRNA encoding the SARS-CoV-2 S protein to the lung[133]. Nasal administration of these polyplexes resulted in high transfection of mRNA throughout the lung, including in epithelial cells and APCs, which led to both cellular and humoral protective immunity against lethal viral challenge in mice. Another study evaluated the immunogenicity and protective efficacy of nasal mRNA encapsulated lipid nanoparticle (mRNA-LNP) vaccines against SARS-CoV-2 in Syrian golden hamsters[134]. Nasal mRNA-LNP vaccination induced S-specific neutralizing IgG and IgA antibodies that reduced respiratory viral loads, decreased lung pathology and prevented weight loss following SARS-CoV-2 challenge. Together, there is reason for optimism regarding induction of protective immunity in humans by nasal mRNA vaccines.

Summary and future issues

As discussed in this Review and elsewhere[135,136], there is increasing evidence that nasal vaccines are safe, prevent pathogen invasion and inhibit severe disease in both mucosal surfaces and systemic sites[12,13,19,20]. A limited number of nasal vaccines are currently licensed and in clinical use[137] and as discussed above, FluMist Quadrivalent, a live-attenuated influenza vaccine[138], is currently the only nasal vaccine licensed by the FDA for healthy individuals aged between 2 and 49 years. However, even if an attenuated vaccine could be created by reducing the virulence of the virus, it is not approved for use in immunologically immature infants or older people with immunosenescence, because nasal administration of live vaccines may cause adverse reactions[139].

The safety of nasal vaccines must be considered during their development[140]. For example, vaccines containing whole pathogens may revert to a replicative state[141]. Recombinant protein-based subunit vaccines appear to be safer than live vaccines, but purified recombinant protein (or subunit) antigens tend to elicit relatively weak immune responses and generally need to be co-administered with an adjuvant as an immunostimulant[142]. Several adjuvants are currently used in injectable vaccine formulations, including alum AS04, MF59, AS01B and CpG 1018 (ref. 143). Other candidates are in clinical trials, such as microbial derivatives (for example, monophosphoryl lipid A and AGP (RC-529, a synthetic acylated monosaccharide)) and particulate adjuvants (for example, ISCOMS (a structured complex of saponins and lipids) and polylactide-co-glycolide)[144]. These candidates could be included in nasal subunit vaccine formulations.

A major concern for nasal vaccine development is the proximity of the nasal cavity to the CNS[19,32]. The superior nasal orifice adjoins the olfactory bulb, which consists of specialized neurons connected to the brain[64,145]. Thus, antigen administered via the nasal route could be transported to the brain via the epithelial cells or the adjacent olfactory bulb and adversely affect neuronal function[145]. Nearly 50% of the nasal surfaces of mice and rats commonly used for vaccine development are covered by olfactory epithelium[19,146,147]. In humans[146,147], respiratory epithelium covers the majority (around 90%) of the nasal cavity lining and olfactory epithelium covers the remaining 10%. Although the surface area of the human olfactory epithelium is small compared with that of rodents, its proximity to the CNS must be considered for the development of safe nasal vaccines. In addition, nasal vaccines should not affect the sustentacular cells in the olfactory epithelium that support olfactory neurons, as it has been shown that these cells cause inflammation by attracting myeloid cells to the olfactory epithelium, leading to proinflammatory cytokine production during pathogen infection[148]. As an example

of the risk, an inactivated nasal influenza vaccine combined with a genetically detoxified mutant of *E. coli* heat-labile enterotoxin as the adjuvant was approved for use[149]. Post-marketing surveillance identified cases of facial nerve palsy as an adverse reaction, leading to the discontinuation of this vaccine[149]. Further, Pandemrix, an inactivated influenza vaccine containing the adjuvant AS03, increased the risk of narcolepsy in children, adolescents[150,151] and adults[152,153]. Cholera toxin, which is similar in structure to the *E. coli* heat-labile enterotoxin, has been used experimentally as a mucosal adjuvant for supporting the induction of mucosal vaccine antigen-specific immune responses. In a mouse model, nasally administered native cholera toxin can be transported to the CNS via the olfactory bulbs, where it induced nerve damage in the CNS[154]. Thus, in parallel with the development of the nasal vaccine, it is essential to verify that there is no transfer of vaccine antigen and/or adjuvant to the CNS via the olfactory bulb epithelium. Candidate nasal vaccines must continue to be carefully evaluated to ensure that all components of the formulation are safe and free of undesirable effects on the CNS prior to initiating clinical trials.

In summary, several key issues need to be considered for nasal vaccine development including: (1) efforts to develop of a safe and stable mRNA and subunit vaccine formulation and delivery system; (2) the identification of the type of immunity—humoral, cell-mediated, tissue-resident and/or circulating memory, alone or in combination—required to combat target pathogens based on human immunoprofiling data generated pre- and post-infection (or immunization); (3) the creation of an optimal nasal vaccine formulation and delivery vehicle and the benefit of an appropriate adjuvant; and (4) the assurance of overall nasal vaccine safety.

1. Sato, S. & Kiyono, H. The mucosal immune system of the respiratory tract. *Curr. Opin. Virol.* **2**, 225–232 (2012).
2. Kiyono, H. & Azegami, T. The mucosal immune system: from dentistry to vaccine development. *Proc. Jpn Acad. B* **91**, 423–439 (2015).
3. Kiyono, H., Bienenstock, J., McGhee, J. R. & Ernst, P. B. The mucosal immune system: features of inductive and effector sites to consider in mucosal immunization and vaccine development. *Reg. Immunol.* **4**, 54–62 (1992).
4. Rusell, W. R. & Ogra, R. L. in *Mucosal Vaccines—Innovation for Preventing Infections Diseases*, 2nd edn (eds Kiyono, H. & Pascual, D. W.) 3–17 (Academic Press, 2020).
This chapter provides a comprehensive summary of the history of research and development of mucosal vaccines including those of nasal vaccines.
5. Okuno, Y. & Nakamura, K. Prophylactic effectiveness of live influenza vaccine in 1965. *Biken J.* **9**, 89–95 (1966).
6. Kiyono, H. & Fukuyama, S. NALT- versus Peyer's-patch-mediated mucosal immunity. *Nat. Rev. Immunol.* **4**, 699–710 (2004).
7. Turner, J. S. et al. SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature* **596**, 109–113 (2021).
8. Bleier, B. S., Ramanathan, M. Jr & Lane, A. P. COVID-19 vaccines may not prevent nasal SARS-CoV-2 infection and asymptomatic transmission. *Otolaryngol. Head Neck Surg.* **164**, 305–307 (2021).
9. Tiboni, M., Casettari, L. & Illum, L. Nasal vaccination against SARS-CoV-2: Synergistic or alternative to intramuscular vaccines? *Int. J. Pharm.* **603**, 120686 (2021).
10. Azzi, L. et al. Mucosal immune response in BNT162b2 COVID-19 vaccine recipients. *eBioMedicine* **75**, 103788 (2022).
11. Tang, J. et al. Respiratory mucosal immunity against SARS-CoV-2 after mRNA vaccination. *Sci. Immunol.* **7**, eadd4853 (2022).
12. Yuki, Y. & Kiyono, H. Mucosal vaccines: novel advances in technology and delivery. *Expert Rev. Vaccines* **8**, 1083–1097 (2009).
13. Kiyono, H., Yuki, Y., Nakahashi-Ouchida, R. & Fujihashi, K. Mucosal vaccines: wisdom from now and then. *Int. Immunol.* **33**, 767–774 (2021).
14. Teijaro, J. R. et al. Cutting edge: tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. *J. Immunol.* **187**, 5510–5514 (2011).
15. Diallo, B. K. et al. Intranasal COVID-19 vaccine induces respiratory memory T cells and protects K18-hACE mice against SARS-CoV-2 infection. *NPJ Vaccines* **8**, 68 (2023).
16. Uddback, I. et al. Prevention of respiratory virus transmission by resident memory $CD8^+$ T cells. *Nature* **626**, 392–400 (2024).
17. Holmgren, J. & Czerkinsky, C. Mucosal immunity and vaccines. *Nat. Med.* **11**, S45–S53 (2005).
18. Dhama, K. et al. COVID-19 intranasal vaccines: current progress, advantages, prospects, and challenges. *Hum. Vaccin. Immunother.* **18**, 2045853 (2022).
19. Nakahashi-Ouchida, R., Fujihashi, K., Kurashima, Y., Yuki, Y. & Kiyono, H. Nasal vaccines: solutions for respiratory infectious diseases. *Trends Mol. Med.* **29**, 124–140 (2023).
20. Tsai, C. J. Y., Loh, J. M. S., Fujihashi, K. & Kiyono, H. Mucosal vaccination: onward and upward. *Expert Rev. Vaccines* **22**, 885–899 (2023).
21. Gizurarson, S. Anatomical and histological factors affecting intranasal drug and vaccine delivery. *Curr. Drug Deliv.* **9**, 566–582 (2012).
22. Mitchison, H. M. & Valente, E. M. Motile and non-motile cilia in human pathology: from function to phenotypes. *J. Pathol.* **241**, 294–309 (2017).
23. Voynow, J. A. & Rubin, B. K. Mucins, mucus, and sputum. *Chest* **135**, 505–512 (2009).
24. Gizurarson, S. The effect of cilia and the mucociliary clearance on successful drug delivery. *Biol. Pharm. Bull.* **38**, 497–506 (2015).
25. Pabst, R. Nose-associated lymphoid tissue (NALT)—structure, function and species differences. *Vaccine* **33**, 4406–4413 (2015).
This review introduces and discusses the basic immunological basis of the NALT as the inductive site for the initiation of antigen-specific immune responses, including species differences.
26. Ramirez, S. I. et al. Immunological memory diversity in the human upper airway. *Nature* **632**, 630–636 (2024).
This study provided evidence that the human nasopharynx, including adenoids, harbours an immunologic environment for IgA B cell induction.
27. Corr, S. C., Gahan, C. C. & Hill, C. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. *FEMS Immunol. Med. Microbiol.* **52**, 2–12 (2008).
28. Stavnezer, J. & Kang, J. The surprising discovery that TGFβ specifically induces the IgA class switch. *J. Immunol.* **182**, 5–7 (2009).
29. Carrasco-Yepez, M. M. et al. *Naegleria fowleri* immunization modifies lymphocytes and APC of nasal mucosa. *Parasite Immunol.* **40**, e12508 (2018).
30. Wellford, S. A. et al. Distinct olfactory mucosal macrophage populations mediate neuronal maintenance and pathogen defense. *Mucosal Immunol.* **17**, 1102–1113 (2024).
31. Liu, J. et al. Turbinate-homing IgA-secreting cells originate in the nasal lymphoid tissues. *Nature* **632**, 637–646 (2024).
This article provides evidence for the existence of a common mucosal immune system through the adoptive transfer of lymphoid B cells leading to their migration to distant and different mucosal tissues.
32. Nakahashi-Ouchida, R., Yuki, Y. & Kiyono, H. Development of a nanogel-based nasal vaccine as a novel antigen delivery system. *Expert Rev. Vaccines* **16**, 1231–1240 (2017).
This review describes how NALT-derived IgA^+ B cells predominantly express CCR10 and migrate to immunological effector tissues with increased CCL28 expression.
33. Hieshima, K. et al. CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. *J. Immunol.* **173**, 3668–3675 (2004).
34. Wang, J. et al. Respiratory influenza virus infection induces intestinal immune injury via microbiota-mediated Th17 cell-dependent inflammation. *J. Exp. Med.* **211**, 2397–2410 (2014).
35. McDermott, M. R. & Bienenstock, J. Evidence for a common mucosal immunologic system: I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. *J. Immunol.* **122**, 1892–1898 (1979).
This paper provided the evidence for the existence of the commensal mucosal immune system.
36. Wellford, S. A. et al. Mucosal plasma cells are required to protect the upper airway and brain from infection. *Immunity* **55**, 2118–2134.e2116 (2022).
37. Moens, L. & Tangye, S. G. Cytokine-mediated regulation of plasma cell generation: IL-21 takes center stage. *Front. Immunol.* **5**, 65 (2014).
38. Corthesy, B. Multi-faceted functions of secretory IgA at mucosal surfaces. *Front. Immunol.* **4**, 185 (2013).
This article summarizes the immunobiological uniqueness of SIgA antibodies, which have crucial roles in creating a balanced state of mutualism and elimination in the harsh environment of mucosal surfaces.
39. Wei, H. & Wang, J. Y. Role of polymeric immunoglobulin receptor in IgA and IgM transcytosis. *Int. J. Mol. Sci.* **22**, 2284 (2021).
40. Brandtzaeg, P. Secretory IgA: designed for anti-microbial defense. *Front. Immunol.* **4**, 222 (2013).
41. Miyamoto, S. et al. Infectious virus shedding duration reflects secretory IgA antibody response latency after SARS-CoV-2 infection. *Proc. Natl Acad. Sci. USA* **120**, e2314808120 (2023).
This paper demonstrated the importance of SARS CoV-2 S protein-specific SIgA antibodies in preventing infectious virus shedding and transmission in humans.
42. Marcotte, H. et al. Conversion of monoclonal IgG to dimeric and secretory IgA restores neutralizing ability and prevents infection of Omicron lineages. *Proc. Natl Acad. Sci. USA* **121**, e2315354120 (2024).
43. Kim, D. Y. et al. The airway antigen sampling system: respiratory M cells as an alternative gateway for inhaled antigens. *J. Immunol.* **186**, 4253–4262 (2011).
44. Lee, H. et al. Phenotype and function of nasal dendritic cells. *Mucosal Immunol.* **8**, 1083–1098 (2015).
45. Tamura, S., Tanimoto, T. & Kurata, T. Mechanisms of broad cross-protection provided by influenza virus infection and their application to vaccines. *Jpn J. Infect. Dis.* **58**, 195–207 (2005).
46. Oh, J. E. et al. Intranasal priming induces local lung-resident B cell populations that secrete protective mucosal antiviral IgA. *Sci. Immunol.* **6**, eabj5129 (2021).
This paper showed that intranasal, but not injection, immunization induces lung-resident IgA B cells, leading to the induction of IgA-mediated protective immunity against influenza virus.
47. Treanor, J. J. et al. Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. *Vaccine* **18**, 899–906 (1999).
48. Thwaites, R. S. et al. Early mucosal events promote distinct mucosal and systemic antibody responses to live attenuated influenza vaccine. *Nat. Commun.* **14**, 8053 (2023).
49. Ali, M. S. & Pearson, J. P. Upper airway mucin gene expression: a review. *Laryngoscope* **117**, 932–938 (2007).
50. Dehghan, M. H., Gaikwad, V. M. & Dandge, B. Nasal absorption of drugs—barriers and solutions. *Res. J. Pharm. Tech.* **2**, 634–641 (2009).
51. Singh, A. K., Singh, A. & Madhv, N. V. S. Nasal Cavity, a promising transmucosal platform for drug delivery and research approaches from nasal to blain targetting. *J. Drug Delivery* https://doi.org/10.22270/jddt.v2i3.163 (2012).

52. Robert-Guroff, M. Replicating and non-replicating viral vectors for vaccine development. *Curr. Opin. Biotechnol.* **18**, 546–556 (2007).
53. Yahalom-Ronen, Y. et al. A single dose of recombinant VSV-ΔG-spike vaccine provides protection against SARS-CoV-2 challenge. *Nat. Commun.* **11**, 6402 (2020).
54. Bezbaruah, R. et al. Developmental landscape of potential vaccine candidates based on viral vector for prophylaxis of COVID-19. *Front. Mol. Biosci.* **8**, 635337 (2021).
55. Ura, T., Okuda, K. & Shimada, M. Developments in viral vector-based vaccines. *Vaccines* **2**, 624–641 (2014).
56. Spearman, P. et al. Intranasal parainfluenza virus type 5 (PIV5)-vectored RSV vaccine is safe and immunogenic in healthy adults in a phase 1 clinical study. *Sci. Adv.* **9**, eadj7611 (2023).
57. Ding, C., Ma, J., Dong, Q. & Liu, Q. Live bacterial vaccine vector and delivery strategies of heterologous antigen: a review. *Immunol. Lett.* **197**, 70–77 (2018).
58. LeCureux, J. S. & Dean, G. A. *Lactobacillus* mucosal vaccine vectors: immune responses against bacterial and viral antigens. *mSphere* **3**, e00061–18 (2018).
59. Li, L. et al. Mucosal IgA response elicited by intranasal immunization of *Lactobacillus plantarum* expressing surface-displayed RBD protein of SARS-CoV-2. *Int. J. Biol. Macromol.* **190**, 409–416 (2021).
60. Sakurai, F., Tachibana, M. & Mizuguchi, H. Adenovirus vector-based vaccine for infectious diseases. *Drug Metab. Pharmacokinet.* **42**, 100432 (2022).
61. De Leo, V., Milano, F., Agostiano, A. & Catucci, L. Recent advancements in polymer/liposome assembly for drug delivery: from surface modifications to hybrid vesicles. *Polymers* **13**, 1027 (2021).
62. Mangla, B. et al. Nanocarriers-assisted needle-free vaccine delivery through oral and intranasal transmucosal routes: a novel therapeutic conduit. *Front. Pharmacol.* **12**, 757761 (2021).
63. Schwendener, R. A. Liposomes as vaccine delivery systems: a review of the recent advances. *Ther. Adv. Vaccines* **2**, 159–182 (2014).
64. Zuglianello, C. & Lemos-Senna, E. The nanotechnological approach for nasal delivery of peptide drugs: a comprehensive review. *J. Microencapsul.* **39**, 156–175 (2022).
65. Yuki, Y. et al. Nanogel-based antigen-delivery system for nasal vaccines. *Biotechnol. Genet. Eng. Rev.* **29**, 61–72 (2013).
66. Nakahashi-Ouchida, R., Yuki, Y. & Kiyono, H. Cationic pullulan nanogel as a safe and effective nasal vaccine delivery system for respiratory infectious diseases. *Hum. Vaccin. Immunother.* **14**, 2189–2193 (2018).
67. Shimizu, T. et al. Nanogel DDS enables sustained release of IL-12 for tumor immunotherapy. *Biochem. Biophys. Res. Commun.* **367**, 330–335 (2008).
68. Ayame, H., Morimoto, N. & Akiyoshi, K. Self-assembled cationic nanogels for intracellular protein delivery. *Bioconjug. Chem.* **19**, 882–890 (2008).
69. Nochi, T. et al. Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. *Nat. Mater.* **9**, 572–578 (2010).
This is the first study to demonstrate that a nanogel consisting of cCHP is a promising nasal vaccine-delivery vehicle for universal protein-based vaccines.
70. Kong, I. G. et al. Nanogel-based PspA intranasal vaccine prevents invasive disease and nasal colonization by *Streptococcus pneumoniae*. *Infect. Immun.* **81**, 1625–1634 (2013).
71. Fukuyama, Y. et al. Nanogel-based pneumococcal surface protein A nasal vaccine induces microRNA-associated Th17 cell responses with neutralizing antibodies against *Streptococcus pneumoniae* in macaques. *Mucosal Immunol.* **8**, 1144–1153 (2015).
72. Nakahashi-Ouchida, R. et al. A nanogel-based trivalent PspA nasal vaccine protects macaques from intratracheal challenge with pneumococci. *Vaccine* **39**, 3353–3364 (2021).
73. Nakahashi-Ouchida, R. et al. Induction of mucosal IgA-mediated protective immunity against nontypeable *Haemophilus influenzae* infection by a cationic nanogel-based P6 nasal vaccine. *Front. Immunol.* **13**, 819859 (2022).
74. Crain, M. J. et al. Pneumococcal surface protein A (PspA) is serologically highly variable and is expressed by all clinically important capsular serotypes of *Streptococcus pneumoniae*. *Infect. Immun.* **58**, 3293–3299 (1990).
75. Nabors, G. S. et al. Immunization of healthy adults with a single recombinant pneumococcal surface protein A (PspA) variant stimulates broadly cross-reactive antibodies to heterologous PspA molecules. *Vaccine* **18**, 1743–1754 (2000).
76. Piao, Z. et al. Protective properties of a fusion pneumococcal surface protein A (PspA) vaccine against pneumococcal challenge by five different PspA clades in mice. *Vaccine* **32**, 5607–5613 (2014).
77. De Chiara, M. et al. Genome sequencing of disease and carriage isolates of nontypeable *Haemophilus influenzae* identifies discrete population structure. *Proc. Natl Acad. Sci. USA* **111**, 5439–5444 (2014).
78. Jain, S. et al. Community-acquired pneumonia requiring hospitalization among U.S. children. *N. Engl. J. Med.* **372**, 835–845 (2015).
79. Shi, T. et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* **390**, 946–958 (2017).
80. Scheltema, N. M. et al. Global respiratory syncytial virus-associated mortality in young children (RSV GOLD): a retrospective case series. *Lancet Glob. Health* **5**, e984–e991 (2017).
81. Yu, X. et al. Antibody and local cytokine response to respiratory syncytial virus infection in community-dwelling older adults. *mSphere* **5**, e00577–20 (2020).
82. Venkatesan, P. First RSV vaccine approvals. *Lancet Microbe* **4**, e577 (2023).
83. Schepens, B. et al. Protection and mechanism of action of a novel human respiratory syncytial virus vaccine candidate based on the extracellular domain of small hydrophobic protein. *EMBO Mol. Med.* **6**, 1436–1454 (2014).
84. Umemoto, S. et al. Cationic-nanogel nasal vaccine containing the ectodomain of RSV-small hydrophobic protein induces protective immunity in rodents. *NPJ Vaccines* **8**, 106 (2023).
This paper demonstrated the feasibility of developing a nasal vaccine that induces both humoral and cell-mediated immunity against RSV infection.
85. van Hoogevest, P. & Wendel, A. The use of natural and synthetic phospholipids as pharmaceutical excipients. *Eur. J. Lipid Sci. Technol.* **116**, 1088–1107 (2014).
86. Sia, Z. R. et al. A liposome-displayed hemagglutinin vaccine platform protects mice and ferrets from heterologous influenza virus challenge. *Proc. Natl Acad. Sci. USA* **118**, e2025759118 (2021).
87. Islam, N. & Ferro, V. Recent advances in chitosan-based nanoparticulate pulmonary drug delivery. *Nanoscale* **8**, 14341–14358 (2016).
88. Sonaje, K. et al. Opening of epithelial tight junctions and enhancement of paracellular permeation by chitosan: microscopic, ultrastructural, and computed-tomographic observations. *Mol. Pharm.* **9**, 1271–1279 (2012).
89. Gong, X., Gao, Y., Shu, J., Zhang, C. & Zhao, K. Chitosan-based nanomaterial as immune adjuvant and delivery carrier for vaccines. *Vaccines* **10**, 1906 (2022).
90. Xing, L. et al. Chemical modification of chitosan for efficient vaccine delivery. *Molecules* **23**, 229 (2018).
91. Mills, K. H. et al. Protective levels of diphtheria-neutralizing antibody induced in healthy volunteers by unilateral priming-boosting intranasal immunization associated with restricted ipsilateral mucosal secretory immunoglobulin a. *Infect. Immun.* **71**, 726–732 (2003).
92. Atmar, R. L. et al. Norovirus vaccine against experimental human Norwalk Virus illness. *N. Engl. J. Med.* **365**, 2178–2187 (2011).
93. El-Kamary, S. S. et al. Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. *J. Infect. Dis.* **202**, 1649–1658 (2010).
94. Patel, D. R. et al. Intranasal SARS-CoV-2 RBD decorated nanoparticle vaccine enhances viral clearance in the Syrian hamster model. *Microbiol. Spectr.* **12**, e0499822 (2024).
95. Wagstaffe, H. R. et al. Mucosal and systemic immune correlates of viral control after SARS-CoV-2 infection challenge in seronegative adults. *Sci. Immunol.* **9**, eadj9285 (2024).
96. Park, H. S. et al. Intranasal immunization with avian paramyxovirus type 3 expressing SARS-CoV-2 spike protein protects hamsters against SARS-CoV-2. *NPJ Vaccines* **7**, 72 (2022).
97. Le Nouen, C. et al. Intranasal pediatric parainfluenza virus-vectored SARS-CoV-2 vaccine is protective in monkeys. *Cell* **185**, 4811–4825.e4817 (2022).
98. Liu, X. et al. A single intranasal dose of a live-attenuated parainfluenza virus-vectored SARS-CoV-2 vaccine is protective in hamsters. *Proc. Natl Acad. Sci. USA* **118**, e2109744118 (2021).
99. van Doremalen, N. et al. Intranasal ChAdOx1 nCoV-19/AZD1222 vaccination reduces viral shedding after SARS-CoV-2 D614G challenge in preclinical models. *Sci. Transl. Med.* **13**, eabh0755 (2021).
100. Ying, B. et al. Mucosal vaccine-induced cross-reactive $CD8^+$ T cells protect against SARS-CoV-2 XBB.1.5 respiratory tract infection. *Nat. Immunol.* **25**, 537–551 (2024).
101. Madhavan, M. et al. Tolerability and immunogenicity of an intranasally-administered adenovirus-vectored COVID-19 vaccine: An open-label partially-randomised ascending dose phase I trial. *eBioMedicine* **85**, 104298 (2022).
102. Mudrick, H. E. et al. Comparison of replicating and nonreplicating vaccines against SARS-CoV-2. *Sci. Adv.* **8**, eabm8563 (2022).
103. Ahi, Y. S., Bangari, D. S. & Mittal, S. K. Adenoviral vector immunity: its implications and circumvention strategies. *Curr. Gene Ther.* **11**, 307–320 (2011).
104. Adler, J. M. et al. An intranasal live-attenuated SARS-CoV-2 vaccine limits virus transmission. *Nat. Commun.* **15**, 995 (2024).
105. Mao, T. et al. Unadjuvanted intranasal spike vaccine elicits protective mucosal immunity against sarbecoviruses. *Science* **378**, eabo2523 (2022).
This paper presented a vaccine strategy of 'prime and spike', which uses the existing systemic immunity induced by primary injection vaccination (prime) to induce mucosal immune memory within the respiratory tract by using unadjuvanted intranasal spike boosters (spike) for protection against SARS-CoV-2 infection.
106. McMahan, K. et al. Mucosal boosting enhances vaccine protection against SARS-CoV-2 in macaques. *Nature* **626**, 385–391 (2024).
This study showed that intratracheal booster vaccination with an Ad26-based bivalent SARS-CoV-2 vaccine in systemically primed non-human primates induced potent mucosal humoral and cellular immunity that provided near-complete protection against a SARS-CoV-2 BQ.1.1 challenge.
107. Gagne, M. et al. Mucosal adenovirus vaccine boosting elicits IgA and durably prevents XBB.1.16 infection in nonhuman primates. *Nat. Immunol.* **25**, 1913–1927 (2024).
108. Chen, J. et al. A live attenuated virus-based intranasal COVID-19 vaccine provides rapid, prolonged, and broad protection against SARS-CoV-2. *Sci. Bull.* **67**, 1372–1387 (2022).
109. Sun, W. et al. A Newcastle disease virus (NDV) expressing a membrane-anchored spike as a cost-effective inactivated SARS-CoV-2 vaccine. *Vaccines* **8**, 771 (2020).
110. Logunov, D. Y. et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet* **396**, 887–897 (2020).
111. Beavis, A. C. et al. Efficacy of parainfluenza virus 5 (PIV5)-vectored intranasal COVID-19 vaccine as a single dose primer and booster against SARS-CoV-2 variants. *J. Virol.* **99**, e01989-24 (2025).
112. An, D. et al. Protection of K18-hACE2 mice and ferrets against SARS-CoV-2 challenge by a single-dose mucosal immunization with a parainfluenza virus 5-based COVID-19 vaccine. *Sci. Adv.* **7**, eabi5246 (2021).
113. Tioni, M. F. et al. Mucosal administration of a live attenuated recombinant COVID-19 vaccine protects nonhuman primates from SARS-CoV-2. *NPJ Vaccines* **7**, 85 (2022).
114. Wang, Y. et al. Scalable live-attenuated SARS-CoV-2 vaccine candidate demonstrates preclinical safety and efficacy. *Proc. Natl Acad. Sci. USA* **118**, e2102775118 (2021).
115. O'Neill, A. et al. Mucosal SARS-CoV-2 vaccination of rodents elicits superior systemic T central memory function and cross-neutralising antibodies against variants of concern. *eBioMedicine* **99**, 104924 (2024).
116. Yunis, J., Short, K. R. & Yu, D. Severe respiratory viral infections: T-cell functions diverging from immunity to inflammation. *Trends Microbiol.* **31**, 644–656 (2023).
117. Schlom, J. & Donahue, R. N. The importance of cellular immunity in the development of vaccines and therapeutics for COVID-19. *J. Infect. Dis.* **222**, 1435–1438 (2020).
118. Kalimuddin, S. et al. Vaccine-induced T cell responses control *Orthoflavivirus* challenge infection without neutralizing antibodies in humans. *Nat. Microbiol.* **10**, 374–387 (2025).

119. Gonzalez Delgado, C. A. *Adaptive phase I / II clinical trial, randomized, of parallel groups, to evaluate the safety and immunogenicity in adults of two vaccine candidates, based on recombinant RBD subunits for the prevention of COVID-19 in regimens that use the nasal route of administration* (RPCEC, accessed 2 March 2024); https://rpcec.sld.cu/en/trials/RPCEC00000345-En.
120. Lam, J. H. et al. Next-generation intranasal Covid-19 vaccine: a polymersome-based protein subunit formulation that provides robust protection against multiple variants of concern and early reduction in viral load of the upper airway in the golden Syrian hamster model. Preprint at *bioRxiv* https://doi.org/10.1101/2022.02.12.480188 (2022).
121. Lam, J. H. et al. Polymersomes as stable nanocarriers for a highly immunogenic and durable SARS-CoV-2 spike protein subunit vaccine. *ACS Nano* **15**, 15754–15770 (2021).
122. Li, J. X. et al. Safety and immunogenicity of heterologous boost immunisation with an orally administered aerosolised Ad5-nCoV after two-dose priming with an inactivated SARS-CoV-2 vaccine in Chinese adults: a randomised, open-label, single-centre trial. *Lancet Respir. Med.* **10**, 739–748 (2022).
123. Zhou, R. et al. Nasal prevention of SARS-CoV-2 infection by intranasal influenza-based boost vaccination in mouse models. *eBioMedicine* **75**, 103762 (2022).
124. Hassan, A. O. et al. A single-dose intranasal ChAd vaccine protects upper and lower respiratory tracts against SARS-CoV-2. *Cell* **183**, 169–184.e113 (2020).
125. Bricker, T. L. et al. A single intranasal or intramuscular immunization with chimpanzee adenovirus-vectored SARS-CoV-2 vaccine protects against pneumonia in hamsters. *Cell Rep.* **36**, 109400 (2021).
126. Banihashemi, S. R. et al. Safety and efficacy of combined intramuscular/intranasal RAZI-COV PARS vaccine candidate against SARS-CoV-2: a preclinical study in several animal models. *Front. Immunol.* **13**, 836745 (2022).
127. Logunov, D. Y. et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet* **397**, 671–681 (2021).
128. Liu, T., Liang, Y. & Huang, L. Development and delivery systems of mRNA vaccines. *Front. Bioeng. Biotechnol.* **9**, 718753 (2021).
129. Yang, L., Tang, L., Zhang, M. & Liu, C. Recent advances in the molecular design and delivery technology of mRNA for vaccination against infectious diseases. *Front. Immunol.* **13**, 896958 (2022).
130. Li, M. et al. Enhanced intranasal delivery of mRNA vaccine by overcoming the nasal epithelial barrier via intra- and paracellular pathways. *J. Control. Release* **228**, 9–19 (2016).
131. Shah, P., Lalan, M. & Barve, K. Intranasal delivery: An attractive route for the administration of nucleic acid based therapeutics for CNS disorders. *Front. Pharmacol.* **13**, 974666 (2022).
132. Dhaliwal, H. K., Fan, Y., Kim, J. & Amiji, M. M. Intranasal delivery and transfection of mRNA therapeutics in the brain using cationic liposomes. *Mol. Pharm.* **17**, 1996–2005 (2020).
133. Suberi, A. et al. Polymer nanoparticles deliver mRNA to the lung for mucosal vaccination. *Sci. Transl. Med.* **15**, eabq0603 (2023).
134. Baldeon Vaca, G. et al. Intranasal mRNA-LNP vaccination protects hamsters from SARS-CoV-2 infection. *Sci. Adv.* **9**, eadh1655 (2023).
135. Waltz, E. How nasal-spray vaccines could change the pandemic. *Nature* **609**, 240–242 (2022).
136. Topol, E. J. & Iwasaki, A. Operation Nasal Vaccine—lightning speed to counter COVID-19. *Sci. Immunol.* **7**, eadd9947 (2022).
137. Lavelle, E. C. & Ward, R. W. Mucosal vaccines—fortifying the frontiers. *Nat. Rev. Immunol.* **22**, 236–250 (2022).
138. US Food and Drug Administration. FluMist Quadrivalent. *FDA* https://www.fda.gov/vaccines-blood-biologics/vaccines/flumist-quadrivalent (2023).
139. Carter, N. J. & Curran, M. P. Live attenuated influenza vaccine (FluMist; Fluenz): a review of its use in the prevention of seasonal influenza in children and adults. *Drugs* **71**, 1591–1622 (2011).
140. Jeyanathan, M. et al. Immunological considerations for COVID-19 vaccine strategies. *Nat. Rev. Immunol.* **20**, 615–632 (2020).
141. Vignuzzi, M., Wendt, E. & Andino, R. Engineering attenuated virus vaccines by controlling replication fidelity. *Nat. Med.* **14**, 154–161 (2008).
142. Aoshi, T. Modes of action for mucosal vaccine adjuvants. *Viral Immunol.* **30**, 463–470 (2017).
143. US Centers for Disease Control and Prevention. Adjuvants and Vaccines. *CDC* https://www.cdc.gov/vaccine-safety/about/adjuvants.html (2024).
144. Zhao, T. et al. Vaccine adjuvants: mechanisms and platforms. *Signal Transduct. Target Ther.* **8**, 283 (2023).
145. Wrobel, B. B. & Leopold, D. A. Olfactory and sensory attributes of the nose. *Otolaryngol. Clin. North Am.* **38**, 1163–1170 (2005).
146. Alvites, R. D. et al. The nasal cavity of the rat and mouse–source of mesenchymal stem cells for treatment of peripheral nerve injury. *Anat. Rec.* **301**, 1678–1689 (2018).
147. Ramvikas, M., Arumugam, M., Chakrabarti, S. R. & Jaganathan, K. S. in *Micro and Nanotechnology in Vaccine Development* (eds Skwarczynski, M. & Toth, I.) 279–301 (Elsevier, 2017).
148. Verma, A. K., Zheng, J., Meyerholz, D. K. & Perlman, S. SARS-CoV-2 infection of sustentacular cells disrupts olfactory signaling pathways. *JCI Insight* **7**, e160277 (2022).
149. Mutsch, M. et al. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *N. Engl. J. Med.* **350**, 896–903 (2004).
150. Miller, E. et al. Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. *Brit. Med. J.* **346**, f794 (2013).
151. Nohynek, H. et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. *PLoS ONE* **7**, e33536 (2012).
152. Stowe, J. et al. Risk of narcolepsy after AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine in adults: a case-coverage study in England. *Sleep* **39**, 1051–1057 (2016).
153. Dauvilliers, Y. et al. Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. *Brain* **136**, 2486–2496 (2013).
154. Fukuyama, Y. et al. Nasal administration of cholera toxin as a mucosal adjuvant damages the olfactory system in mice. *PLoS ONE* **10**, e0139368 (2015).

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Additional information

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