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Promises and challenges of mucosal COVID-19 vaccines

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ABSTRACT

Coronavirus disease-2019 (COVID-19) is an ongoing pandemic caused by the newly emerged virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Currently, COVID-19 vaccines are given intramuscularly and they have been shown to evoke systemic immune responses that are highly efficacious towards preventing severe disease and death. However, vaccine-induced immunity wanes within a short time, and booster doses are currently recommended. Furthermore, current vaccine formulations do not adequately restrict virus infection at the mucosal sites, such as in the nasopharyngeal tract and, therefore, have limited capacity to block virus transmission. With these challenges in mind, several mucosal vaccines are currently being developed with the aim of inducing long-lasting protective immune responses at the mucosal sites where SARS-COV-2 infection begins. Past successes in mucosal vaccinations underscore the potential of these developmental stage SARS-CoV-2 vaccines to reduce disease burden, if not eliminate it altogether. Here, we discuss immune responses that are triggered at the mucosal sites and recent advances in our understanding of mucosal responses induced by SARS-CoV-2 infection and current COVID-19 vaccines. We also highlight several mucosal SARS-COV-2 vaccine formulations that are currently being developed or tested for human use and discuss potential challenges to mucosal vaccination.

1. Mucosal immunity and its functions

Mucus membranes cover body cavities, forming the linings of the respiratory, digestive and reproductive organs. At these sites, foreign matter, including pathogens, can be cleared through distinctive physical properties including the presence of ciliated cells and the production of mucus. Apart from their chemical (e.g., soluble mediators, lysozyme, defensins etc.) and mechanical properties (such as mucociliary transport), the mucosae are also rich immunologically, with specialized cell populations and tissue structures that are key for protection against invading pathogens at each unique mucosal site [1,2]. The respiratory mucosal tissues are comprised of the upper respiratory tract (nostrils, nasal cavity and pharynx) and the lower respiratory tract (trachea, bronchi, bronchioles and alveoli) (Fig. 1). At these tissue sites a single layered pseudostratified columnar epithelium forms the first line of defense containing cilia on their apical surface, which facilitate movement of mucus, innocuous substances and pathogens out of the airways [3]. The secretory club cells, mucus producing goblet cells and sensory pulmonary neuroendocrine cells are some of the examples of specialized epithelial cells that are present within the airways, which can coordinate a response to injury or infection with the professional immune cells [3,4]. Various innate, antigen presenting and/or phagocytic immune cells including dendritic cells (DCs), macrophages, mast cells, NK cells and neutrophils are present just under the mucosal epithelial surface [5]. These cells are equipped with innate defenses, can provide early protection against an invading pathogen, and can help initiate the development of an adaptive immune response [5,6]. Although they bear many similarities to immune cell subsets at other sites, there are several examples of immune cells that have developed specialized mucosal-specific phenotypes or activation programs. For example, NK cells, which are a major component of innate host defense at mucosal surfaces, display unique properties in the lungs, tonsils and Peyer's patches, marked by low cytotoxic functionality and increased production of the cytokine IL-22 in humans [7,8]. These cells were shown to promote epithelial cell release of antimicrobial peptides and epithelial survival [8]. Another example, DCs in the gut have been shown to lack significant expression of TLR4 at steady state, likely because of the high concentrations of LPS produced by host commensal bacteria in the gut [9]. Mast cells also have a unique mucosal-specific subset of cells that contain gran-

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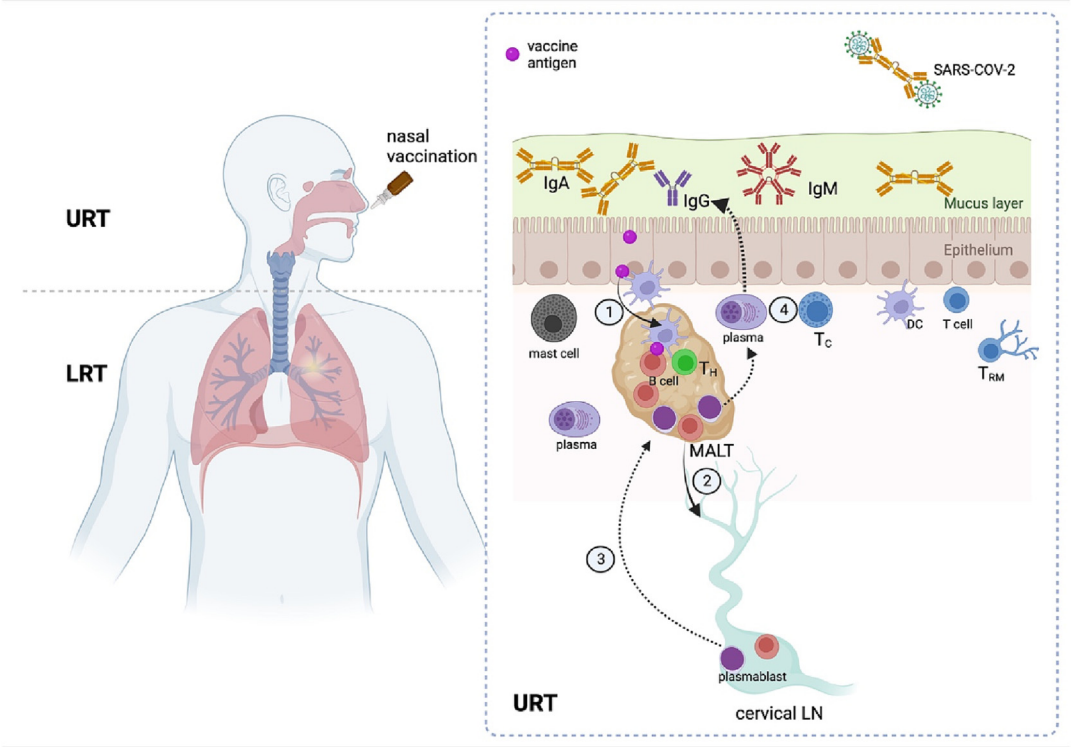

Fig. 1. A simplified view of immune responses at the respiratory mucosae. During an exposure to natural or vaccine antigens via nasal delivery, (1) antigens can be taken up by DCs that reside in the respiratory mucosae and presented to the specialized B and T_H cells located in the MALT, a lymphoid structure unique to each mucosal site. Certain antigens may also activate mast cells, which can then degranulate and release an array of immune mediators with diverse functions. (2) Antigen-specific lymphocytes that are activated in the MALT can traffic to the nearest draining cervical LN for their proliferation and differentiation. These mucosal tissue draining LNs provide a connecting link between peripheral sites and the systemic immune system. (3) Antigen-specific plasmablasts and/or effector T cells, Tc/T_{RM} can then either directly home in to the mucosae or transit through MALT for their effector functions, (4) such as secretion of IgA, and IgM antibodies. IgA is the most abundantly secreted antibody at mucosal sites and has the capacity to neutralize SARS-CoV-2 at the first exposure site where infection initially begins. URT-upper respiratory tract, LRT-lower respiratory tract, MALT-mucosa associated lymphoid tissue, T_H-helper T cell, Tc-cytotoxic T cell, T_{RM}-tissue resident memory T cell, LN-lymph node.

ules and promote pro-inflammatory responses to pathogens and vaccines [6], which are different from connective tissue resident mast cells, defined by their granule contents [10]. Plasma cells localized in the gut also mostly secrete IgA, the most important antibody for protection of mucosal surfaces [11]. These are only some examples of the unique attributes of mucosal immune cells which allow the immune response in the tissue to be site-specific and for the resulting adaptive immune response generated to retain a phenotype optimized for mucosal protection.

Some mucosal tissues are supported by distinct structures that facilitate the transition from innate to adaptive immune responses. This includes the highly compartmentalized mucosal associated lymphoid tissues (MALT) in which immune cells, including lymphocytes, accumulate in and/or traffic through [12]. In particular, MALT are uniquely enriched with B cells that differ both phenotypically and functionally from the B cells that are present in lymphoid tissues such as in the spleen or lymph nodes (LNs) [11]. For example, B cells that are activated in the Peyer's patches or in the respiratory MALT often class-switch to become predominantly IgA-producing plasma cells [11]. These cells express $\alpha4\beta7$ integrins and CCR9 or $\alpha4\beta1$ and CCR10 to facilitate their preferential homing into the gut or respiratory mucosae, respectively [13,14]. Although having similarities, these MALT tissues are often specific to the organ or mucosal surface that they protect, with the NALT (nasal associated lymphoid tissue, for rodents) or the analogous human structure, the palatine tonsils and adenoids, being the draining site for antigens exposed to the nasal or oral mucosae, while the Peyer's patches serve this role for the gut [15]. Although these MALT tissues are connected to draining LNs which, themselves, can have tissue-draining specific properties [15], they have a function of being a dedicated site of immune activation more proximal to the mucosal tissue. This may both allow improved efficiency of immune activation, while also imprinting the environment in which activation occurred on the adaptive immune response and subsequent memory response.

As highlighted above, a major aspect of the adaptive immune response at the mucosae is the production of secretory immunoglobulin, IgA, although IgM and IgG are also produced after mucosal challenges [16]. While IgA and IgM have an active transport mechanism across epithelial barriers and into the mucus layer, IgG and, to a lesser extent, other subclasses of antibodies can leak across blood vessels and may be found in saliva or other secretions [17]. Circulating IgA is monomeric while secretory IgA (sIgA) is mainly dimeric and mostly produced by plasma cells that are present in the subepithelial space or lamina propria of the mucosal tissue and transported across into the lumen by polymeric Ig receptor expressed on epithelial cells [18]. Although mice have one form of IgA, two types of IgAs exist in humans, IgA1 and IgA2 and their effector functions are different [19]. Structurally, IgA1 has an extended hinge region compared to IgA2, which is attributed to the insertion of a duplicated amino acid sequence stretch in IgA1 [20]. Functionally, IgA2 antibodies are found to be pro-inflammatory in nature, by activating neutrophils and macrophages more strongly compared to IgA1. These differences result from varied glycosylation profiles between IgA1 and IgA2 antibodies [19]. It is believed that the sIgA dimeric state and glycosylation

provide protection from the degradation by high levels of proteases that are present in the mucosal fluids [21].

sIgA is important for blocking the pathogens or irritants that may be present within mucosal cavities. Interestingly, in the gut associated lymphoid tissues, including Peyer's patches and mesenteric LNs, IgA can be formed and secreted in a T cell-independent manner, as shown by the production of IgA in T cell-deficient mouse models [22]. This T cell-independent IgA often binds to commensal bacteria and it can be induced through T cell independent class-switch recombination that is influenced by dietary factors [23–25]. IgA is more broadly cross-reactive compared to other subclasses of antibodies [26,27], meaning it sometimes can bind to antigens that did not specifically trigger its production. In the context of Influenza, this has been shown to result in more cross-reactive antibodies that bind to different antigenically and evolutionarily divergent strains [28], which could be of particular relevance to SARS-CoV-2, which has evolved into multiple subvariants [29]. sIgA is also found in breast milk, and may play a role in protection against infections in early life. For example, Influenza-specific IgA levels in breast milk were found to be associated with reduced viral respiratory infections in infants [30]. However, although IgA is key in preventing invasion of pathogens at the mucosal surface, selective IgA deficiency is one of the most common primary immune deficiencies in humans. Although individuals are largely asymptomatic, some may be more prone to recurrent infections [31]. This fact that humans are minimally effected by IgA-deficiency emphasizes the importance of other less-studied aspects of mucosal immunity to immune defense in these tissues, beyond the role of IgA.

2. Mucosal immune responses to SARS-CoV-2

Respiratory pathogens such as SARS-CoV-2 invade through the nasal and oral passages by droplets and aerosols, resulting in infection of the upper and lower respiratory tracts [32]. SARS-CoV-2 emerged as a novel coronavirus in 2019, and is a member of the family *Coronaviridae* with antigenic similarities and phylogenetic relationships with both seasonal coronaviruses and highly pathogenic coronaviruses such as SARS-CoV-1 and Middle East respiratory syndrome (MERS) viruses [33–35]. Upon exposure to mucosal surfaces, SARS-CoV-2 infects multiciliated airway epithelial cells expressing ACE2/TMPRSS2 receptors [36,37]. However, SARS-CoV-2 is not confined to the upper and lower airways, it also has been shown to infect the gut and other tissues upon autopsy [38–40]. Gut infection by SARS-CoV-2 is characterized by the detection of viral antigen in epithelial cells of the intestine and glands, which is consistent with the expression of ACE2 by these cells [38,40]. Viral RNA is shed in the fecal matter for prolonged periods with longer shedding periods reported for children than adults [41], although it remains to be proven whether shed virus is replication competent and infectious, with literature suggesting contradictory results [42–44]. This, nevertheless, supports that the gut mucosa is a site of SARS-CoV-2 antigen exposure.

During natural SARS-CoV-2 infection, IgA levels were elevated in mucosal fluids including bronchoalveolar lavage (BAL) and saliva beginning with the onset of symptoms and were more neutralizing than circulating IgG [45,46]. Antibody-producing plasmablasts with mucosal homing characteristics were identified in the circulation [45]. These cells expressed CCR10, a chemokine receptor and marker for lung homing [45,47,48], and produced antibodies directed against the Spike protein as well as the nucleocapsid protein [45]. It will be important to understand IgA subclasses, IgA1 vs IgA2, and specificities, that are evoked after natural SARS-CoV-2 infection or after vaccinations and their associations with acute COVID-19 disease and its sequelae.

3. Limited mucosal immune responses to first generation COVID-19 vaccines

There are multiple approved vaccines against SARS-CoV-2, reviewed elsewhere [49,50], that use varying approaches or platforms, including mRNA vaccines (e.g., BNT162b2/ Pfizer, mRNA-1273/Moderna), viral vector platforms (Oxford/AstraZeneca/AZD1222/ChAdOx1, Janssen vaccine/Ad26.COV2.S), inactivated vaccines (e.g., CoronaVac/Sinovac, Covaxin/Bharat biotech) and subunit vaccines (e.g., Nuvaxovid/Novavax, Covovax/Serum Institute). These are all given intramuscularly in current approved formats [50]. Of these platforms, mRNA vaccines have shown the highest levels of protection, at least in short-term studies evaluating outcomes within months of vaccination [51]. While current COVID-19 vaccines that are given intramuscularly provide excellent protection against severe disease and death, they do not efficiently limit re-infection and transmission. This may be, in part, due to the fact that systemic immunizations evoke weaker immune responses at mucosal sites, such as in the upper respiratory tract. Limited induction of mucosal immunity coupled with lower accessibility of serum IgG to the upper respiratory tract likely leaves one vulnerable for re-infection. Indeed, intramuscular COVID-19 vaccinations, so far, have failed to induce a sustained IgA response in the nasal and oral cavities [52–54]. In a matched case control study, breakthrough infections assessed within 2–4 weeks after the second dose mRNA vaccination were shown to be associated with lower levels of serum IgA (but not IgG) in study participants compared to those who remain uninfected, suggesting IgA responses may be important in preventing breakthrough infections [52]. During natural SARS-CoV-2 infection, nasal IgA was shown to persist for up to 9 months post-infection [54]. However, it is not known if the neutralizing activity of this IgA persisted after acute infection resolution since, for example, Omicron infections occurred in both vaccinated and previously infected individuals who had received first-generation vaccines based on the Spike protein from the ancestral strain of SARS-CoV-2 [55,56]. Moreover, when convalescent individuals were boosted with the ChAdOx1 vaccine, only the serum IgG response was boosted and nasal IgA titers remained largely unchanged [54]. Mucosal IgA responses were boosted by mRNA vaccination primarily in those who had previous infections [57]. Similarly, unlike those who had prior immunity to SARS-CoV-2 obtained through natural infection, SARS-CoV-2 naïve individuals who received mRNA vaccines lacked virus-specific resident T cells in the nasal secretions and BAL [58,59]. It was also shown that mRNA-vaccinated individuals had lower levels of the Spike receptor binding domain (RBD)-specific IgG or neutralizing antibodies in the BAL compared to convalescent individuals [58]. RBD-specific B cells and Spike-specific CD8 and CD4 T cells in the BAL were also higher in convalescent individuals compared to the vaccines [58]. In contrast to the picture at mucosal sites, vaccine-induced responses were more robust in plasma and PBMCs compared to convalescent individuals [58], although the differences in cellularity could be reflective of recent infection clearance from the upper and lower respiratory tract in convalescent individuals. Nevertheless, these studies have highlighted that the nasal IgA response following natural infection is potentially distinct from that of plasma IgA/IgG responses and not boosted upon further immunization using an intramuscular route.

Given the limitations of current COVID-19 vaccines, efforts to develop the next generation vaccine platforms and formulations

have begun. It is thought that immunization via the nasal route would induce antigen-specific immune responses in the upper respiratory tract including the nose and oral cavity where SARS-CoV-2 infection begins [60] (Fig. 1). This could have the potential not only to limit infection spread from the upper respiratory tract to lower respiratory tract but may also provide sterilizing immunity, which could reduce virus transmission within the population.

4. Mucosal vaccines for SARS-COV-2: Current knowledge and preclinical pipeline

Several vaccine strategies are currently being pursued at various stages of preclinical and clinical development with the aim of improving vaccine-induced mucosal immune responses against SARS-CoV-2. Most pre-clinical studies of SARS-CoV-2 mucosal vaccines have been performed in mice or hamsters. These use various strategies, including subunit vaccines and viral vector-based platforms (Fig. 2).

In one study, inhalable virus-like particles (VLPs) were used to generate mucosal immune responses. These VLPs consist of recombinant RBD conjugated to lung-derived exosomes [61], which is thought to enhance retention of vaccine in both mucus airways and also in the lung parenchyma. In mice, this vaccine induced RBD-specific IgG in the serum and IgA in the nasopharyngeal lavage and BAL fluids, and CD4 and CD8 T cells in the lung with a strong induction of Th1 cytokines [61]. This strategy also protected hamsters from severe pneumonia after virulent SARS-CoV-2 challenge [61]. Furthermore, the particles comprising the vaccine were stable after lyophilization at room temperature for 3 months [61], which is interesting since stability is a key concern for mRNA vaccines. Other vaccines that are protein-based also have the potential advantage of stability. In another strategy, unadjuvanted Spike was given intranasally as a boost, following a prime with an mRNA vaccine (delivered i.m.). This resulted in induction of resident memory B and T cells and IgA in the respiratory mucosa, and also boosted systemic immunity, which protected hamsters from lethal SARS-CoV-2 infection [62]. These vaccines advance the idea that antigen alone can serve as a powerful inducer of mucosal immunity as either a first dose or booster, with successful outcomes in short-term animal studies.

Contrasting these unadjuvanted challenges, there are also strategies that aim to develop adjuvanted vaccines for mucosal delivery (Fig. 2). For example, using three different adjuvants, carbomer-based nanoemulsion adjuvant Adjuplex (ADJ) with CpG or TLR4 agonist glucopyranosyl lipid A (GLA), K18-hACE2 mice (i.e. mice that have been engineered to express the human ACE2 receptor for SARS-CoV-2) were vaccinated intranasally against Spike using a prime-boost strategy. This evoked both respiratory tract-resident and systemic CD4 and CD8 memory T cells and protected against virulent homologous and heterologous SARS-CoV-2 challenges [63]. Those data also suggested protective role for T cells, since viral titers were higher in the lungs after antibody depletion of CD4 or CD8 T cells [63]. We also observed the potential of adjuvants to improve mucosal T cell responses after vaccination when we compared the T cell profile of animals vaccinated intranasally with Spike protein with or without the use of the experimental mucosal adjuvant mastoparan-7 (M7), compared to a standard subcutaneous challenge with Alum [64]. Nasal vaccination with M7 induced heightened T central memory (T_{CM}) cells in the draining lymphoid tissues and spleen compared to unadjuvanted antigen or adjuvanted antigen delivered via a peripheral route [64]. T_{CM} cells are characteristic of long-lived and systemic memory immune responses [65,66]. Memory T cells also showed improved lung and brachial lymph node homing after antigen challenge to the lungs following vaccination against RBD adjuvanted with M7 when delivered to the nasal mucosa, compared to peripheral sub-cutaneous injection [64]. Furthermore, this vaccine strategy induced more broadly cross-protective antibodies that showed enhanced neutralization against multiple SARS-CoV-2 variants of concern [64]. These studies suggest that adjuvants could be used to improve mucosal vaccine responses. Although promising in preclinical testing, to-date, few adjuvants have been tested for mucosal delivery in humans, which has been a limitation to mucosal vaccine development [67].

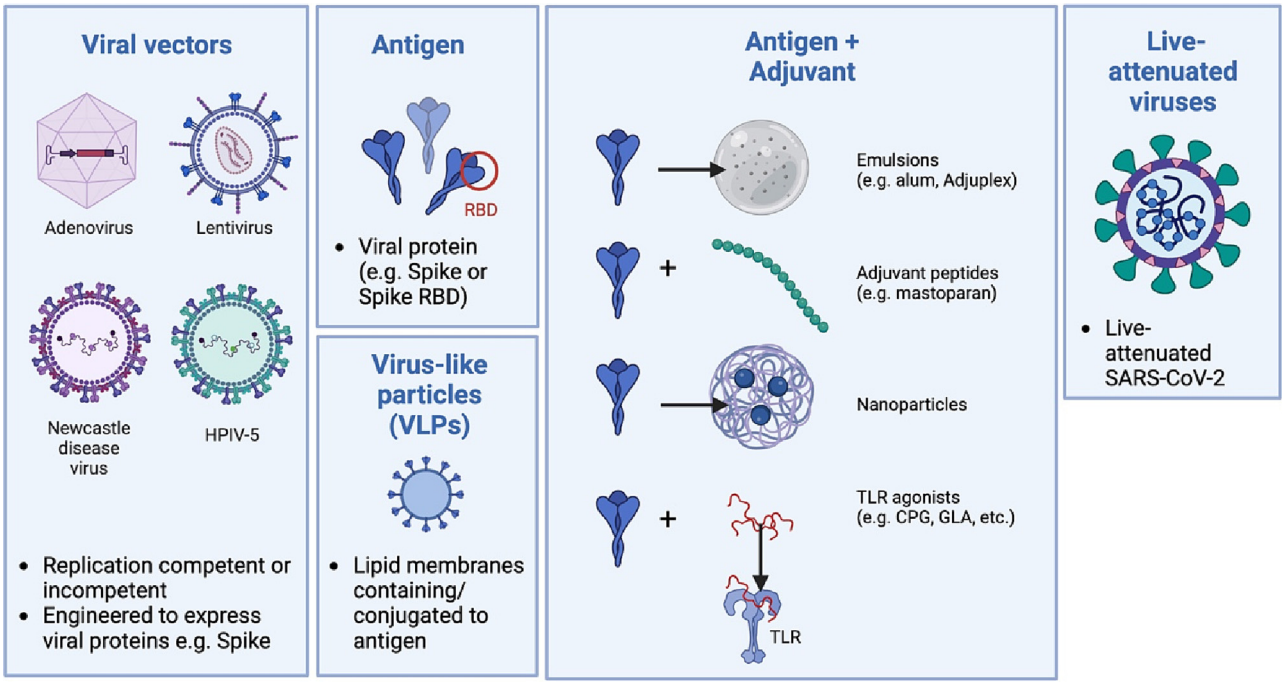

Fig. 2. Strategies currently being employed for the development of mucosal SARS-CoV-2 vaccines.

Table 1
Select mucosal SARS-CoV-2 vaccines at advanced stages of clinical development.

Mucosal vaccine	Type, delivery method	Status
iNCOVACC (Bharat Biotech)	Non replicating viral vector, nasal drops	Approved for use in India, (phase 3 trial completed with non-peer reviewed data available as preprint (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4342771)
CoviLiv (Codagenix)	Live attenuated, nasal drops	Ongoing phase 3 trial as a part of the WHO-sponsored solidarity trial vaccines
Razi Vaccine	Protein subunit, nasal spray	Ongoing phase 3 trial (https://www.irct.ir/trial/58143)
Convidecia (CanSino Biologics)	Non replicating viral vector, nasal drops	Approved for use as emergency use booster in China, clinical data not available
VXA-CoV2-1.1-S (Vaxart)	Non replicating viral vector, oral pill	Ongoing phase 2 trial (https://clinicaltrials.gov/ct2/show/NCT05067933)

Alternatively to subunit vaccines, viral-vectored and live-attenuated vaccines are also in development for SARS-CoV-2 infection control. An advantage of live-attenuated vaccines or replicating viral vector platforms is that they could possibly provide higher levels of antigen availability [68]. In one strategy, a live-attenuated Newcastle disease virus encoding Spike (rNDV-S) was administered intranasally in mice, which induced high levels of SARS-CoV-2-specific neutralizing antibodies in the serum, higher IgA and IgG2a antibodies in the pleural fluid and increased CD4 and CD8 T cells in the lung [69]. Hamsters immunized with two doses of this vaccine showed protection from lung infection, inflammation, and pathological lesions following SARS-CoV-2 challenge. Importantly, administration of two doses of intranasal rNDV-S vaccine also significantly reduced SARS-CoV-2 shedding in nasal turbinates and lungs of hamsters [69]. Similarly, intranasal administration of single dose vaccine containing parainfluenza virus 5 (PIV5) expressing Spike protected K18-hACE2 mice from lethal challenge and protected against infection and contact-based transmission in ferrets [70]. Lentivirus vectors have also been used and have demonstrated protection against SARS-CoV-2 in either hamster or mouse models [71,72]. In one study, lentivirus encoding a stabilized Spike, in a non-integrating, non-replicative, non-cytopathic lentivirus was given to K18-hACE2 mice which had previously been given a prime and boost using an mRNA vaccine (i.m.). After waiting 4 months for natural waning of immunity, the animals were cross-immunized intranasally with a lentivirus-expressing Spike from the Beta variant. A strong boost of immunity was detected in terms of increased IgG, IgA and activated immune cells and the vaccine was also shown to provide cross-protection against Delta and Omicron VOCs [72]. A systemic prime followed by intranasal boost strategy was also effective when non-human primates (NHPs) were primed with Spike + Alum, followed by a boost using Spike plus an adjuvant cocktail containing CpG, polyIC and IL15, known as C15 nanoparticles [73]. Although, this formulation generated weaker systemic immune responses, it boosted dimeric IgA and IFNα production in the BAL and virus was cleared faster in NHPs boosted with Spike + CP15 upon challenge [73].

It is also suggested that vaccine formulations involving more than one viral antigen could induce a broader immune response. In this regard, one study utilized a trivalent vaccine containing the viral proteins Spike, nucleocapsid and RdRp, engineered in human or chimpanzee adenoviral vectors [74]. When given intranasally in mice, this formulation generated better humoral and cellular immune responses compared to intramuscular immunization and provided protection from challenges using the ancestral strain and VOCs [74]. It has been suggested that when Spike antigens were expressed in a non-replicating adenovirus type 5 vector as vaccines, there were differences in the cross-reactivity against multiple SARS-CoV-2 strains induced by different Spike sequences. Spike from the ancestral strain induced greater cross-reactive antibodies than Spike from Delta or Omicron, although all of these provided *in vivo* protection in a hamster model [75]. Together, these studies support that viral vector-based platforms could be viable as mucosal vaccines against SARS-CoV-2. Some of these rely on viruses that can replicate *in vivo* but are attenuated, while others are engineered for safety considerations to be non-replicating, which can affect antigen persistence [76]. These viral vector based approaches have potential benefits, such as not requiring an adjuvant for immune stimulation, as well as drawbacks, including the potential of off-target immune responses to the vector, or pre-existing immunity to the vector possibly limiting their efficacy or developmental potential. These and additional pros and cons of viral vector strategies have been reviewed elsewhere [76] and additional studies are needed comparing strategies such as viral vector-based platforms to sub-unit vaccines, side-by-side.

There are numerous mucosal vaccine candidates, or mucosal boost protocols in clinical trials (Table 1) [77], which show varying degrees of success and highlight some of the potential pitfalls that can meet mucosal vaccine development. In fact, several mucosal vaccine candidates showed limited efficacy in clinical trials. For example, recently, ChAdOx1 was evaluated in individuals who had previously been vaccinated intramuscularly with either ChAdOx1 or approved mRNA vaccines. A small cohort of 30 patients were boosted with a single intranasal ChAdOx1 dose, of multiple concentrations [78]. In this study, the intranasal boost failed to induce mucosal antibody responses that were higher than natural SARS-CoV-2 infection and further testing is currently on hold [79]. Although supporting data are not publicly available, Bharat Biotech's iNCOVACC vaccine, a nasal spray also containing an adenovirus vector expressing Spike protein, was also recently approved for use in India [79].

In contrast to the testing of a nasally-delivered vaccines or boosts, oral vaccines have also been considered for SARS-CoV-2 [80]. In a single site, dose ranging, open labelled clinical trial, an oral SARS-CoV-2 vaccine comprised of a non-replicating adenoviral vector expressing Spike and nucleocapsid genes combined with a TLR3 agonist generated a cross-reactive IgA response in the nasal secretions and saliva [80]. These antibodies persisted for up to a year and neutralized the delta and omicron VOCs [80]. Supported by data that an orally-administered Adv5-nCOV vaccine was effective in boosting immunity in previously-vaccinated study participants, this strategy promoted by CanSino was recently approved for human use in China [79,81].

Together these data provide evidence that nasal vaccines could be a tool to improve mucosal vaccine protection, while also suggesting that the vaccine platform, route of mucosal delivery and antigen could require optimization. Furthermore, these early attempts at mucosal vaccines against SARS-CoV-2 have provided evidence for potential mechanisms of protection or correlates of protection that could be used to monitor human responses in subsequent clinical trials. Other studies are ongoing, for example human testing of chimpanzee adenovirus-vectored vaccine encod-

ing a stabilized Spike protein (ChAd-SARS-CoV-2-S) [77,82] and a live Newcastle disease virus vector expressing a stabilized Spike protein (AVX/COVID-12-HEXAPRO) [77,83], and these will be likely to provide more information regarding the viability of nasal vaccines in humans.

5. Challenges, historical perspective, and future goals

SARS-CoV-2 mucosal vaccine development is built on a long history of efforts to improve vaccines at mucosal surfaces. There have been examples of safe and at least partially successful mucosal vaccines, including against polio virus, *V. cholera*, *S. typhi*, rotavirus, and influenza virus [84]. We are reminded of the precedent set by the Salk and Sabin Polio vaccines, where it became apparent that the oral vaccine induced superior mucosal responses including IgA, which protects from infection, while the intramuscular vaccine resulted in limited defense against infection of the gut and polio virus replication and transmission, even though they both strongly protected against poliomyelitis. These vaccines demonstrated the principle that mucosal administration can evoke both localized and systemic protective immune responses [85]. It is possible that SARS-CoV-2 vaccines may also eventually support the importance of mucosal immune protection to transmission, as the polio vaccines have. Although there are recent examples of mucosal vaccines against SARS-CoV-2 being given fast-track approval or emergency approval for use in certain countries, Flumist, comprised of live-attenuated influenza, is the only fully approved nasal vaccine for humans with wide-spread use [86].

In spite of examples of highly successful, safe and protective mucosal vaccines, there are also limitations and clinical development risks. First, there is a concern that mucosal surfaces are more prone to tolerogenic responses, by virtue of their constant exposure to commensals and innocuous foreign substances [84]. These tolerogenic immune programs, often site-specific, have the potential to lead to weak induction of pro-inflammatory responses. In the context of vaccines, breaking tolerance can be aided through the use of effective adjuvants. However, as discussed above, there are limited mucosal adjuvants that have a sufficient safety profile for development as human vaccines, and many of the most promising ones require further testing to demonstrate their safety and efficacy in humans. At mucosal surfaces where ciliated cells quickly clear away debris and pathogens in a protective mucous layer, antigen dilution could be an obstacle to vaccine delivery. This may necessitate optimizing the delivery of vaccine, as well as the dose of antigen/adjuvant. Multiple studies have demonstrated that soluble antigens are slowly absorbed and may promote tolerance [87–89]. Therefore, strategies to generate particulate antigens that could be better absorbed are also in development [90–93] and may be applicable for mucosal vaccines.

Furthermore, there may be limitations to our ability to provide mucosal protection against SARS-CoV-2, even with an optimal vaccine formation. Even though natural route infection with SARS-CoV-2 apparently induces superior immune protection at mucosal surfaces than intra-muscular vaccination [52–54,58,59], it also provides limited protection against re-infection. Indeed, many individuals experience multiple re-infections with SARS-CoV-2 [94,95], some only months after their initial infection [94]. This outcome was observed prior to the emergence of SARS-CoV-2 in the frequent reinfections caused by seasonal coronaviruses [96,97]. We also do not yet know how repeated antigen exposure at mucosal sites would alter long-term protective immunity. In some contexts, such as allergen immunotherapies, repeated exposure to antigens can re-establish tolerance [98]. Therefore, care must be taken when designing strategies for boosting vaccines in general, and mucosal vaccines as well. And finally, we know that mucosal vaccines, like other vaccine and therapeutic strategies, will also be at risk of becoming obsolete in the face of a highly host-adapted and mutating virus, which will require continued monitoring of newly emerging strains and assessments of long-term vaccine efficacy. Strategies that are currently in development towards targeting cross-reactive epitopes capable of neutralizing multiple coronaviruses could be applicable in this context for limiting the mutation of currently circulating strains as well as preventing the emergence of novel coronaviruses in the future.

Yet, vaccines hold the promise of not only being able to induce immunity, but also to be engineered for optimal immunity, which can potentially overcome pathogen antagonism of immune responses, or other mechanisms that result in limited or non-protective immune responses during infection. Mucosal vaccines may also have advantages for vaccine compliance, being relatively easier to administer and noninvasive [99].

6. Conclusions

The information still emerging from research on the basic biology of SARS-CoV-2 and also clinical outcomes of infections and vaccinations, likely, will allow us to design second generation vaccines that are superior to natural immunity, either through vaccine design and/or vaccine schedule. Mucosal vaccines show great promise in solving the limitations of first-generation vaccines and providing needle-free alternatives to the vaccine hesitant. Rational vaccine design utilizing adjuvants and or immunomodulators may further improve the lasting efficacy and durability of vaccine-induced immune responses, an issue that afflicts current COVID-19 vaccines.

Author contributions

The authors contributed equally to all aspects of the article.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ashley St. John has a patent pending to Duke-NUS Medical School.

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