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REVIEW ARTICLE

Dealing with a mucosal viral pandemic: lessons from COVID-19 vaccines

Violette Mouro[1,2 ✉] and Alain Fischer[3,4,5,6]

The development and deployment of vaccines against COVID-19 demonstrated major successes in providing immunity and preventing severe disease and death. Yet SARS-CoV-2 evolves and vaccine-induced protection wanes, meaning progress in vaccination strategies is of upmost importance. New vaccines directed at emerging viral strains are being developed while vaccination schemes with booster doses and combinations of different platform-based vaccines are being tested in trials and real-world settings. Despite these diverse approaches, COVID-19 vaccines are only delivered intramuscularly, whereas the nasal mucosa is the primary site of infection with SARS-CoV-2. Preclinical mucosal vaccines with intranasal or oral administration demonstrate promising results regarding mucosal IgA generation and tissue-resident lymphocyte responses against SARS-CoV-2. By mounting an improved local humoral and cell-mediated response, mucosal vaccination could be a safe and effective way to prevent infection, block transmission and contribute to reduce SARS-CoV-2 spread. However, questions and limitations remain: how effectively and reproducibly will vaccines penetrate mucosal barriers? Will vaccine-induced mucosal IgA responses provide sustained protection against infection?

INTRODUCTION

In December 2019, the emergence of a new human pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the spread of the associated highly contagious disease left the world in chaos. Drastic non-pharmaceutical interventions were mobilized to control the pandemic, but vaccination against COVID-19 soon emerged as an indispensable solution to the global health crisis. Vaccine development was exceptionally fast and clinical trials showed efficacy results beyond initial hopes. Thanks to previous progress on vaccine platforms and incredible effort into biomedical research for COVID-19 vaccines, several candidate vaccines were rapidly designed, evaluated, manufactured and deployed. Over 10.5 billon doses of vaccines have been administered in the world in a little more than a year[1]. As of March 2022, ten vaccines have been authorized for emergency or full use by WHO-recognized regulatory authorities. These are the BNT162b2 vaccine (Pfizer/BioNTech), the mRNA-1273 vaccine (Moderna), the AZD1222 vaccine (AstraZeneca/University of Oxford) and its counterpart Covishield (Serum Institute of India), the Ad26.COV-2.S vaccine (Janssen), the CoronaVac vaccine (Sinovac Biotech), the BBIBP-CorV vaccine (Sinopharm), the Covaxin BBV152 vaccine (Bharat Biotech) and the NVX-CoV2372 vaccine (Novavax) as well as its counterpart Covovax (Serum Institute of India). In addition, several other vaccines have shown encouraging efficacy results and received authorizations in a number of countries, including the Gam-COVID-Vac Sputnik V (Gamaleya Research Institute), the Ad-nCoV Convidicea (Cansino

Biologics), the WIBP-CorV vaccine (Sinopharm) and the COVIFENZ vaccine (Medicago and GSK)[2]. 346 candidate COVID-19 vaccines are still in development, 151 of which are currently in clinical trials[3]. The many candidate vaccines against SARS-CoV-2 rely on various platforms, including mRNA-based vaccines, viral-vectored vaccines, inactivated virus-based vaccines and recombinant proteins.

Despite major successes in vaccine development and implementation, the COVID-19 pandemic is far from being over. As of March 2022, 65% of the world population received at least one dose of a COVID-19 vaccine, unequally distributed among countries. Making vaccines available in all parts of the world (including in low- and middle-income countries) remains a challenge. Even in populations with large access to SARS-CoV-2 vaccines, some issues still need to be addressed. The variability of the SARS-CoV-2 virus and its variants of concern (VOCs) are a threat to vaccine-induced protection. Concerns on the durability of the immune response induced by vaccines have led several countries to engage in campaigns to administer booster doses of vaccine to parts or all of their population. Studies are ongoing to determine the durability of vaccine-induced immunity and define indications for booster doses. In the meantime, research on new vaccine candidates continues, investigating different routes of administration. While all COVID-19 vaccines in use and the vast majority of vaccines in clinical development are delivered intramuscularly, the route of infection of the SARS-CoV-2 virus makes mucosal vaccination approaches particularly relevant.

[1]Université Paris Cité, Paris, France. [2]Sorbonne Université, Paris, France. [3]Imagine Institute, Paris, France. [4]Immunology and Pediatric Hematology Department, Assistance Publique-Hôpitaux de Paris, Paris, France. [5]Institut National de la Santé et de la Recherche Médicale UMR 1163, Paris, France. [6]Collège de France, Paris, France. ✉email: violette.mouro@sciencespo.fr
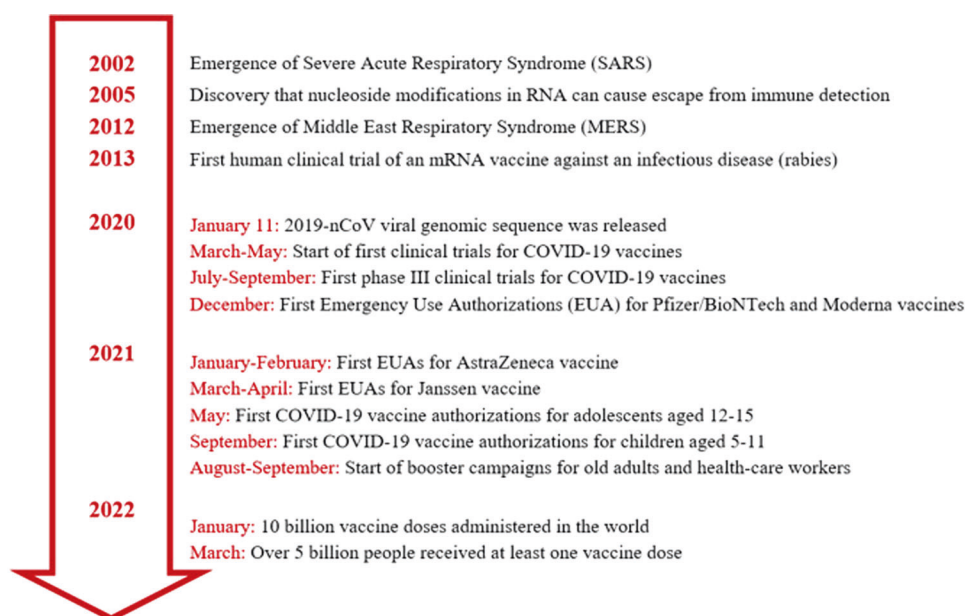
Fig. 1 A history of COVID-19 vaccines development. The genome sequence of the SARS-CoV-2 virus was released in January 2020 and followed by rapid design, evaluation, manufacturing and deployment of vaccines against COVID-19. 10 billion vaccine doses were administered in one year.

THE DEVELOPMENT OF SARS-COV-2 VACCINES

From the beginning of the COVID−19 pandemic, after the first release of the genome sequence of the SARS-CoV-2 virus on January 11, 2020, amazing effort was put into the development of vaccines to prevent infection and disease, with two major goals for vaccine candidates: the induction of a protective immunity and the obtention of a satisfactory safety profile (Fig. 1).

The spike protein: a major vaccine target

Spike is a large glycoprotein present at the surface of SARS-CoV-2 virions, which plays a major role in the attachment to target cells and entry of the viral genome into the cell. It consists of a surface ectodomain containing a receptor binding domain (RBD) and a transmembrane domain. The RBD is mostly responsible for viral attachment via ACE-2 (angiotensin-converting enzyme 2), a receptor displayed at the surface of target cells. The RBD, as well as the N-terminal domain (NTD) of the protein are particularly immunogenic: the immunodominant trimeric Spike protein is a triggering agent for the elaboration of an immune response via neutralizing antibodies[4] and through the T-cell epitopes it contains[5]. Its functions make it a target antigen of choice for the development of COVID-19 vaccines. Guided by the choice of the Spike protein, the first step in vaccine development was therefore to design and produce a highly immunogenic Spike protein. This was done by introducing two proline mutations in the sequence of the SARS-CoV-2 Spike protein, stabilizing the protein in its natural prefusion conformation and thus improving immunogenicity[6].

Multiple vaccine platforms

Trials on multiple vaccine platforms have been launched by many companies in developing COVID-19 vaccines. Vaccines based on recombinant proteins, non-replicating viral vectors, DNA, inactivated viruses or RNA represent together over 90% of candidate vaccines.

Gene-based platforms allow to encode the antigen of interest and induce its production by the body. Among them, synthetic RNA platforms were a major breakthrough in the fight against the COVID-19 pandemic, allowing for rapid cell-free manufacturing of safe and highly immunogenic prophylactic mRNA vaccines. The RNA sequence of the viral Spike protein is enclosed in a lipid nanoparticle and delivered in the body, allowing human cells to produce and display the protein for the immune system to respond. The candidate vaccines of Moderna (mRNA-1273) and Pfizer/BioNTech (BNT162b2) were the first to demonstrate impressive results starting in November 2020. DNA vaccines, consisting of a DNA plasmid encoding the Spike protein, are another type of nucleic acid-based vaccines, although in the case of SARS-CoV-2 not one has yet been approved for use. Non-replicating viral-vectored vaccines also use the genomic sequence of the SARS-CoV-2 Spike protein: another virus, the vector, is manipulated to express the protein of interest. Because part of its genome was deleted, the viral vector is unable to replicate. AstraZeneca/Oxford, Janssen and Gamaleya Research Institute used recombinant adenoviruses for their COVID-19 vaccines.

Other vaccines rely on an inactivated virus (SARS-CoV-2 is grown in cell culture and chemically inactivated). Interestingly, such vaccines are the most used COVID-19 vaccines in the world in terms of number of doses administered: almost half of vaccine doses delivered in the world are doses of CoronaVac (Sinovac Biotech) or BBIBP-CorV (Sinopharm)[7]. The distribution of these vaccines plays a particularly important role for vaccination in low- and middle-income countries.

Over one third of candidate vaccines in development are based on recombinant protein subunits. Among them, the NVX-CoV2373 (Novavax) vaccine candidate, made from the full-length Spike protein, started to be distributed in December 2021. Recombinant protein technologies benefit from robust safety and immunogenicity data in the history of vaccines.

Viral-like particles, resembling an empty virus displaying the Spike protein on its surface, and live attenuated viruses (genetically weakened viruses) are other possible platforms for the development of vaccines, but they only represent 4% and 1% of candidate vaccines in clinical phase, respectively[3].

EFFICACY AND EFFECTIVENESS OF SARS-COV-2 VACCINES

Phase 3 trials demonstrated strong efficacy against symptomatic infection for mRNA vaccines, as reported in Table 1. The BNT162b2 (BioNTech/Pfizer) was shown to have a 95% efficacy after the

Table 1. COVID-19 vaccines in use and in clinical development with reported efficacy.

Vaccine	Manufacturer	Platform	Efficacy against infection	Efficacy against severe disease	Predominant viral strains at time of trials
BNT162b2	Pfizer–BioNTech	mRNA	95.0 (7 days post second dose)[8]	100	B.1, B.1.1.7
mRNA-1273	Moderna	mRNA	94.1 (14 days post second dose)[9]	100	B.1, B.1.1.7
AZD1222	AstraZeneca–Oxford	Viral vector	80.7 (14 days post second dose)[10]	100	B.1, B.1.1.7, B.1.351
Ad26.COV-2-S	Janssen	Viral vector	66 (28 days post first dose)[111]	85.4	B.1.1.7, B.1.351
CoronaVac	Sinovac Biotech	Inactivated virus	51–91[14]	100	P.1, P.2
Covaxin	Bharat Biotech	Viral vector	78[112]	100	B.1.617.2, B.1.617.1
BBIBP-CorV	Sinopharm	Inactivated virus	78[13]	79	
NVX-CoV2373	Novavax	Protein subunit	89.7 (7 days post second dose)[113]	100[114]	B.1.1.7, B.1.351
Sputnik V	Gamaleya	Viral vector	92[12]		B.1.1.7
Convidecia	CanSino Biologics	Viral vector	64[115]	96	
WIBP-CorV	Sinopharm	Inactivated virus	73[13]	100	
COVIFENZ	Medicago; GSK	Viral-like particle	71[116]		B.1.1.7, P.1, B.1.617.2

Reported efficacy of COVID-19 vaccines after primo vaccination (percentages).

administration of two doses[8]. The mRNA-1273 (Moderna) exhibited similar results: 94% efficacy after two doses[9]. The efficacy results from phase 3 trials for viral-vectored vaccines were less homogeneous. AstraZeneca/Oxford published data revealing a 70% efficacy of their AZD1222 vaccine after two doses[10,11]. A comparable efficacy of 66.9% against moderate to severe disease was obtained with the Ad26.COV-2.S vaccine (Janssen) after one dose[11], while the Sputnik V vaccine developed by the Gamaleya Research Institute claimed a 90% efficacy after two doses[12]. Inactivated virus BBIBP-CorV vaccine developed by Sinopharm demonstrated a 78% efficacy[13], while several phase 3 trials on CoronaVac (Sinovac) conducted in Brazil, Chile, Indonesia and Turkey gave efficacy results ranging from 50 to 91%[14,15] (Table 1).

Following assessment of the data obtained in clinical trials, evaluation of safety and efficacy for COVID-19 vaccines must continue in real-world settings. According to multiple case-control studies, BNT162b2 (BioNTech/Pfizer) has a real-world efficacy of 94–96% against symptomatic infection after two doses[16] and 86–92% against any infection after two doses. The effectiveness of mRNA-1273 (Moderna) was evaluated around 90% against symptomatic infection after the administration of two doses[17]. mRNA vaccines therefore demonstrate a very satisfactory effectiveness in real-world settings, comparable to efficacy levels observed in phase 3 clinical trials. The safety profile of mRNA vaccines is also very satisfactory. Although events of myocarditis in adolescent males after receiving the second dose of an mRNA vaccine garnered media attention, post-mRNA-vaccine-myocarditis remain rare and usually resolve within days[18,19]. The effectiveness of the AZD1222 vaccine was evaluated around 70%[20]. Concerns regarding the safety of AZD1222 were also raised after a few vaccinated people developed unusual clotting events. Evaluations revealed that vaccination with ADZ122 can result in the rare development of immune thrombotic thrombocytopenia[21,22] and led several countries to withdraw or restrain the use of the AZD1222 vaccine to people aged over 50 years.

Interestingly, heterologous vaccination schemes were proved to be safe and highly immunogenic, both when using two different COVID-19 vaccines for the first and second doses of a primary vaccination (heterologous primary vaccination) and when using a different COVID-19 vaccine as a booster a few months after a primary vaccination (heterologous boosting). Data from an increasing number of clinical studies suggest that a combination of viral vector-based vaccines and mRNA vaccines elicits the production of high levels of antibodies[23], while several immunogenicity studies showed that a combination between ADZ1222 and BNT162b2 induces a higher CD4+ and CD8+ T lymphocyte response against the Spike protein of SARS-CoV-2 than using the same vaccine[24,25].

All vaccines discussed above demonstrated high levels of protection (over 90%) against severe forms of the disease in all age groups, including protection against lethality. But the capability of COVID-19 vaccines to prevent the transmission of the virus by vaccinated individuals remains a substantial question. Transmission of SARS-CoV-2 between individuals via aerosol particles and droplets is made possible by viral replication following infection of the respiratory epithelia. In addition to protective immunity against severe disease, most vaccines demonstrated levels of protection against asymptomatic infection by SARS-CoV-2. This suggests that vaccination could induce at least transiently a sterilization of the nasopharynx and provides good chances that vaccinated people have a lower probability of transmitting the virus. However, since the protection against infection remains incomplete and transient—further detailed below, lifting non-pharmaceutical protective measures for vaccinated people has been a public health policy challenge.

IMMUNE RESPONSES TO SARS-COV-2 VACCINES
Vaccines developed to target SARS-CoV-2 aim at inducing protective immunity that should at least match the immunity induced by SARS-CoV-2 infection, despite their non-physiological intramuscular route of administration. The development of an immune response following SARS-CoV-2 infection has been characterized from the serum and blood cells of convalescent patients.

Immune responses during and after SARS-CoV-2 infection
Antibody and cell-mediated immunity are involved in recovery from SARS-CoV-2 infection[26]. Neutralizing antibodies targeting the Spike protein (whether it be the RBD or other regions of the protein) can be detected in most individuals following infection,

and the degree of antibody responses appears to be correlated with viral load[27]. The antibody response to infection by SARS-CoV-2 is both systemic, mostly through high levels of IgG detected in the blood, and mucosal, through IgA found in the upper respiratory tract[28]. Titers of IgG and IgM antibodies significantly decrease over time although they remain detectable in the majority of individuals up to 6 months after infection[29]. After a first large secretion of antibodies following antigen encounter, Spike-specific B cells undergo somatic hypermutation in germinal centers in the months following infection. Antibodies mutated to display higher affinity to the RBD of the Spike protein are positively selected[30]. Memory B cells against the Spike protein persist and even increase between 1 month and 8 months after infection[29,31]. In addition to high titers of neutralizing antibodies, many studies identified a contributing role for CD8+ T cells and CD4+ T cells, both detectable in the blood of recovered patients up to 1 year after SARS-CoV-2 infection[32]. CD4+ T-cell responses to SARS-CoV-2 appear to be more prominent than CD8+ T-cell responses[32,33]. SARS-CoV-2 specific CD4+ T cells differentiate into T helper 1 (Th1) cells which produce and release interferon γ (IFNγ) and other anti-viral cytokines. Th1 cells were found to be associated with milder infection in COVID-19 patients[26]. CD4+ T cells can also differentiate into T-follicular helper cells (Tfh), specialized in helping B cells in germinal centers and crucial for the establishment of long-term humoral immunity. SARS-CoV-2-specific circulating Tfh cells are produced during SARS-CoV-2 infection[34]. Specific CD8+ T-cell responses also develop during SARS-CoV-2 infection and release cytolytic molecules such as granzyme B and perforin, as well as anti-viral cytokines like IFNγ. Memory CD8+ and CD4+ T cells were also identified in convalescent patients[34], both as circulating and as tissue-resident memory T cells. Studies on the local implication of B and T cells in respiratory tissues showed a tissue coordination between local mucosal and systemic immune responses following SARS-CoV-2 infection[35], which might allow for site-specific protection against future challenges by the virus, and shed light on the role of the nasopharyngeal microbiome in the regulation of local immunity[35]. Priming of the upper airways by the virus induces the formation of a local compartmentalized network of immune cells in the tracheobronchial epithelium and nearby lymph nodes, organized within the nasopharyngeal-associated lymphoid tissue (NALT)[36]. Interconnection with gut-associated immunity (in gut-associated lymphoid tissue; GALT) is also likely, as intestinal viral pools of SARS-CoV-2 were observed in recovered COVID-19 patients with nasal swabs negative for SARS-CoV-2[37], and could stimulate the sustained production of neutralizing IgA in mucosal tissues. Finally, the role of innate immunity in the response to SARS-CoV-2 infection also seems critical. Type 1 and type 3 interferon innate responses appear to be important especially in early infection[38]. Impaired and delayed type 1 and type 3 IFN responses have been associated with a higher risk of developing a severe form of COVID-19[39]. Different roles of interferon responses have been identified depending on the site within the respiratory tract. High IFN levels were identified in the lower airways of patients with severe COVID-19, whereas IFN responses in the upper airways were associated with milder disease[40].

Immune responses induced by Covid-19 vaccines

As most COVID-19 vaccines target the SARS-CoV-2 Spike protein, vaccination is expected to induce a Spike-directed immunity, ideally combining neutralizing antibodies and effector T-cell responses against the Spike protein. Individuals vaccinated with mRNA vaccines BNT162b2 or mRNA-1273 indeed demonstrated levels of neutralizing antibodies against Spike protein, yet they were mainly detectable after the administration of a second dose of vaccine. Thus, 14 days after the first dose, serum levels of binding antibodies against the RBD or the entire Spike protein are

equivalent to those observed in the serum of convalescent patients[41], but they are non-neutralizing antibodies. Via their fragment constant (Fc) region, they may activate natural killer cells and trigger antibody-dependent phagocytosis mediated by monocytes or neutrophils[42]. Yet, the contribution of non-neutralizing antibodies to protective immunity against SARS-CoV-2 is yet to be completely understood. Neutralizing antibodies are barely detectable before the administration of the second dose of vaccine, but their importance in protective immunity has been better characterized. mRNA vaccines and Novavax protein subunit vaccine elicit higher levels of neutralizing antibodies than viral-vectored vaccines, which persist longer. After two doses, mRNA vaccines induce up to tenfold higher levels of neutralizing antibodies than titers observed in human convalescent serum, peaking 7 days post second dose and persisting at these levels at least 28 days post second dose for the mRNA-1273 vaccine[41].

Vaccines against SARS-CoV-2 were developed with limited data and knowledge on what would constitute effective protective immunity. Following the delivery of vaccines, determining correlates of protection has been a challenge in the crisis management, and no precise correlate of protection can be used at the individual scale to predict disease outcomes. However, the accumulation of data on immune responses following infection or immunization tends to provide information at the collective scale. Concentrations of neutralizing antibodies in the serum have been associated with lower probability of disease and higher likeliness of survival. Therefore, titers of neutralizing antibodies directed at the Spike protein emerged as the most robust correlate of protection. In particular, Feng et al. showed that antibody titers above a threshold of 264 BAU per mL were associated with 80% protection against SARS-CoV-2 Alpha variant[43].

mRNA vaccines exhibit some levels of efficacy only 10–12 days after the administration of the first dose[41], despite almost undetectable levels of neutralizing antibodies at that stage, implying that other components of the immune system, likely innate and cell-mediated response, are involved in the early vaccine-induced immunity. The mRNA-1273 (Moderna) vaccine was found to elicit a Th1 cell response characterized by production of TNF and IFNγ by CD4+ T cells[44]. CD8+ cytotoxic T cells are known to be important effector cells in vaccine-induced responses. Studies showed a rapid and stable mobilization of CD8+ T cells by mRNA vaccine BNT162b2[45], conferring an early protection: Spike-specific CD8+ T cells were already detected at 6 days post first dose, with a peak at 9–12 days. This CD8+ T-cell response was shown to be maintained after the second dose, peaking at 5 to 6 days post second dose. Levels of B-cell immune memory were also investigated in response to COVID-19 vaccines. The administration of one dose of BNT162b2 vaccine allowed for priming of memory B cells as efficiently as with infection by SARS-CoV-2. Administering a second dose of vaccine proved to induce a boosting of B memory cells response[46]. This boost was higher when administering one dose of vaccine after recovery from a SARS-CoV-2 infection.

COVID-19 vaccines and in particular mRNA vaccines therefore manage to induce diverse immune mechanisms involved in protective immunity against SARS-CoV-2. After one dose, mRNA vaccines elicit significant levels of binding antibodies but low levels of neutralizing antibodies as well as significant but relatively low levels of circulating specific T cells. Neutralizing antibody levels rise significantly after the second dose, and memory B and T cells become detectable, persisting for at least 6 months (Fig. 2). The kinetics of these events are overall similar to those seen following vaccination against influenza virus[47,48].

While SARS-CoV-2 vaccines have demonstrated satisfactory efficacy and safety both in clinical trials and in real-world settings, research must continue to assess the safety and immunogenicity of vaccines in specific populations not initially included in the clinical trials. Because of their medical condition putting them at
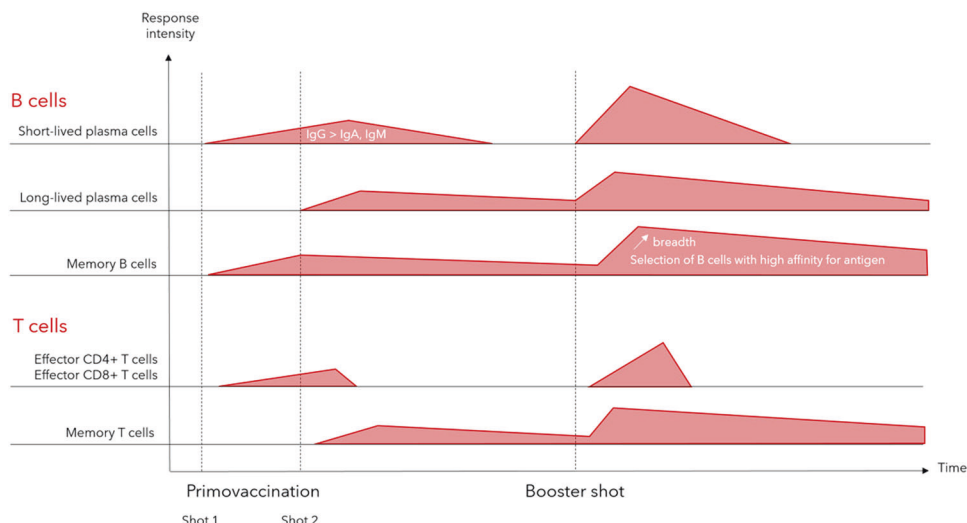
Fig. 2 Schematic representation of immune responses generated by vaccination against SARS-CoV-2. Vaccination against COVID-19 induces the differentiation of IgG-producing plasma cells and elicits SARS-CoV-2-specific CD4+ and CD8+ effector T cell responses. Both Memory B and T cells become detectable after primovaccination and are further increased by the administration of a booster dose. B cells with increased breadth and potency are selected and mount a diverse memory repertoire able to respond rapidly to subsequent viral challenge.

very high risk of severe COVID-19, immunocompromised people (people on immune-modulating medication or suffering from an immune deficiency) were among the top-priority targets of vaccine delivery once vaccines became available. Unfortunately, the levels of neutralizing antibodies induced remained low for many[49]. Studies were launched to evaluate modified doses and vaccination schedules for immunocompromised patients with the objective to maximize protective immunity. Protective immunity for immunocompromised patients has been hard to define. First results suggest variable responses between patients and call for personalized strategies.

DURABILITY OF VACCINE-INDUCED IMMUNE RESPONSES

Over one year after the administration of the first vaccine doses, the durability of the protection conferred by vaccination must be investigated and further vaccination strategies adapted. Durability seems to vary depending on the assessed responses. mRNA vaccines demonstrated a maintained efficacy (91% efficacy 6 months after the second dose for the mRNA vaccine BNT162b2). Neutralizing antibodies are detected in the serum of vaccinated individuals for most authorized vaccines at least 6 months post vaccination[29,50,51], but progressively decline following a biphasic curve[41]. There is hope that cellular immunity mediated by T-cell subsets induced after vaccination persist for longer[52], but the decrease in neutralizing antibodies raises concerns regarding sustained protection against SARS-CoV-2 infection. In fact, several studies showed a decline of vaccine efficacy against SARS-CoV-2 infections after 6 months, whatever the type of vaccine used[53–57]. This waning immunity is more important in older people but is significant in all age groups and justifies, especially for fragile populations, the inoculation of a booster vaccine dose to further stimulate immunity. Several countries launched booster vaccination campaigns for their population regardless of age. Interestingly, the need to administer booster doses might reveal comparative advantages between vaccine platforms: inoculating a viral-vectored vaccine several times might attenuate the response to SARS-CoV-2 antigens in booster doses because of the possible development of an anti-vector immunity. In comparison, mRNA vaccines would not suffer from such technical difficulties. Results from the first booster campaigns conducted in Israel show that neutralizing antibody titers after the administration of a booster dose of mRNA vaccine BNT162b2 are increased

by a factor of 10 compared with neutralizing antibody titers one month after the completion of a two doses vaccination scheme, and that receiving a booster dose reduces by 10-12 times the risk of infection. mRNA-1273 vaccine demonstrated similar results[58]. The administration of a booster dose induces a significant reduction of severe covid cases (by a factor of 18.7 among people over 60 years old, and by a factor of 22 among people aged 40 to 60, after a BMT162b2 booster dose)[59], with an efficacy of over 90% against hospitalization and severe disease[60].

SARS-COV-2 VARIANTS AND IMPACTS ON VACCINE-INDUCED IMMUNE RESPONSES

The sustainability of vaccine-induced immune responses is further challenged by the antigenic variation of the SARS-CoV-2 virus. Because of the low fidelity of the viral RNA polymerase, random mutations can occur in the viral genome and introduce changes in amino-acid sequences of viral proteins, some of which can confer advantages to the virus in terms of viral spread and are therefore favored by selection pressures. Mutations can impact the severity of the disease and the efficiency of viral transmission with potential consequences on public health. Importantly in the context of COVID-19 vaccination, significant mutations in the sequence of the Spike protein can reduce its recognition by neutralizing antibodies and therefore decrease vaccine-induced immunity (Table 2). An accumulation of mutations that confer significant advantages to the virus result in what has been referred by WHO as variants of concern (VOCs). Four of such variants— Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2)— emerged and spread in the first half of 2021, raising concerns regarding possibilities of immune escape. Fortunately, efficacy against symptomatic disease remained high against these VOCs for most vaccines[61]. VOC Beta is the most antigenically diverse compared with the original strain, and neutralization of this variant appears to be decreased 5 to 8-fold for most vaccines[62]. Vaccine-induced immune responses have been shown to retain partial neutralizing capacities (decreased by a factor 5) against the Delta variant, without significant loss of efficacy against severe disease. In late 2021, a more divergent variant called Omicron (B.1.1.529) emerged in South Africa and spread to become now dominant in most parts of the world. Although it appears to cause less severe COVID-19 cases than the Delta variant[63], Omicron is at least three times more contagious and seems to evade immunity

Table 2. Efficacy of COVID-19 vaccines against four SARS-CoV-2 VOCs.

Vaccine		BNT162b2	mRNA-1273	AZD1222	Ad26.COV-2-S	CoronaVac	BBIBP-CorV	NVX-CoV2373
Manufacturer		Pfizer–BioNTech	Moderna	AstraZeneca–Oxford	Janssen	Sinovac Biotech	Sinopharm	Novavax
Platform		mRNA	mRNA	Viral vector	Viral vector	Inactivated virus	Inactivated virus	Protein subunit
Alpha (B.1.1.7)	Severe disease	95	97	94	86	50	73	89
	Infection	78–95	84–99	75	72	47	68	86
Beta (B.1.351)	Severe disease	95	97	N.R	76	N.R	N.R	86
	Infection	75	96	10	65	N.R	N.R	86–93
Delta (B.1.617.2)	Severe disease	94	97	92	76	N.R	N.R	N.R
	Infection	42–79	76–84	67	64	59.0	67	N.R
Omicron (B.1.1.529)	Severe disease	72 (primo vaccination) 90 (booster)	73 (primo vaccination) 90 (booster)	71 (primo vaccination)	57	N.R	N.R	65
	Infection	44 (primo vaccination) 75 (booster)	48 (primo vaccination)	36 (primo vaccination) 75 (mRNA booster)	33	N.R	N.R	43

Reported short term efficacy of COVID-19 vaccines against VOCs Alpha, Beta, Delta, and Omicron (percentages).

much more efficiently than Delta[64]. Several mutations in the RBD (K417N, N440K, G446S, S477N, T478K, and E484A), as well as mutations, deletions and insertions in the NTD of Omicron Spike protein are likely to substantially decrease the neutralizing capacity of antibodies obtained post infection or vaccination. Immunity conferred by primo vaccination seems relatively low against infection by Omicron variant[65–70]. However, the administration of a booster dose of mRNA vaccine showed increased protection levels against infection by Omicron, although it remains lower than protective immunity against Delta. Data suggest that booster doses of vaccines allow for protection levels of about 90% against severe forms of COVID-19 caused by Omicron[71]. This booster-induced protection decreases to 44% with BNT162b2 vaccine after 10 weeks, but remains high and stable concerning protection against hospitalization[71,72]. According to a recent study from Muecksch et al. such protection against variant-induced severe disease is obtained due to an increase of neutralizing antibody potency: individuals who received three doses of vaccine mount a diverse memory B-cell repertoire, able to respond rapidly and capable of producing neutralizing antibodies against diversified variants such as Omicron[73].

Although it has not been observed as of early 2022, pressures exerted by vaccination at the populational level could theoretically contribute to the selection of viral strains more susceptible to evade vaccine-induced immunity. Another significant risk lies in the fact that sustained high transmission levels within naïve or only partially immune populations substantially increase the likelihood of new variants emerging, and have led to the emergence and spread of the D614G strain in 2020, followed with several VOCs (Alpha, Beta, Gamma, Delta, and Omicron) in 2021. Vaccination coverage must be and remain high to limit the impact of emerging variants on public health. One implication is that solutions developed in high income countries only do not allow for a control of the worldwide transmission of the virus, revealing the major necessity to make COVID-19 vaccines available in all parts of the world.

To keep up with the evolution of SARS-CoV-2 virus, the future of COVID-19 vaccines could rely on combinations of different mutated Spike proteins, allowing for the induction of immunity against several viral strains. Such a strategy was adopted by Moderna with the development of two bivalent vaccines: mRNA-1273.211 (comprising a mix of original mRNA-1273 and mRNA-1273.351 which encodes for the Spike protein found in VOC Beta) and mRNA-1273.213 (mix of Beta and Delta variant mRNAs). Additionally, the evaluation of an Omicron specific candidate mRNA-1273.529 started in February 2022[74]. Pfizer also started a clinical trial to test the efficacy of a multivalent vaccine combining the ancestral D214G strain with the Omicron VOC in at-risk individuals. Difficulties in forecasting future viral mutations are the main obstacle to such strategies. Overcoming the problem of VOCs with universal vaccines containing identified immunogenic sequences of conserved antigens could be easier, should they allow for sufficient protective immunity.

Most vaccines currently in use rely on the induction of a B-cell mediated antibody response. On the other hand, specifically targeting T cells with synthetic peptides could strengthen CD4+ and CD8+ cellular immunity against COVID-19: primed T cells could tackle different fragments of SARS-CoV-2, recognize viral variants and more effectively target infected cells to clear the infection. T-cell vaccines could also substantially help to obtain protection in immunocompromised people who cannot mount strong B-cell responses[75].

While well tolerated and highly immunogenic mRNA vaccines have been an important breakthrough in the fight against the COVID-19 pandemic, new RNA-based technologies could further improve vaccine strategies. Existing RNA vaccines (Pfizer/BioNTech and Moderna) are based on conventional mRNA, the induced Spike antigen expression is therefore proportional to the number

of mRNA transcripts that are successfully delivered during vaccination. An implication is that large doses or repeated administrations may be required to achieve sufficient antigen expression for efficient protection. Another synthetic RNA strategy relies on self-amplifying RNAs (saRNA) and has shown promising results regarding protective immunization in preclinical studies against multiple infectious diseases[76]. It consists of genetically engineered replicons derived from self-replicating single-stranded RNA viruses[77], whose self-replicating properties allow for enhanced antigen expression in situ[78]. In addition, saRNAs can be circular and therefore benefit from a higher stability. saRNA vaccines could therefore achieve comparable antigen expression at lower doses compared to conventional mRNA vaccines.

DELIVERY ROUTES FOR SARS-COV-2 VACCINES—AN OPPORTUNITY FOR MUCOSAL IMMUNIZATION

Exposure to SARS-CoV-2 occurs by inhalation of small droplets and aerosol particles or by deposition of particles containing the virus on exposed mucous membranes in the nose, mouth or eye. SARS-CoV-2 infects the respiratory tract by binding to ACE-2 receptors on the surface of epithelial cells. Therefore, immunity at mucosal sites likely plays a key role in the response to SARS-CoV-2 infection and might be particularly relevant for the prevention of re-infection. Despite infection sites of SARS-CoV-2 being mucosal membranes of the upper airways, the vast majority of COVID-19 vaccines are delivered intramuscularly. Intramuscular immunization primarily elicits IgG responses in the blood, with low concentrations of IgG in the upper respiratory tract and nasal passages and does not recruit local resident memory lymphocytes.

Mucosal immunity holds a front-line status within the immune system and is therefore investigated for use in vaccines. Stimulation of the nasal mucosa (by infection or immunization) induces the production of secretory IgA, actively transported across the epithelium at nasal passages and released in respiratory fluids in the lumen[52,79]. IgA is secreted as a dimer joined by a J chain and bound to a secretory component. This configuration was shown to be more stable, allowing effective neutralization of viruses at mucosal surfaces. The presence of secretory IgA has been associated with resistance to infection by various pathogens, through prevention of viral adherence to epithelial cells, mediation of pathogen excretion and prevention of viral particles assembly[80]. The history of coronaviruses indicates that levels of IgA present at the nasal site are correlated with protection against infection[81]. In addition to antibody responses, priming B and T cells at the respiratory mucosa can promote their residency in mucosal sites of the respiratory tract as long-lived cells or tissue-resident memory cells[82], able to respond rapidly to potential re-infection. This suggests that local immunization may be more effective than peripheral immunization for the prevention of mucosal infections[82].

Mucosal responses to SARS-CoV-2 in the upper respiratory tract are mediated by adaptive and innate immune components. Upon entry in the upper respiratory tract, SARS-CoV-2 replicates in mucosal surfaces of the nasopharynx and rapidly induces a neutralizing secretory IgA response detected in saliva, nasal swabs or broncho-alveolar lavage[83]. Antigen specific tissue-resident memory B and T cells are formed early following mucosal infection[84]. Innate immunity factors also play a role in mucosal responses to SARS-CoV-2, including MAIT cells which act as critical components of the epithelial barrier protection. Alterations in MAIT cells activation and cytotoxicity positively correlate with the severity SARS-CoV-2 infection[85].

It has been observed that IgG concentrations in the serum and lung of patients treated with IgG prevents pulmonary infection, but that IgG diffusion into mucosal membranes of the upper airways is insufficient to prevent sinus infection. Thus, the complementarity between a strong systemic response mediated
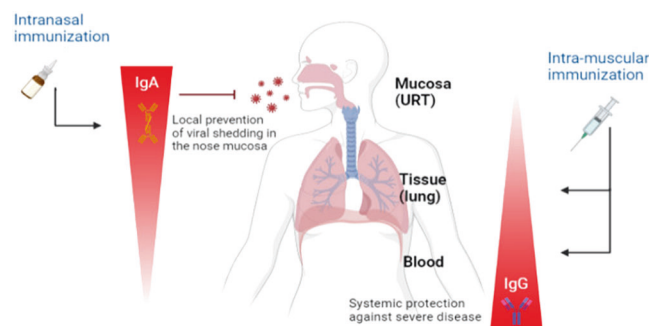

Fig. 3 Gradients of Ig in SARS-CoV-2 infection and expected vaccine-induced humoral immunity. Vaccines administered intra-muscularly result in significant levels of IgG in the blood and in the lower respiratory tract, however poorly diffusing into mucosal membranes of the upper respiratory tract. Intranasal immunization could allow for the local production of IgA in the mucosa of the upper respiratory tract and hopefully prevent SARS-CoV-2 infection (created with BioRender.com).

by IgG in the lung and a mucosal response at infection sites mediated by IgA could explain why a better protection has been observed in individuals who were vaccinated after a previous SARS-CoV-2 infection[86]. Delivered intramuscularly, available SARS-CoV-2 vaccines prevent the development of COVID-19 once the pathogen has crossed mucosal barriers, and mostly result in the systemic production of antibodies and recruitment of T lymphocytes in the lower respiratory tract, but not in the upper respiratory tract. On the contrary, stimulation of the mucosal immune system and the development of a robust IgA-mediated mucosal immunity could confer early protection against SARS-CoV-2 infection even before the virus reaches the lungs (Fig. 3). This opens the potential for the development of vaccines designed to stimulate mucosal membranes of the upper airways, with the objective to induce an effective barrier against infection and, in the case of an infection, reduce viral replication and shedding with a stronger inhibitory effect on the transmission of the virus.

A historical example of intranasal vaccine is the live attenuated influenza vaccine (LAIV) which has been used as a nasal spray since the 1960s in the United States. Several other mucosal vaccines are currently authorized for use against poliovirus, cholera, salmonella and rotavirus, and are administered orally[87].

Among the 334 COVID-19 vaccine candidates in development[3], there are seven intranasal SARS-CoV-2 vaccines in clinical trials: BBV154, CIBG-669, COVI-VAC, CVXGA1, ChAdOx-1S, DelNS1-nCoV-RBD LAIV, and MV-014-212 (Table 3). Two of them, COVI-VAC (developed by Codagenix, Inc) and DelNS1-nCoV-RBD LAIV (developed by the University of Hong Kong) use live attenuated viruses and are in phase 3 trials[88,89]. MV-014-212, developed by Meissa Vaccines, Inc., also relies on a live attenuated virus (respiratory syncytial virus) expressing SARS-CoV-2 Spike protein[90], while BBV154 candidate of Bharat Biotech and CVXGA1 candidate of CyanVac LLC are viral-vectored vaccines using a chimpanzee adenovirus or parainfluenza virus 5 (PIV5), respectively[91,92]. Additionally, a phase 1 clinical trial in progress evaluates the intranasal administration of University of Oxford's ChAdOx-1S chimp adenovirus-vectored vaccine[93]. Only one of intranasal candidate vaccines is a protein subunit: CIBG-669 developed by the Center for Genetic Engineering and Biotechnology[94]. Oral vaccines are also being developed; in particular, VXA-CoV-2-1 Ad5 (viral-vectored) adjuvanted vaccine developed by Vaxart is administered orally and has reached phase 2 trials[95].

Several animal studies using intranasal vaccinations against SARS-CoV-2 demonstrated favorable results regarding protection from infection in the upper respiratory tract and suggested that

Table 3. Mucosal vaccines in clinical development.

Vaccine candidate	Vaccine platform	Route of administration	Developer
BBV154	Adenoviral vector (Non-replicating)	Intranasal	Bharat Biotech International Limited
CIGB-669 (RBD + AgnHB)	Protein subunit	Intranasal	Center for Genetic Engineering and Biotechnology (CIGB)
COVI-VAC	Live attenuated virus	Intranasal	Codagenix/Serum Institute of India
CVXGA1	Viral vector (Non-replicating)	Intranasal	CyanVac LLC
ChAdOx-1S	Adenoviral vector	Intranasal	University of Oxford
DelNS1-2019-nCoV-RBD-OPT1	Viral vector (Replicating)	Intranasal	University of Hong Kong, Xiamen University and Beijing Wantai Biological Pharmacy
MV-014-212	Live attenuated virus	Intranasal	Meissa Vaccines, Inc.
VXA-CoV-2-1 Ad5 adjuvanted Oral Vaccine platform	Viral vector (Non-replicating)	Oral	Vaxart
CoV-2-OGEN1, protein-based vaccine	Protein subunit	Oral	USSF/Vaxform
bacTRL-Spike oral DNA vaccine	DNA based vaccine	Oral	Symvivo Corporation
COVID-19 Oral Vaccine Consisting of Bacillus Subtilis Spores	Bacterial antigen-spore expression vector	Oral	DreamTec Research Limited

intranasal immunization could also provide systemic immunity. While intramuscular injection of ChAdOx1 n-CoV vaccine in rhesus macaques reduced viral load in the lung but not the upper respiratory tract[96], its intranasal administration was able to efficiently prevent nasal shedding of SARS-CoV-2[97]. Another study on macaques showed that administration of Ad5-S-nb2 vaccine in the nasal cavity induced both local and systemic protective antibody responses[98]. Intranasal adenovirus-vectored vaccines ChAd-SARS-CoV-2-S and Ad5.SARS-CoV-2-S1 induced high levels of mucosal IgA and robust T-cell immune responses in mice and prevented SARS-CoV-2 infection almost entirely[99,100]. A live Newcastle disease virus vector expressing a prefusion conformation stabilized SARS-CoV-2 Spike protein (AVX/COVID-12-HEXA-PRO; Patria) tested in pigs showed strong serum neutralizing antibody responses when administered intramuscularly, intranasally, or with a combination of the two[101]. hAd5 S-Fusion + N-ETSD vaccine (SARS-CoV-2 Spike and nucleocapsid proteins delivered with a human adenovirus) was also tested in mice using different delivery methods and results showed that a subcutaneous prime immunization followed by an intranasal booster elicited high T-cell responses[102]. The nasal administration of a lentivirus vector containing the SARS-CoV-2 Spike protein protected mice from SARS-CoV-2 infection thanks to an IgA response[103]. An adenoviral-vectored trivalent vaccine expressing the Spike protein but also nucleocapsid and RdRp antigens was found to induce local and systemic antibodies and lung tissue-resident T lymphocytes in mice 4 to 8 weeks post-immunization[104]. Humoral responses obtained after intranasal administration of this vaccine were superior compared to intramuscular immunization and induced protective mucosal immunity against ancestral and variant (Alpha, Beta) strains of SARS-CoV-2.

In addition to viral vectors, other vaccine platforms are being tested for mucosal immunization. A recombinant RBD-based subunit vaccine adjuvanted with alum and administered intranasally was sufficient to induce protective local and systemic antibodies in mice[105]. Another subunit vaccine that uses lyophilized Spike protein adjuvanted with a liposomal STING agonist, tested in mice, elicited IgA responses in the nasal cavity and the lung as well as coordinated activation of T- and B-cell responses within the NALT[106]. Finally, intranasal administration is not the only option for mucosal immunization against SARS-CoV-2. An oral delivery route was tested in mice with a S. Cerevisiae-based vaccine (EBYY100/pYD1-RBD) expressing the spike protein on the surface of the yeast, and showed significant humoral and mucosal responses as well as a robust Th1/Th2 cellular response[107].

To conclude, results regarding the capacity of intranasal vaccines to prevent infection by SARS-CoV-2, shedding of viral particles and therefore inter-human transmission are very encouraging. Further studies are needed to confirm that mucosal vaccines can be sufficient to induce systemic immune response. This might depend on vaccine platforms, dosage and immunization schedule. Rather than subunit vaccines which often require adjuvants, viral vectors and attenuated viruses might be more suitable to trigger mucosal immune responses because their infection process involves pathogen-associated molecular patterns (PAMPs) and is intrinsically immunogenic[82].

Despite exciting possibilities, mucosal vaccination and its adaptation to SARS-CoV-2 raise significant difficulties. The major challenge is to obtain a durable immune response, as IgA responses are known to be relatively short-lived. According to studies in primates, maintaining high levels of neutralizing antibodies was proved necessary for protection in the upper respiratory tract and minimization of transmission[108]. Another struggle resides in the route of administration itself: delivering a vaccine at the nasal mucosa is challenging mainly because it requires nasal clearing[109]. In the nose, cilia and sticky mucus act as protective barriers to prevent entry of chemicals and pathogens. The mucociliary clearance in the nose and time an antigen stays in the mucosa influence its absorption and the success of nasal immunization. Proteolytic enzymes present in the mucosa could also be a challenge to antigen absorption with mucosal immunization. Because of these challenges, mucosal vaccines could require repeated delivery. But their non-invasive needle-free administration process, low-cost production and easier storage and transport logistics represent considerable advantages that may ease mass immunization and delivery of vaccines in low- and middle-income countries[83,110]. Therefore, research on mucosal vaccine-mediated immunity to SARS-CoV-2 is of great clinical and practical relevance.

Should safe and efficient COVID-19 vaccines targeting nasal mucosa be successfully developed in the upcoming months,

vaccine strategies in the fight against the pandemic could accordingly be modified. Administering an intranasal vaccine to people previously vaccinated intramuscularly could be a way to boost immunity, by combining a systemic stimulation of the immune system with a local stimulation of the mucosal immune system, allowing for more efficient viral clearance in the nasal cavity and maximizing protection against SARS-CoV-2 infection.

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V.M. drafted the article and designed the figures. A.F. contributed to the conception and design of the article and revised the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Violette Mouro.

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