Immune Netw. 2021 Feb;21(1):e4
https://doi.org/10.4110/in.2021.21.e4
pISSN 1598-2629·eISSN 2092-6685

IMMUNE NETWORK

Review Article

Check for updates

Current Status of COVID-19 Vaccine Development: Focusing on Antigen Design and Clinical Trials on Later Stages

Pureum Lee [1,2,†], **Chang-Ung Kim** [1,3,†], **Sang Hawn Seo** [4], **Doo-Jin Kim** [1,2,3,*]

[1]Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Korea
[2]Department of Bioscience, University of Science and Technology (UST), Daejeon 34113, Korea
[3]Department of Biochemistry, Chungnam National University, Daejeon 34134, Korea
[4]International Vaccine Institute, Seoul 08826, Korea

OPEN ACCESS

Received: Jan 11, 2021
Revised: Feb 17, 2021
Accepted: Feb 17, 2021

***Correspondence to**
Doo-Jin Kim
Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Korea.
E-mail: golddj@kribb.re.kr

[†]Pureum Lee and Chang-Ung Kim contributed equally to this work.

ORCID iDs
Pureum Lee
https://orcid.org/0000-0001-9477-8531
Chang-Ung Kim
https://orcid.org/0000-0002-8245-3315
Sang Hawn Seo
https://orcid.org/0000-0002-4518-8858
Doo-Jin Kim
https://orcid.org/0000-0003-4934-4931

ABSTRACT

The global outbreak of coronavirus disease 2019 (COVID-19) is still threatening human health, economy, and social life worldwide. As a counteraction for this devastating disease, a number of vaccines are being developed with unprecedented speed combined with new technologies. As COVID-19 vaccines are being developed in the absence of a licensed human coronavirus vaccine, there remain further questions regarding the long-term efficacy and safety of the vaccines, as well as immunological mechanisms in depth. This review article discusses the current status of COVID-19 vaccine development, mainly focusing on antigen design, clinical trials in later stages, and immunological considerations for further study.

Keywords: COVID-19; Vaccines; Prefusion-stabilized; VAERD; Antibody-dependent enhancement; Pre-existing immunological memory

INTRODUCTION

Since its first reported case in winter 2019, coronavirus disease 2019 (COVID-19) has been spreading at an alarming rate worldwide. As of February 16, 2021, more than 100 million confirmed cases and 2.4 million deaths were reported worldwide. In addition to health problems, COVID-19 poses a significant threat to the global economy and social life. Although there has been no licensed human coronavirus (HCoV) vaccine to date, numerous vaccine candidates for pathogenic human viruses have been investigated in animal models as well as in clinical trials, including the vaccines against respiratory syncytial virus (RSV), influenza virus, HIV and Ebola virus. Information and new technologies accumulated from these previous studies have been accelerating the development of current COVID-19 vaccines. As of December 2020, 61 and 172 candidates based on diverse vaccine platform technologies are being tested in clinical and preclinical stages, respectively (1).

IMMUNE NETWORK

Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
2P, Two proline substitution; ACE2, Angiotensin-converting enzyme 2; Ad, Adenovirus; ADE, antibody-dependent enhancement; COVID-19, coronavirus disease 2019; DC, dendritic cell; E, envelope; FIPV, feline infectious peritonitis virus; FP, fusion peptide;HCoV, human coronavirus; ICP, immune correlates of protection; M, membrane; MERS, Middle East respiratory syndrome; modRNA, nucleoside-modified mRNA; N, nucleocapsid; NHP, non-human primate; QIV, quadrivalent influenza vaccine; RBD, receptor binding domain; RSV, respiratory syncytial virus; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VAERD, vaccine-associated enhanced respiratory disease; VLP, virus-like particle; WHO, World Health Organization.

Author Contributions
Conceptualization: Kim DJ, Seo SH; Funding acquisition: Kim DJ; Supervision: Kim DJ; Visualization: Kim DJ; Writing - original draft: Lee P, Kim CU, Kim DJ; Writing - review & editing: Lee P, Seo SH, Kim DJ.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-CoV-2)

SARS-CoV-2, a causative agent of COVID-19, is a single-stranded positive-sense RNA virus belonging to the genus *Betacoronavirus*. The genome is composed of replicase genes encoded within the 5' end and structural protein genes in the 3' end. The structural proteins include spike (S), membrane (M), and envelope (E) proteins that are displayed on the envelop of SARS-CoV-2 virion, and the nucleocapsid (N) protein that form a helical ribonucleocapsid structure by binding to genomic RNA inside the virion. The S protein protrudes on the viral surfaces, forming trimeric structures (**Fig. 1**) (2).

SPIKE: A MAJOR TARGET ANTIGEN FOR COVID-19 VACCINES

SARS-CoV-2 gains entry into target cells by binding its S to angiotensin-converting enzyme 2 (ACE2) on host cells (3,4). ACE2 is expressed in various human organs including oral and nasal epithelium, nasopharynx, lung, small intestine, kidney, spleen, liver, colon and brain (5). SARS-CoV-2 primarily infects respiratory airway, despite its relatively low levels of ACE2 expression compared to other organs. Since SARS-CoV-2 enters target cells through the interaction between S and ACE2, S is considered as a primary target antigen for COVID-19 vaccine development.

The S protein is composed of a S1 domain containing the N-terminal domain and receptor binding domain (RBD), and a S2 domain containing a fusion peptide (FP) and the transmembrane and cytoplasmic domains (4) (**Fig. 2**). Various forms of S protein, including full-length, ectodomain, S1, and RBD, have been investigated as target antigens, as shown in the SARS and Middle East respiratory syndrome (MERS) vaccine studies (2). Full-length S

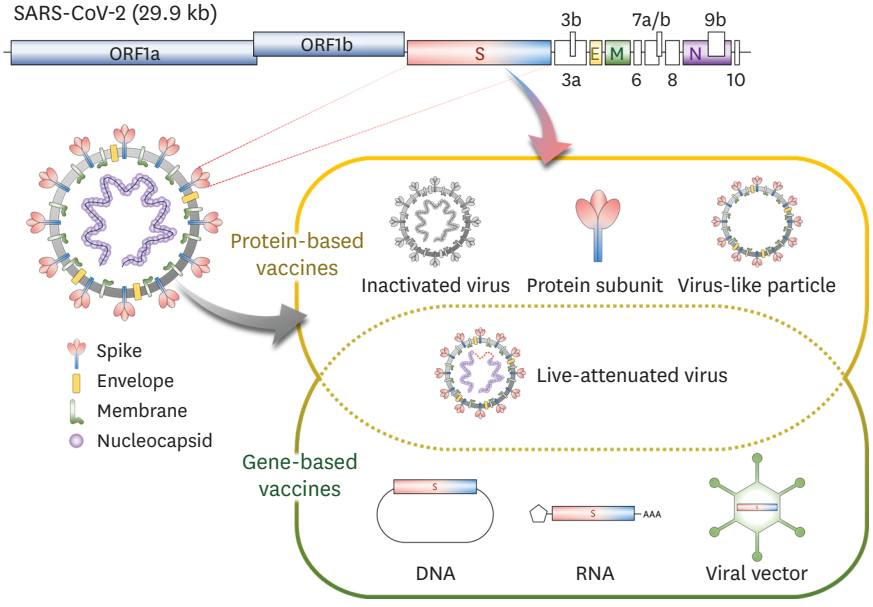

Figure 1. Genome structure of SARS-CoV-2 and the general classification of the vaccine platforms platforms. Modified from Lee et al. (2).
ORF, open-reading frame; S, spike; E, envelope; M, membrane; N, Nucleocapsid.

https://doi.org/10.4110/in.2021.21.e4

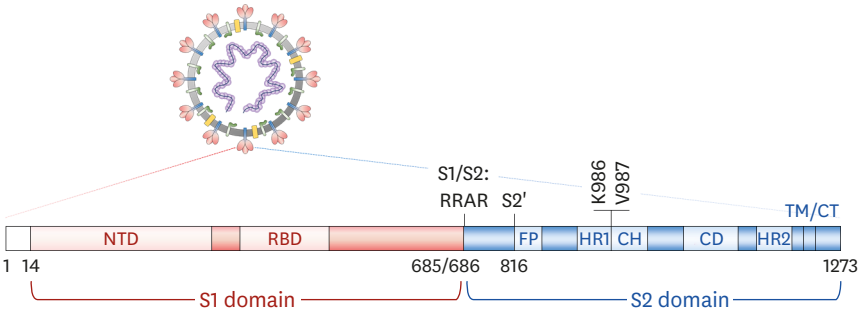

Figure 2. Schematic diagram of a SARS-CoV-2 S protein.
CD, connector domain; CH, central helix; CT, cytoplasmic domain; HR1, heptad repeat 1; HR2, heptad repeat 2; NTD, N-terminal domain; S1/S2, S1/S2 protease cleavage site; S2', S2 protease cleavage site; TM, transmembrane domain.

is one of the most frequently used antigens in COVID-19 vaccine development, especially for gene-based vaccines. The final candidates for mRNA vaccines of Moderna/National Institutes of Health (6) and Pfizer/BioNTech (7), a DNA vaccine of Inovio (8), and adenoviral-vectored vaccines of AstraZeneca/Oxford University (9), Janssen (10) and Gamaleya Research Institute (11) contain full-length S as an antigenic component. In these vaccines, the S protein is expressed in a M-bound form on the surface of transfected or infected cells. It is relatively easy to handle antigens containing hydrophobic transmembrane domains in genetic vaccines compared to recombinant protein vaccines. Novavax is investigating its full-length S recombinant protein-based COVID-19 vaccine in a phase 3 clinical trial (12).

An important feature introduced to full-length S-based vaccines is prefusion-stabilizing mutations. S protein is firstly expressed as a single polypeptide and then is readily cleaved by furin-like protease into S1 and S2 fragments in the host cells (13,14). These 2 fragments exist in a metastable prefusion conformation on the viral M. Once S1 binds to hACE2, transmembrane protease serine subtype 2, a serine protease on the host cells, cleaves the S2' site (15). This additional proteolytic cleavage triggers a conformational change in the S2 domain, leading to the dissociation of the S1 fragment. Finally, the S2 undergoes an irreversible 'jack-knife transition', resulting in a stable postfusion structure (**Fig. 3**) (14,16). Previous studies have reported that proline substitutions in the loop between the first heptad repeat and the central helix stabilize the prefusion structure of M fusion proteins such as HIV-1 gp160, RSV fusion, and influenza virus hemagglutinin proteins (17-19). Similarly, 2 consecutive mutations in MERS-CoV S (V1060P and L1061P) resulted in a stable prefusion form of S, increasing the immunogenicity and efficacy of the recombinant protein antigen (20). Based on these previous studies, the efficacy of COVID-19 vaccine candidates that harbor 2 proline substitutions (2P) in the S2 loop (K986P and V987P) or mutations in the S1/S2 furin cleavage site have been extensively evaluated. In many preclinical studies, prefusion-stabilized S provided increased neutralizing Ab responses and protective efficacy against SARS-CoV-2 and MERS-CoV, compared to wild-type S (6,10,20). Currently, the final products or candidates of Moderna, Pfizer/BioNTech, Janssen CureVac, and Novavax include this prefusion-stabilized S protein as a target antigen (**Fig. 3**).

RBD is another promising vaccine target as the most antibodies with neutralizing activities bind to RBD (21). Moreover, RBD is glycosylated at a relatively low level within S protein and therefore potentially immunogenic (22). However, RBD exhibits lower immunogenicity than S, possibly due to its smaller molecular weight and lower stability *in vivo*. There have been

https://doi.org/10.4110/in.2021.21.e4

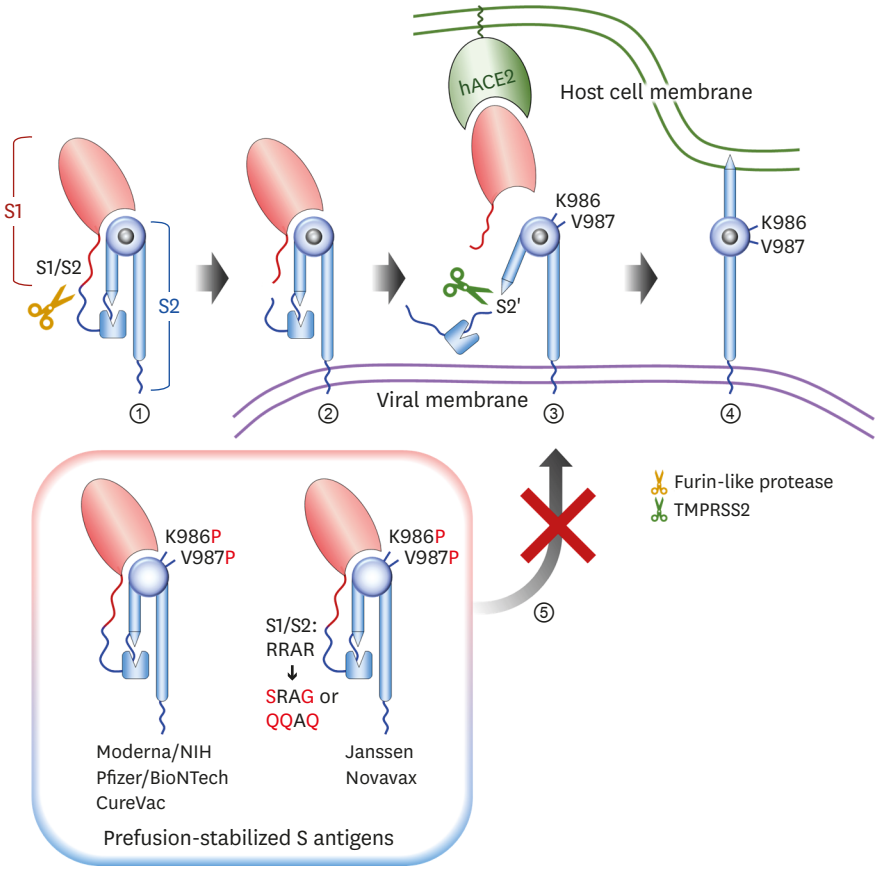

Figure 3. Proteolytic activation of S and prefusion-stabilized S antigens. S protein is expressed as a single polypeptide and cleaved by a furin-like protease into S1 and S2 (①). The two fragments exist in a metastable prefusion conformation on the viral membrane (②). Upon binding of S1 to hACE2, a TMPRSS2 cleaves the S2′ site. The proteolytic cleavage triggers a conformational change in S2 and then S1 dissociates from S2 (③). Finally, the S2 undergoes an irreversible 'jack-knife transition' into a stable postfusion structure (④). Substitution of K986 and V987 into two prolines and/or mutation in S1/S2 cleavage site prevent the S protein from changing into a postfusion conformation, resulting in enhanced immunogenicity and efficacy of COVID-19 vaccines (⑤). TMPRSS2, transmembrane protease serine subtype 2; S1/S2, S1/S2 protease cleavage site; S2′, S2 protease cleavage site.

several approaches to overcome such limitation, including Fc fusion (3) or multimerization of RBD (23). In particular, compared to RBD monomer, RBD dimer or RBD protein nanoparticles significantly increased neutralizing Ab responses and improved protective efficacy in a murine model (23,24). These results may be attributed to the increase in the molecular weight of RBD as well as the induction of efficient B cell receptor cross-linking by the repeated structure of a multivalent antigen (25,26).

Besides the S protein, SARS-CoV-2 has other structural proteins such as M, E and N. As the sera immunized with SARS-CoV-2 M and E failed to neutralize the virus (27), these 2 proteins are currently not considered as target antigens for COVID-19 vaccines. On the other hand, N is highly immunogenic and induces robust humoral and cellular immune responses (28). Since the amino acid sequence of N is highly conserved among HCoVs (29,30), N-specific immunity can induce cross-reactive responses. A prior study has shown that N-specific cellular immune responses in the respiratory mucosa could provide partial cross-protective immunity between SARS-CoV and MERS-CoV. Additionally, vaccination with MERS-CoV N induced cross-reactive cellular immune responses against various coronaviruses,

including SARS-CoV (31). However, another study has reported that the immunization with recombinant vaccinia virus expressing SARS-CoV N caused severe pneumonia accompanied by infiltration of eosinophils, neutrophils, and lymphocytes into the lungs upon subsequent viral infection in mice (32). It remains unclear whether the pneumonia was caused by SARS-CoV N and SARS-CoV-2 N has a potential of inducing such a side effect. Accordingly, the utilization of N as a COVID-19 vaccine antigen requires careful consideration. Currently, several candidates containing the N antigen are being evaluated in the preclinical development of the COVID-19 vaccine (1).

COVID-19 VACCINES IN LATER STAGES OF DEVELOPMENT

As of December 2020, over 200 COVID-19 vaccine candidates are in development based on several different platforms: inactivated virus, live-attenuated virus, protein subunit, virus-like particle (VLP), DNA, RNA, and viral vectored vaccines (**Fig. 1**). Among them, 13 candidates are being assessed in phase 3 clinical trials, and a few of them have been approved for human use in several countries as of December 2020. **Tables 1** and **2** summarize the features of each vaccine platform and information about COVID-19 vaccines in phase 3 trials and beyond, respectively (**Tables 1** and **2**).

As summarized in **Table 1**, the magnitude and quality of the immune responses following vaccination varies depending on the type and composition of each vaccine. However, the immune system utilizes multiple types of cells and molecules in common for inducing efficient immune responses. Among them, dendritic cells (DCs) play an indispensable role in the recognition of danger signal provided by adjuvants, the processing of ingested antigens and the activation of T cells. Follicular DCs, which originate from stromal cells, are also critical in the induction of Ab responses to conformational epitopes. Once antigen-specific

Table 1. Characteristics of each vaccine platform

Vaccine platform	Advantages	Limitations	Human-approved vaccines (except COVID-19)
Inactivated virus	Stable and no risk of reversion Strong antibody response Cost-effective	Biosafety issue Usually requires adjuvants Weak cellular immune response	Influenza (injection), polio (injection), hepatitis A
Live attenuated virus	Strong immune responses No adjuvant required Cost-effective	Biosafety issue Risk of reversion to virulence Time-consuming development	Influenza (nasal), polio (oral), measles
Recombinant protein subunit	No risk of infection and reversion Fewer side effects Easy antigen modification	Low immunogenicity Requires adjuvants High cost	Hepatitis B, influenza (injection)
VLPs	No risk of infection and reversion Fewer side effects Good antibody response	Complicated manufacturing process Requires adjuvants High cost	Cervical cancer by human papillomavirus
DNA	Rapid development and production Stable in room temperature High producibility	Low immunogenicity Requires a delivery device (electroporator or jet-injector)	-
mRNA	Cell-free Rapid development and production Good immunogenicity	Unstable High cost Requires low temperature storage	-
Viral-vectored	Strong immune responses Various viral vectors Large-scalable	Pre-existing immunity against the vector	Ebola

VLP, virus-like particle.

https://doi.org/10.4110/in.2021.21.e4

Table 2. COVID-19 vaccines in phase 3 clinical trials and beyond (as of December 2020)

Platform	Developer (product name)	Target antigen	Comments
mRNA	Moderna (mRNA-1273)	S protein with 2P (K986P and V987P)	LNP-encapsulated
	Pfizer/BioNTech (BNT-162b2)	S protein with 2P (K986P and V987P)	LNP-encapsulated
	CureVac AG (CVnCoV)	S protein	LNP-encapsulated
Viral-vectored	CanSino Biological Inc vaccine (Ad5-nCoV)	S protein	Human Ad5
	Oxford/AstraZenaca (AZD-1222)	S protein	Chimpanzee adenovirus
	Gamaleya Research Institutes (Gam-COVID-Vac)	S protein	rAd5 and rAd26 prime-boost
	Janssen Pharmaceutical Companies (Ad26.COV2.5)	S protein with 2P (K986P and V987P) and 2 mutations at furin cleavage site (R682S and R685G)	Ad26
Inactivated virus	Wuhan Institute of Biological Products/Sinopharm (NA)	Whole pathogen	Alum adjuvant
	Beijing Institute of Biological Products/Sinopharm (BBIBP-CorV)	Whole pathogen	Alum adjuvant
	Sinovac Life Sciences (CoronaVac)	Whole pathogen	Alum adjuvant
Recombinant protein subunit	Novavax (NVX-CoV2373)	S protein with 2P (K986P and V987P) and 3 mutations at furin cleavage site (R682Q, R683Q and R685Q)	Protein nanoparticle, matrix-M™ adjuvant
	Anhui Zhifei Longcom Biopharmaceutical (NA)	RBD	RBD-dimer, alum adjuvant
DNA	Inovio (INO-4800)	S protein	Electroporation, intradermal injection
	Osaka University/AnGes/Takara Bio (AG0301-COVID19)	S protein	Alum adjuvant, intramuscular injection

LNP, lipid nanoparticle; NA, not available.

T and B cells experience the cognate vaccine antigen(s), they are activated and differentiated into the specialized subset for the optimal function. The general cellular mechanisms of immune induction following vaccination are shown in **Fig. 4**.

mRNA vaccines

mRNA vaccines have induced optimal protective immunity against infectious pathogens in various animal models (33-35). Before COVID-19 outbreak, mRNA vaccines targeting infectious viruses, including influenza virus, Zika virus and HIV, have been investigated in clinical stages (36,37). Moderna performed a phase 3 clinical trial (NCT04470427) of COVID-19 vaccine using the mRNA encoding full-length S with 2P substitutions (mRNA-1273) and showed 94.1% vaccine efficacy compared to the placebo group (38). In the preclinical stage, mRNA-1273 induced neutralizing antibodies against pseudovirus expressing SARS-CoV-2 S protein in BALB/c, C57BL/6, B6C3 mouse models and rhesus macaques (6,39). After vaccination with mRNA-1273, participants in phase 1 clinical trial showed neutralizing Ab titers similar to that of convalescent serum (40). Currently, the emergency use of mRNA-1273 has been authorized in several countries, including the US and Canada. Pfizer/BioNTech is investigating the mRNA vaccine BNT162 in clinical trials. BNT162 is divided into 4 mRNA types: 1) BNT162a1, unmodified mRNA encoding RBD; 2) BNT162b1, nucleoside-modified mRNA (modRNA) encoding trimeric RBD; 3) BNT162b2, modRNA encoding full-length S with 2P or prefusion-stabilized S; 4) BNT162c2, self-amplifying mRNA encoding full-length S. Among them, BNT162b1 and BNT162b2 have entered into phase 2/3 clinical trials (NCT04368728), based on safety and immunogenicity tests in preclinical and clinical phase 1 studies (41). Preclinical results showed that BNT162b2 effectively induced neutralizing antibodies against SARS-CoV-2 pseudovirus and authentic SARS-CoV-2 in BALB/c mice and rhesus macaques, respectively. T cell responses were also induced in both animal models. More importantly, the induction of immune responses by BNT162b2 resulted in potent protection against SARS-CoV-2 in rhesus macaques (42). In clinical results, although both BNT162b1 and BNT162b2 induced similar levels of neutralizing antibodies, BNT162b2 showed lower incidence and severity of adverse events, especially in the elderly (41). Neutralizing Ab levels induced by BNT162b2 in the participants were similar to the

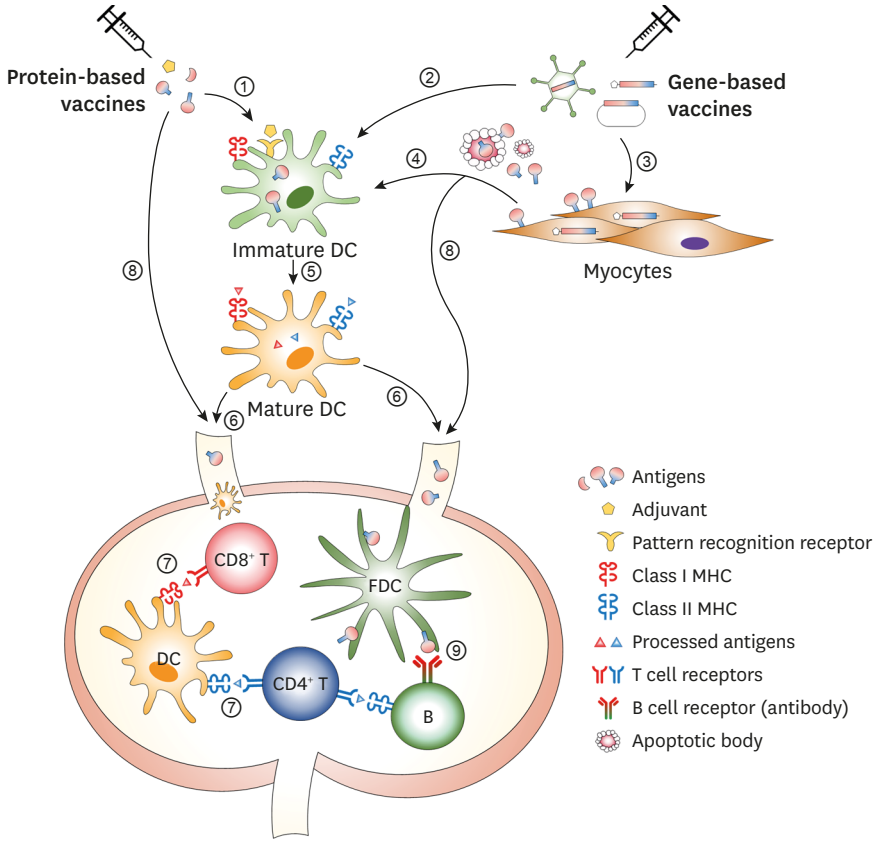

Figure 4. Cellular mechanisms of the induction of vaccine-specific immune responses. DCs can uptake protein vaccine antigen(s) (①) or be transfected with gene-based vaccines to express the vaccine antigen inside the cells (②). Gene-based vaccines can be also transfected to or infected into myocytes (③). The expressed antigens in the myocytes are either secreted or released from the cells and taken up by DCs (④). DCs then process the antigen into the antigenic peptides and present them on the MHC I or II molecules (⑤). Then, DCs migrate into the draining LNs (⑥) where the mature DCs prime antigen-specific $CD4^+$ or $CD8^+$ T cells (⑦). Vaccine antigens also can be directly drained into LNs through the lymphatic vessels (⑧). In the draining LNs, FDCs trap the soluble antigens and present them to antigen-specific B cells, leading to an antibody response to conformational epitopes (⑨).
FDC, follicular dendritic cell; LN, lymph node.

convalescent serum samples (41). The results of a phase 3 trial of BNT162b2 showed 94.8% efficacy compared to that of the placebo group (43). Based on its outstanding safety and efficacy, BNT162b2 has been authorized in the EU and US and recently been pre-qualified by World Health Organization (WHO). CureVac AG, another company developing mRNA vaccine, is currently investigating a vaccine encoding full-length S protein called CVnCoV in a phase 2/3 trial (NCT04652102) after completing a phase 1 study (NCT04449276), where potent SARS-CoV-2-binding antibodies and neutralizing Ab responses were observed in immunized participants. Based on these results, the immunization dose was determined for a phase 3 trials, which is currently being conducted (44).

Adenoviral-vectored vaccines

The safety and efficacy of an adenovirus (Ad), a non-replicating viral vector, has been already investigated in phase 3 trials and beyond. CanSino Biological Inc. developed the human Ad5-vectored vaccine expressing full-length S. No severe adverse effects were observed and neutralizing antibodies were effectively induced in the vaccinated group. However, as the titer of pre-existing neutralizing Ab against Ad5 was higher, seroconversion and T cell immune

responses showed decreasing tendency (45). Despite these phenomena, the vaccine induced a higher titer of neutralizing antibodies than did the placebo control. CanSino Biological Inc. is further investigating a mucosal vaccine using the Ad5 vector expressing full-length S in a phase 1 clinical trial (NCT04552366). The University of Oxford/AstraZenaca has developed a chimpanzee Ad-vectored vaccine expressing full-length S (AZD1222, formerly named ChAdOx1 nCoV-19) that can bypass pre-existing vector-specific immunity. In preclinical stages, AZD1222 induced neutralizing antibodies and T cell responses in BALB/c mice and rhesus macaques and protected rhesus macaques from SARS-CoV-2 infection (9). In phase 1/2 trials, the participants immunized with ADZ1222 exhibited robust T cell responses as well as neutralizing antibodies, similar to convalescent plasma (46). Although the neutralizing Ab titer was increased by booster immunization, there were no changes in the T cell responses, likely due to the immune responses toward homologous viral vector vaccine. In a phase 3 trial, participants administered with half dose at the first vaccination showed 90% vaccine efficacy, while those receiving the full dose at the first vaccination showed 62.1% vaccine efficacy (70.4% efficacy on average) (NCT04400838, ISRCTN89951424) (47). AZD1222 has been approved in several countries including the UK, India, Argentina, El Salvador, and South Korea. Gamaleya Research Institutes tested the safety of rAd5 or rAd26 expressing full-length S in a phase 1 trial and investigated their immunogenicity after prime-boost vaccination in a phase 2 study (NCT04436471, NCT04437875). Neutralizing antibodies and T cell responses were detected in all participants (11). Participants receiving the heterologous vaccination elicited a similar titer of neutralizing antibodies compared to convalescent individuals. Gamaleya Research Institutes is currently conducting a phase 3 clinical trial with rAd5/rAd26 prime-boost immunization (NCT04530396). Interim results are showing 91.4% efficacy in the vaccinated group compared to placebo group. Janssen Pharmaceutical Companies completed preclinical and phase 1/2 clinical tests using the rAd26 vectored vaccine expressing full-length S with 2P and mutations in furin cleavage site (NCT04436276) and entered a phase 3 study (NCT04505722). In a preclinical study comparing the immunogenicity of various S mutants in mice, rAd26.S.PP with a furin cleavage site mutation and 2P in the S2 hinge region was selected and named rAd26.CoV.S (48). A single immunization with rAd26.CoV.S was sufficient to inhibit viral replication in the lungs and nasal region of non-human primates (NHPs), and this was well-correlated with the increased neutralizing Ab titer (10). In line with a preclinical study, the results from a phase 1/2a trial showed that a single immunization induced high levels of neutralizing antibodies similar to those in patients recovered from SARS-CoV-2 infection (49). After vaccination with rAd26.CoV.S, the Th1/Th2 ratio in participants was 28.9, indicating Th1-skewed responses, and potent $CD8^+$ T cell responses were also induced.

Inactivated virus vaccines

The Wuhan Institute of Biological Products/Sinopharm tested the COVID-19 vaccine using inactivated SARS-CoV-2. In a phase 1/2 trial, participants immunized with inactivated SARS-CoV-2 with alum adjuvant showed higher titers of neutralizing antibodies and increased T cell responses compared to those of the placebo group. Moreover, the SARS-CoV-2-specific Ab titer was increased with the number of vaccinations (ChiCTR2000031809) (50). The Beijing Institute of Biological Products/Sinopharm has also investigated inactivated SARS-CoV-2 (BBIBP-CorV) with alum adjuvant. In a phase 1/2 clinical trial (ChiCTR2000032459), BBIBP-CorV generated high titers of antibodies in immunized participants (51). BBIBP-CorV achieved 79.3% efficacy in preventing SARS-CoV-2 infection, and participants immunized with this vaccine showed 99.5% seroconversion after 2 doses as shown by interim clinical results. BBIBP-CorV received conditional approval in China for emergency use. Also, Sinovac

Life Sciences has investigated CoronaVac, an inactivated SARS-CoV-2. In preclinical studies, CoronaVac effectively induced neutralizing antibodies and T cell responses in BALB/c mice and rhesus macaques (52). Subsequently, they tested the safety and immunogenicity of CoronaVac in multiple phase 1/2 clinical trials (NCT04383574, NCT04352608, NCT04551547). Participants immunized with CoronaVac with alum adjuvant showed seroconversion without serious adverse events (53). They are currently conducting phase 3 clinical trials (NCT04456595, NCT04508075, NCT04582344, NCT04617483, NCT04617483). CoronaVac has been approved in China for emergency use in high-risk groups. It has been reported that vaccination with formalin-inactivated SARS-CoV increases, rather than decreases, lesions induced by SARS-CoV challenge (52). This result raised concerns that inactivated virus-based COVID-19 vaccines could cause Ab-dependent enhancement (ADE) or vaccine-associated enhanced respiratory disease (VAERD), but such phenomenon has not been reported to date.

Recombinant protein subunit vaccines

Novavax is investigating a protein nanoparticle vaccine consisting of prefusion-stabilized full-length S in combination with Matrix-M™ (NVX-CoV2373). Preclinical results using cynomolgus macaques showed that NVX-CoV2373 effectively induced S-specific neutralizing Ab responses (12). This vaccine also effectively induced neutralizing antibodies that exceeded the levels of convalescent individuals and predominantly induced Th1 responses with mild or no side effects in phase 1/2 trials (NCT04368988, NCT04533399) (54). Multiple phase 3 studies are being conducted in the US, Mexico, and Peru (NCT04611802, NCT04583995, EUCTR2020-004123-16). Anhui Zhifei Longcom Biopharmaceutical designed a disulfide bonded RBD-dimer vaccine purified from mammalian cells. In preclinical studies, immunization with RBD-dimer with alum adjuvant effectively induced Ab responses with neutralizing activity against pseudovirus or live SARS-CoV-2, but did not induce T cell responses, in a BALB/c mouse model (23). Although they have completed phase 1/2 trials, the results have not been reported yet (NCT04445194, NCT04466085, ChiCTR2000035691, NCT04550351). Currently, they are recruiting volunteers for a phase 3 trial (ChiCTR2000040153).

An adjuvant is one of the most critical factors affecting the efficacy of protein-based vaccines. In most prophylactic vaccines, neutralizing antibodies has been considered critical, but the importance of the cellular immune response is also increasingly being emphasized. Recently, many novel adjuvants have been developed that can simultaneously enhance humoral and cellular immune responses. Therefore, in addition to alum, various adjuvants such as MF-59, Matrix-M™, AS03, and GpG1018, are also combined with the COVID-19 vaccine (1). Adjuvants mainly stimulate pattern recognition receptors directly or indirectly to provide an "infection-like signal" in the host. Thus, innate immune responses induced by adjuvants significantly affect the quality, intensity and persistence of antigen-specific immune responses. When two cervical cancer vaccines with similar antigenic preparation and different adjuvants were tested, a significant difference was observed between the those two vaccines in the long-term immune response (55). Considering the possibility of COVID-19 recurrence after the current global pandemic, an adjuvant inducing long-term immunity could be a critical determinant for the efficacy of the protein-based vaccines.

DNA vaccines

DNA vaccines have been applied to various diseases, such as cancer, autoimmune diseases, allergies and infectious diseases. Several DNA vaccine candidates against influenza, hepatitis B virus and HIV-1 have been tested in clinical trials. In the context of SARS-CoV-2, 2 DNA

vaccine candidates are being tested in phase 3 trials. Inovio tested the immunogenicity of a DNA vaccine construct encoding a full-length S (INO-4800) in mice and guinea pigs in preclinical studies (8). Intramuscular injection of INO-4800 effectively induced SARS-CoV-2-specific Ab and T cell responses. Sera from BALB/c and C57BL/6 mice immunized with INO-4800 effectively neutralized the pseudovirus expressing SARS-CoV-2 S protein and live SARS-CoV-2. Intradermal immunization of INO-4800 induced robust SARS-CoV-2 S-specific Ab and T cell responses in NHPs and the immunized sera effectively neutralized both wild-type and D614G variant SARS-CoV-2 (56). INO-4800 also elicited neutralizing antibodies and T cells that are comparable with those of convalescent samples in phase 1/2 trials (NCT04336410) (57). Inovio is currently conducting a phase 3 trial (NCT04642638). Osaka University/AnGes/Takara Bio developed a DNA vaccine expressing a full-length S (AG0301-COVID19) and tested its safety and efficacy in a phase 1/2 trial (NCT04463472), but the results have not been reported. They have recently initiated a phase 3 clinical study (NCT04655625). In a preclinical study, AG0301-COVID19 with an alum adjuvant effectively induced neutralizing antibodies and T cell responses in the rats, with the complete absence of toxic reactions to various organs (58). To date, no DNA vaccine has completed phase 3 trials or has been approved.

EFFICACY AND SAFETY OF COVID-19 VACCINES

Immune correlates of protection (ICP)

ICP or correlates of protective immunity is a specific immune marker or response that is associated with protection against infection (59). As neutralizing antibodies are the most critical ICP in many infectious diseases such as influenza and hepatitis A and B, the primary objective of most prophylactic vaccines is inducing potent neutralizing Ab responses (60-62). In the case of COVID-19, neutralizing antibodies have been considered as the primary ICP as well. Passive immunization of convalescent sera or antibodies purified from convalescent patients and S-specific monoclonal antibodies effectively inhibited SARS-CoV-2 infection or alleviated disease symptoms in preclinical or clinical studies (63-65). It has also been shown that vaccine candidates whose efficacy has been validated in phase 3 trials exhibited higher neutralizing Ab titers than convalescent patient sera (49). Recently, beyond neutralizing potential, polyfunctionality of the antibodies is considered as a substantial factor affecting protective immunity. It has been reported that polyfunctional antibodies are closely associated with disease outcomes in patients infected with human immunodeficiency, influenza and Ebola viruses (66-68). Several COVID-19 vaccine studies have also demonstrated Ab functions such as Ab-dependent cellular phagocytosis, Ab-dependent neutrophil phagocytosis, Ab-dependent NK cell degranulation and Ab-dependent complement deposition (69,70). These Ab features are differently presented in convalescent and deceased individuals infected with SARS-CoV-2 (71). However, little is known about the direct correlation of polyfunctional antibodies with protection against infectious agents including SARS-CoV-2, raising the need for further investigation.

The importance of the cellular immune response in protective immunity has also been discussed. Virus-specific T cells are crucial for the clearance of SARS-CoV or MERS-CoV (72-74). In a recent study, $CD4^+$ and $CD8^+$ T cells played an important role in protection against SARS-CoV-2 infection in a murine model (27). Moreover, strong virus-specific T cell responses were observed in asymptomatic or mild COVID-19 patients, suggesting the potential of cellular immune responses in the protection or clearance of SARS-CoV-2 (75,76).

Therefore, a vaccine that can simultaneously induce neutralizing antibodies and cellular immune responses is thought to be ideal. Currently, most COVID-19 vaccine candidates are validating both parameters.

Recently, a WHO international standard serum for SARS-CoV-2 has been developed by the National Institute for Biological Standards and Control, UK (77). However, in the aspect of cellular immunity, it is relatively difficult to prepare a standard sample or quantify the immune response compared to the Ab response. The role of antigen-specific T cells in the protection from SARS-CoV-2 is also controversial (78). Further studies are required to develop a standard assay or establish surrogate markers to quantify the cellular immune response and understand the correlation between cellular immunity and protective immunity.

VAERD and ADE of disease

In some cases, virus-specific immune responses generated by prior infection or vaccination can increase viral pathogenicity when subsequent infection occurs. There are 2 different mechanisms; VAERD and ADE of the disease. VAERD is allergic inflammation in the respiratory tract caused by excessive Th2-biased immune responses induced by prior vaccination. In a clinical trial of the RSV vaccine in the 1960s, a significant portion (16 out of 20) of children administered with a formalin-inactivated RSV vaccine developed severe symptoms following natural infection with RSV, whereas only one out of 21 children in the placebo group was hospitalized (79). Subsequently, it was revealed that the inactivated RSV vaccine induced strong Th2-biased immune responses and caused hyper-production of IL-4, IL-5, and IL-13, and excessive lung inflammation by eosinophils (80). ADE is an event in which a suboptimal concentration of neutralizing antibodies or cross-reactive non-neutralizing antibodies increases viral infection through interaction with Fc receptors (81,82). ADE is well known in flaviviruses such as dengue virus and Zika virus (83,84), but inactivated SARS-CoV or recombinant viral vectored-SARS vaccines also increased liver or respiratory lesions during subsequent SARS-CoV infection (85,86). ADE was also observed in cats immunized with a feline infectious peritonitis virus (FIPV) vaccine or passively administered with FIPV-specific antibodies (87,88). The possibility of ADE may generate serious concerns in the development of the COVID-19 vaccine. However, no severe side effects have been reported in any of the following cases: passive transfer of convalescent plasma to COVID-19 patients (89), infection of vaccinated animals with SARS-CoV-2, and large-scale phase 3 trials. It is required to investigate the potential adverse effects of COVID-19 vaccines depending on the antigen design and vaccine formulation.

Pre-existing memory response cross-reactive to SARS-CoV-2

Recently, T cell and Ab responses reactive to SARS-CoV-2 have been observed in people who have not been exposed to SARS-CoV-2 (collected before the COVID-19 outbreak or seronegative for SARS-CoV-2). This cross-reactivity was presumed to be induced by infection with seasonal HCoVs. In many cases, the cross-reactive responses recognize epitopes in the S2 domain (90,91). Meanwhile, pre-existing cross-reactive immunity may affect the immune response following vaccination as well as viral infection. Influenza virus-specific pre-existing memory $CD4^+$ T cells increased the influenza vaccine-induced Ab response in clinical trials (92). Pre-existing memory B cells also affect the outcome of a quadrivalent influenza vaccine (QIV); when pre-existing B cell memory exhibits a dominant response to a particular subtype (subtype immunodominance), Ab response to QIV was positively correlated with the preexisting memory (93). Also, it was recently reported that the kinetics and magnitude of Ab response to a hepatitis B vaccine were significantly increased in the presence of hepatitis

B vaccine-specific memory $CD4^+$ T cells (94). Ab response to HCoV can be observed in most adults (95) and the S2 domain of SARS-CoV-2 S exhibits relatively high amino acid sequence homology with those of seasonal HCoVs (up to 42%) (90). In particular, epitopes in the FP are highly conserved among various coronaviruses (96). Therefore, seasonal HCoV-induced pre-existing immunological memory that is cross-reactive to SARS-CoV-2 may affect the immune responses induced by a COVID-19 vaccine, especially those containing the S2 domain. Considering that most COVID-19 vaccines in later stages of development have the S2 domain, it is necessary to study the influence of cross-reactive pre-existing immunity on the efficacy of the vaccine and the diversity of immune responses.

CONCLUDING REMARKS

Paradoxically, COVID-19, a serious threat to human health and the economy, is accelerating the advancement of the vaccine field. Technologies that have been utilized in preclinical studies and clinical trials are being integrated into the development of COVID-19 vaccines. mRNA and viral vectored vaccines have been approved and are now being used in several countries. Some of these vaccines harbor a prefusion-stabilized antigen that has never been observed in conventional licensed vaccines. Various types of vehicles such as Ad5, Ad26, chimpanzee Ad, and other replication-competent viruses are being widely studied for viral vectored vaccines. Recombinant protein vaccines have also made significant advancements combined with self-assembling nanoparticle technology, which is also considered promising. Safety is the most crucial factor to be considered in vaccine development. Considering that a substantial portion of the population needs to be COVID-19-vaccinated, it needs to be further investigated the long-term study on the potential adverse effects, such as VAERD and ADE, and the immunological correlation with seasonal HCoVs.

ACKNOWLEDGEMENTS

This work was supported by grants of the Bio & Medical Technology Development Program (2016M3A9B6918675 and 2018M3A9H4077992) of the National Research Foundation of Korea (NRF) and the KRIBB Initiative program (KGM9942112), funded by the Korean government (Ministry of Science & ICT). We would like to thank Dr. SW Kim and Editage (www.editage.co.kr) for language editing.

REFERENCES

1. World Health Organization. Draft landscape of COVID-19 candidate vaccines [Internet]. Available at https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines [accessed on 22 December 2020].
2. Lee P, Kim DJ. Newly emerging human coronaviruses: animal models and vaccine research for SARS, MERS, and COVID-19. *Immune Netw* 2020;20:e28.
PUBMED | CROSSREF
3. Gu H, Chen Q, Yang G, He L, Fan H, Deng YQ, Wang Y, Teng Y, Zhao Z, Cui Y, et al. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* 2020;369:1603-1607.
PUBMED | CROSSREF
4. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367:1260-1263.
PUBMED | CROSSREF

5. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004;203:631-637.
PUBMED | CROSSREF

6. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, Himansu S, Schäfer A, Ziwawo CT, DiPiazza AT, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* 2020;586:567-571.
PUBMED | CROSSREF

7. Rauch S, Roth N, Schwendt K, Fotin-Mleczek M, Mueller SO, Petsch B. mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents [Internet]. Available at https://doi.org/10.1101/2020.10.23.351775 [accessed on 10 January 2021].

8. Smith TR, Patel A, Ramos S, Elwood D, Zhu X, Yan J, Gary EN, Walker SN, Schultheis K, Purwar M, et al. Immunogenicity of a DNA vaccine candidate for COVID-19. *Nat Commun* 2020;11:2601.
PUBMED | CROSSREF

9. van Doremalen N, Lambe T, Spencer A, Belij-Rammerstorfer S, Purushotham JN, Port JR, Avanzato VA, Bushmaker T, Flaxman A, Ulaszewska M, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature* 2020;586:578-582.
PUBMED | CROSSREF

10. Mercado NB, Zahn R, Wegmann F, Loos C, Chandrashekar A, Yu J, Liu J, Peter L, McMahan K, Tostanoski LH, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* 2020;586:583-588.
PUBMED | CROSSREF

11. Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatulin AI, Shcheblyakov DV, Dzharullaeva AS, Grousova DM, Erokhova AS, Kovyrshina AV, Botikov AG, 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* 2020;396:887-897.
PUBMED | CROSSREF

12. Guebre-Xabier M, Patel N, Tian JH, Zhou B, Maciejewski S, Lam K, Portnoff AD, Massare MJ, Frieman MB, Piedra PA, et al. NVX-CoV2373 vaccine protects cynomolgus macaque upper and lower airways against SARS-CoV-2 challenge. *Vaccine* 2020;38:7892-7896.
PUBMED | CROSSREF

13. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J Virol* 2003;77:8801-8811.
PUBMED | CROSSREF

14. Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* 2020;117:11727-11734.
PUBMED | CROSSREF

15. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181:271-280.e8.
PUBMED | CROSSREF

16. Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh RM Jr, Rawson S, Rits-Volloch S, Chen B. Distinct conformational states of SARS-CoV-2 spike protein. *Science* 2020;369:1586-1592.
PUBMED | CROSSREF

17. Sanders RW, Vesanen M, Schuelke N, Master A, Schiffner L, Kalyanaraman R, Paluch M, Berkhout B, Maddon PJ, Olson WC, et al. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J Virol* 2002;76:8875-8889.
PUBMED | CROSSREF

18. Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, van Meersbergen R, Huizingh J, Wanningen P, Verspuij J, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science* 2015;349:1301-1306.
PUBMED | CROSSREF

19. Kong L, He L, de Val N, Vora N, Morris CD, Azadnia P, Sok D, Zhou B, Burton DR, Ward AB, et al. Uncleaved prefusion-optimized gp140 trimers derived from analysis of HIV-1 envelope metastability. *Nat Commun* 2016;7:12040.
PUBMED | CROSSREF

20. Pallesen J, Wang N, Corbett KS, Wrapp D, Kirchdoerfer RN, Turner HL, Cottrell CA, Becker MM, Wang L, Shi W, et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc Natl Acad Sci U S A* 2017;114:E7348-E7357.
PUBMED | CROSSREF

21. Shi R, Shan C, Duan X, Chen Z, Liu P, Song J, Song T, Bi X, Han C, Wu L, et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* 2020;584:120-124.
PUBMED | CROSSREF

22. Grant OC, Montgomery D, Ito K, Woods RJ. Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition. *Sci Rep* 2020;10:14991.
PUBMED | CROSSREF

23. Dai L, Zheng T, Xu K, Han Y, Xu L, Huang E, An Y, Cheng Y, Li S, Liu M, et al. A universal design of *Betacoronavirus* vaccines against COVID-19, MERS, and SARS. *Cell* 2020;182:722-733.e11.
CROSSREF

24. Walls AC, Fiala B, Schäfer A, Wrenn S, Pham MN, Murphy M, Tse LV, Shehata L, O'Connor MA, Chen C, et al. Elicitation of potent neutralizing antibody responses by designed protein nanoparticle vaccines for SARS-CoV-2. *Cell* 2020;183:1367-1382.e17.
PUBMED | CROSSREF

25. Bennett NR, Zwick DB, Courtney AH, Kiessling LL. Multivalent antigens for promoting b and t cell activation. *ACS Chem Biol* 2015;10:1817-1824.
PUBMED | CROSSREF

26. Irvine DJ, Swartz MA, Szeto GL. Engineering synthetic vaccines using cues from natural immunity. *Nat Mater* 2013;12:978-990.
PUBMED | CROSSREF

27. Sun J, Zhuang Z, Zheng J, Li K, Wong RL, Liu D, Huang J, He J, Zhu A, Zhao J, et al. Generation of a broadly useful model for COVID-19 pathogenesis, vaccination, and treatment. *Cell* 2020;182:734-743.e5.
PUBMED | CROSSREF

28. Sariol A, Perlman S. Lessons for COVID-19 immunity from other coronavirus infections. *Immunity* 2020;53:248-263.
PUBMED | CROSSREF

29. Zuwała K, Golda A, Kabala W, Burmistrz M, Zdzalik M, Nowak P, Kedracka-Krok S, Zarebski M, Dobrucki J, Florek D, et al. The nucleocapsid protein of human coronavirus NL63. *PLoS One* 2015;10:e0117833.
PUBMED | CROSSREF

30. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. *Cell Host Microbe* 2020;27:671-680.e2.
PUBMED | CROSSREF

31. Zhao J, Zhao J, Mangalam AK, Channappanavar R, Fett C, Meyerholz DK, Agnihothram S, Baric RS, David CS, Perlman S. Airway memory CD4$^+$ T cells mediate protective immunity against emerging respiratory coronaviruses. *Immunity* 2016;44:1379-1391.
PUBMED | CROSSREF

32. Yasui F, Kai C, Kitabatake M, Inoue S, Yoneda M, Yokochi S, Kase R, Sekiguchi S, Morita K, Hishima T, et al. Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV. *J Immunol* 2008;181:6337-6348.
PUBMED | CROSSREF

33. Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, Dowd KA, Sutherland LL, Scearce RM, Parks R, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* 2017;543:248-251.
PUBMED | CROSSREF

34. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, Laska ME, Smith M, Almarsson Ö, Thompson J, et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. *Mol Ther* 2017;25:1316-1327.
PUBMED | CROSSREF

35. Schnee M, Vogel AB, Voss D, Petsch B, Baumhof P, Kramps T, Stitz L. An mRNA vaccine encoding rabies virus glycoprotein induces protection against lethal infection in mice and correlates of protection in adult and newborn pigs. *PLoS Negl Trop Dis* 2016;10:e0004746.
PUBMED | CROSSREF

36. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* 2018;17:261-279.
PUBMED | CROSSREF

37. Jackson NA, Kester KE, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. *NPJ Vaccines* 2020;5:11.
CROSSREF

38. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021;384:403-416.
PUBMED | CROSSREF

39. Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu-Barnum S, Werner AP, Flach B, O'Connell S, Bock KW, Minai M, et al. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. *N Engl J Med* 2020;383:1544-1555.
PUBMED | CROSSREF

40. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, McCullough MP, Chappell JD, Denison MR, Stevens LJ, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. *N Engl J Med* 2020;383:1920-1931.
PUBMED | CROSSREF

41. Walsh EE, Frenck RW JrFalsey AR, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, Bailey R, et al. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. *N Engl J Med* 2020;383:2439-2450.
PUBMED | CROSSREF

42. Vogel AB, Kanevsky I, Che Y, Swanson KA, Muik A, Vormehr M, Kranz LM, Walzer KC, Hein S, Güler A, et al. A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and prevents lung infection in non-human primates [Internet]. Available at https://doi.org/10.1101/2020.09.08.280818 [accessed on 10 January 2021].

43. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med* 2020;383:2603-2615.
PUBMED | CROSSREF

44. Kremsner P, Mann P, Bosch J, Fendel R, Gabor JJ, Kreidenweiss A, Kroidl A, Leroux-Roels I, Leroux-Roels G, Schindler C, et al. Phase 1 assessment of the safety and immunogenicity of an mRNA-lipid nanoparticle vaccine candidate against SARS-CoV-2 in human volunteers [Internet]. Available at https://doi.org/10.1101/2020.11.09.20228551 [accessed on 10 January 2021].

45. Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX, Wu SP, Wang BS, Wang Z, Wang L, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet* 2020;395:1845-1854.
PUBMED | CROSSREF

46. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, Bellamy D, Bibi S, Bittaye M, Clutterbuck EA, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020;396:467-478.
PUBMED | CROSSREF

47. Voysey M, Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, Angus B, Baillie VL, Barnabas SL, Bhorat QE, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021;397:99-111.
PUBMED | CROSSREF

48. Bos R, Rutten L, van der Lubbe JE, Bakkers MJ, Hardenberg G, Wegmann F, Zuijdgeest D, de Wilde AH, Koornneef A, Verwilligen A, et al. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 spike immunogen induces potent humoral and cellular immune responses. *NPJ Vaccines* 2020;5:91.
PUBMED | CROSSREF

49. Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, Stoop J, Tete S, Van Damme W, Leroux-Roels I, et al. Safety and immunogenicity of the Ad26.COV2.S COVID-19 vaccine candidate: interim results of a phase 1/2a, double-blind, randomized, placebo-controlled trial [Internet]. Available at https://doi.org/10.1101/2020.09.23.20199604 [accessed on 10 January 2021].

50. Xia S, Duan K, Zhang Y, Zhao D, Zhang H, Xie Z, Li X, Peng C, Zhang Y, Zhang W, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. *JAMA* 2020;324:951-960.
PUBMED | CROSSREF

51. Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, Tan W, Wu G, Xu M, Lou Z, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis* 2021;21:39-51.
PUBMED | CROSSREF

52. Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, Li Y, Zhu L, Wang N, Lv Z, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 2020;369:77-81.
PUBMED | CROSSREF

53. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, Han W, Chen Z, Tang R, Yin W, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2020;21:181-92.
PUBMED | CROSSREF

54. Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, Plested JS, Zhu M, Cloney-Clark S, Zhou H, et al. Phase 1-2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N Engl J Med* 2020;383:2320-2332.
PUBMED | CROSSREF

55. Nicoli F, Mantelli B, Gallerani E, Telatin V, Bonazzi I, Marconi P, Gavioli R, Gabrielli L, Lazzarotto T, Barzon L, et al. HPV-specific systemic antibody responses and memory b cells are independently maintained up to 6 years and in a vaccine-specific manner following immunization with cervarix and gardasil in adolescent and young adult women in vaccination programs in Italy. *Vaccines (Basel)* 2020;8:26.
PUBMED | CROSSREF

56. Patel A, Walters J, Reuschel EL, Schultheis K, Parzych E, Gary EN, Maricic I, Purwar M, Eblimit Z, Walker SN, et al. Intradermal-delivered DNA vaccine provides anamnestic protection in a rhesus macaque SARS-CoV-2 challenge model [Internet]. Available at https://doi.org/10.1101/2020.07.28.225649 [accessed on 10 January 2021].

57. Tebas P, Yang S, Boyer JD, Reuschel EL, Patel A, Christensen-Quick A, Andrade VM, Morrow MP, Kraynyak K, Agnes J, et al. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: a preliminary report of an open-label, phase 1 clinical trial. *EClinicalMedicine* 2020;31:100689.
CROSSREF

58. Hayashi H, Sun J, Yanagida Y, Otera T, Kubota-Kotetsu R, Shioda T, Ono C, Matsuura Y, Arase H, Yoshida S, et al. Preclinical study of DNA vaccines targeting SARS-CoV-2 [Internet]. Available at https://doi.org/10.1101/2020.10.21.347799 [accessed on 10 January 2021].

59. Plotkin SA. Vaccines: correlates of vaccine-induced immunity. *Clin Infect Dis* 2008;47:401-409.
PUBMED | CROSSREF

60. Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, Chan P, Wong KC, Leung CB, Cheng G. Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis* 2005;24:44-46.
PUBMED | CROSSREF

61. Yeh KM, Chiueh TS, Siu LK, Lin JC, Chan PK, Peng MY, Wan HL, Chen JH, Hu BS, Perng CL, et al. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital. *J Antimicrob Chemother* 2005;56:919-922.
PUBMED | CROSSREF

62. Wang W, Vassell R, Song HS, Chen Q, Keller PW, Verma S, Alvarado-Facundo E, Wan H, Schmeisser F, Meseda CA, et al. Generation of a protective murine monoclonal antibody against the stem of influenza hemagglutinins from group 1 viruses and identification of resistance mutations against it. *PLoS One* 2019;14:e0222436.
PUBMED | CROSSREF

63. Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, van Buskirk C, Grossman BJ, Joyner M, Henderson JP, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest* 2020;130:2757-2765.
PUBMED | CROSSREF

64. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, Wang F, Li D, Yang M, Xing L, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA* 2020;323:1582-1589.
PUBMED | CROSSREF

65. Liu X, Cao W, Li T. High-dose intravenous immunoglobulins in the treatment of severe acute viral pneumonia: the known mechanisms and clinical effects. *Front Immunol* 2020;11:1660.
PUBMED | CROSSREF

66. Tomaras GD, Haynes BF. HIV-1-specific antibody responses during acute and chronic HIV-1 infection. *Curr Opin HIV AIDS* 2009;4:373-379.
PUBMED | CROSSREF

67. Cobey S, Hensley SE. Immune history and influenza virus susceptibility. *Curr Opin Virol* 2017;22:105-111.
PUBMED | CROSSREF

68. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. *Nat Immunol* 2018;19:1169-1178.
PUBMED | CROSSREF

69. Barrett JR, Belij-Rammerstorfer S, Dold C, Ewer KJ, Folegatti PM, Gilbride C, Halkerston R, Hill J, Jenkin D, Stockdale L, et al. Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. *Nat Med* 2021;27:279-288.
PUBMED | CROSSREF

70. Yu J, Tostanoski LH, Peter L, Mercado NB, McMahan K, Mahrokhian SH, Nkolola JP, Liu J, Li Z, Chandrashekar A, et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science* 2020;369:806-811.
PUBMED | CROSSREF

71. Atyeo C, Fischinger S, Zohar T, Slein MD, Burke J, Loos C, McCulloch DJ, Newman KL, Wolf C, Yu J, et al. Distinct early serological signatures track with SARS-CoV-2 survival. *Immunity* 2020;53:524-532.e4.
PUBMED | CROSSREF

72. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, Subbarao K. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. *J Virol* 2010;84:1289-1301.
PUBMED | CROSSREF

73. Zhao J, Zhao J, Perlman S. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. *J Virol* 2010;84:9318-9325.
PUBMED | CROSSREF

74. Zhao J, Li K, Wohlford-Lenane C, Agnihothram SS, Fett C, Zhao J, Gale MJ Jr, Baric RS, Enjuanes L, Gallagher T, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proc Natl Acad Sci U S A* 2014;111:4970-4975.
PUBMED | CROSSREF

75. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, Alanio C, Kuri-Cervantes L, Pampena MB, D'Andrea K, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science* 2020;369:eabc8511.
PUBMED | CROSSREF

76. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, Llewellyn-Lacey S, Kamal H, Bogdanovic G, Muschiol S, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* 2020;183:158-168.e14.
PUBMED | CROSSREF

77. World Health Organization. Establishment of the who international standard and reference panel for anti-SARS-CoV-2 antibody [Internet]. Available at https://www.who.int/publications/m/item/WHO-BS-2020.2403 [accessed on 11 January 2021].

78. Altmann DM, Boyton RJ. SARS-CoV-2 T cell immunity: specificity, function, durability, and role in protection. *Sci Immunol* 2020;5:eabd6160.
PUBMED | CROSSREF

79. Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, Parrott RH. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969;89:422-434.
PUBMED | CROSSREF

80. De Swart RL, Kuiken T, Timmerman HH, van Amerongen G, Van Den Hoogen BG, Vos HW, Neijens HJ, Andeweg AC, Osterhaus AD. Immunization of macaques with formalin-inactivated respiratory syncytial virus (RSV) induces interleukin-13-associated hypersensitivity to subsequent RSV infection. *J Virol* 2002;76:11561-11569.
PUBMED | CROSSREF

81. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 1977;146:201-217.
PUBMED | CROSSREF

82. Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S, Limpitikul W, Puttikhunt C, Edwards C, Duangchinda T, Supasa S, et al. Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 2010;328:745-748.
PUBMED | CROSSREF

83. Kliks SC, Nimmanitya S, Nisalak A, Burke DS. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am J Trop Med Hyg* 1988;38:411-419.
PUBMED | CROSSREF

84. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat Rev Immunol* 2011;11:532-543.
PUBMED | CROSSREF

85. Weingartl H, Czub M, Czub S, Neufeld J, Marszal P, Gren J, Smith G, Jones S, Proulx R, Deschambault Y, et al. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *J Virol* 2004;78:12672-12676.
PUBMED | CROSSREF

86. Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, Funkhouser W, Gralinski L, Totura A, Heise M, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides

incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. *J Virol* 2011;85:12201-12215.
PUBMED | CROSSREF

87. Takano T, Kawakami C, Yamada S, Satoh R, Hohdatsu T. Antibody-dependent enhancement occurs upon re-infection with the identical serotype virus in feline infectious peritonitis virus infection. *J Vet Med Sci* 2008;70:1315-1321.
PUBMED | CROSSREF

88. Weiss RC, Scott FW. Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. *Comp Immunol Microbiol Infect Dis* 1981;4:175-189.
PUBMED | CROSSREF

89. Joyner MJ, Bruno KA, Klassen SA, Kunze KL, Johnson PW, Lesser ER, Wiggins CC, Senefeld JW, Klompas AM, Hodge DO, et al. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. *Mayo Clin Proc* 2020;95:1888-1897.
PUBMED | CROSSREF

90. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, Hippenstiel S, Dingeldey M, Kruse B, Fauchere F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020;587:270-274.
PUBMED | CROSSREF

91. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, Rawlings SA, Sutherland A, Premkumar L, Jadi RS, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020;181:1489-1501.e15.
PUBMED | CROSSREF

92. Nienen M, Stervbo U, Mölder F, Kaliszczyk S, Kuchenbecker L, Gayova L, Schweiger B, Jürchott K, Hecht J, Neumann AU, et al. The role of pre-existing cross-reactive central memory CD4 T-cells in vaccination with previously unseen influenza strains. *Front Immunol* 2019;10:593.
PUBMED | CROSSREF

93. Abreu RB, Kirchenbaum GA, Clutter EF, Sautto GA, Ross TM. Preexisting subtype immunodominance shapes memory B cell recall response to influenza vaccination. *JCI Insight* 2020;5:e132155.
PUBMED | CROSSREF

94. Elias G, Meysman P, Bartholomeus E, Neuter ND, Keersmaekers N, Suls A, Jansens H, Souquette A, Reu HD, Smits E, et al. Preexisting memory CD4 T cells in naïve individuals confer robust immunity upon vaccination [Internet]. Available at https://doi.org/10.1101/2020.08.22.262568 [accessed on 10 January 2021].

95. Gorse GJ, Patel GB, Vitale JN, O'Connor TZ. Prevalence of antibodies to four human coronaviruses is lower in nasal secretions than in serum. *Clin Vaccine Immunol* 2010;17:1875-1880.
PUBMED | CROSSREF

96. Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, Ulferts R, Earl C, Wrobel AG, Benton DJ, et al. Preexisting and *de novo* humoral immunity to SARS-CoV-2 in humans. *Science* 2020;370:1339-1343.
PUBMED | CROSSREF