BIOCELL
2020 44(2): 127-135

Tech Science Press

Updated thoughts on SARS-CoV-2 and coronavirus therapies, fighting and surviving

Wenjuan LI*; Ge SONG

Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, USA

Key words: SARS-CoV2, Cellular mechanism, Therapies, Antibody, Vaccine

Abstract: From late December 2019 a new human-adapted coronavirus, SARS-CoV-2, was observed and isolated in clustered patients in Wuhan, China. It has been proved to be able to transmit human-to-human and cause pneumonia, leading to about 2% fatality. Its genome characteristics, immune responses and related potential treatments, such as chemical drugs, serum transfusion and vaccines including DNA vaccines, are discussed in this review for a brief summary.

Introduction

In December 2019, several clustered l cases of pneumonia (Chan *et al.*, 2020) were reported in Wuhan, Hubei province, China. Shortly later on January 7th, 2020, a novel virus was observed in the throat swab sample from one patient under electronic microscopy. Its characteristics appeared obviously corona-like, thus it was identified as a coronavirus. WHO declared this outbreak a global health emergency, and named it as SARS-CoV-2 subsequently in February. Since several coronaviruses have hit human society previously, among which the most severe ones were SARS-CoV-1 in 2003–2004 and MERS-CoV in 2012, their information and most recent researches have provided us the opportunity to know this new virus better. Here we take a brief view about SARS-CoV-2, its related symptoms, and therapies along with its other family members.

New virus and coronavirus family

SARS-CoV-2's genome later on was sequenced and determined to belong to betacoronaviruses, whose higher Coronavirus genus belongs to the *Coronaries* family. Members of this family hold one single positive-sense RNA of around 30 kb as their genetic material. SARS-CoV and MERS-CoV are two well-known betacoronaviruses with respectively 27.9 kb and 30.1 kb lengths for their genomes. At the time of writing of this review, the genome of SARS-CoV-2 has been sequenced and uploaded to virological.org, by the research group of Edward C. Holmes and Yongzheng Zhang, *et al.* Summarized and briefly analyzed in other works (Dong *et al.*, 2020; Paraskevis *et al.*, 2020; Xu *et al.*, 2020), SARS-CoV-2 has a 29,903 bp-long genome, containing 10 genes, with higher homology to SARS-CoV than to MERS-CoV. The amino acid sequence identity of their receptor binding proteins, the Spike protein, is around 76%. More analysis and interpretations by researchers are currently accelerating in order to understand this virus better and to develop better therapies.

Usually, the order *Nidovirales* RNA genome has a unique gene distribution pattern, with two-thirds of the genome from the 5' end encodeding two large polyproteins, and the rest transcribed into a set of subgenomic mRNAs (Pasternak *et al.*, 2006; Perlman and Netland, 2009) (Fig. 1). The polyproteins are pp1a and pp1ab, including 16 non-structural proteins (nsp1-nsp16) that form the viral replicase-transcriptase complex. Among these 16 proteins, there are two proteases including a papain-like protease (PLpro) and a main protease, 3C-like protease (3CLpro), corresponding to nsp3 and nsp5. For the rest of the genome, SARS-CoV and MERS-CoV respectively transcribe to 12 and 9 subgenomic RNAs encoding the four important structural proteins as spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as several accessory proteins that interfere with the host immune response and maybe harbor other unclear functions.

The single-stranded RNA (ssRNA) genomes of betacoronavirus. Normally, the genome starts with two large polyproteins, reading frames as ORF1a and ORF1b. After transcription and translation, these two large polyproteins would be cleaved into 16 non-structural proteins (nsps), including the 3rd one to papain-like protease (PLpro), 3C-like protease (3CLpro). Normally the Spike protein, envelope (E) protein, membrane (M) and nucleocapsid (N) are encoded in sequential order, but the genes between them would change among different strains, not indicated in this graph.

When the coronavirus particles enter the hosts (Fig. 2), their envelope spike glycoprotein binds to the cellular receptors and leads to membrane fusion, either directly with the host cell

*Address correspondence to: Wenjuan Li, wenjuan@scripps.edu
Received: 04 February 2020; Accepted: 08 April 2020

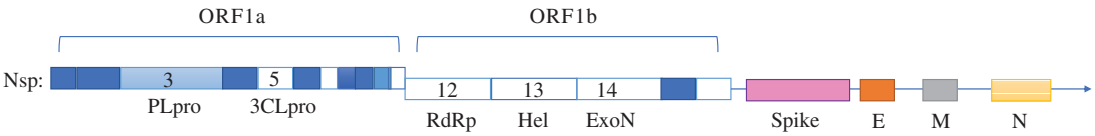

FIGURE 1. Betacoronavirus genome graphic illustration.

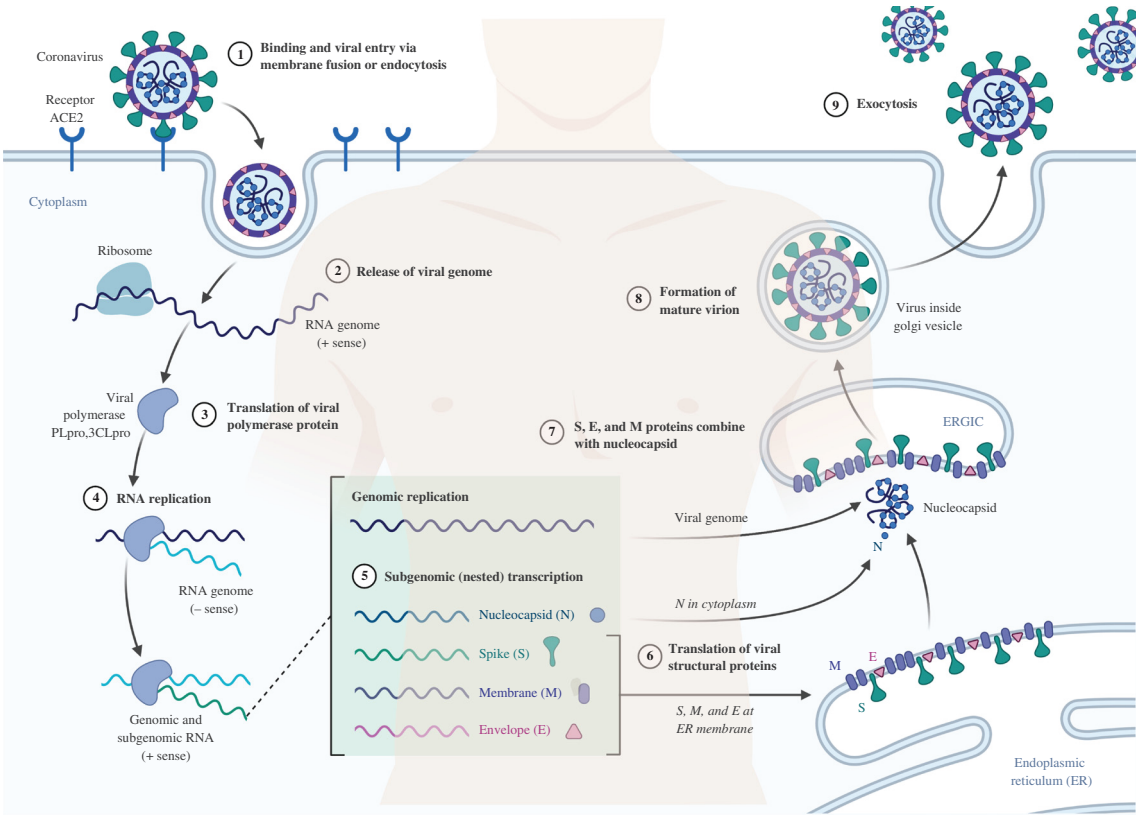

FIGURE 2. Betacoronavirus life cycle in host cell.

membrane or with endosome membrane after being engulfed. At this point, the viral RNA genome is released into the cytoplasm and becomes uncoated. Translation of the first two-thirds of genes into two large polyproteins starts. The two proteases PLpro and 3CLpro from nsp3 and nsp5 cleave the two polyproteins and allow them to pack into replicase-transcriptase complexes, which largely amplify the replication and transcription process. Positive-sense RNA genomes are replicated into negative-sense RNAs, then transcribed into subgenomic positive-sense mRNAs, followed by translation into structural proteins including spike, membrane, envelope and nucleocapsid proteins, which are prepared for the packaging and release of new viral particles.

After the spike protein on virus envelope binds to responding host cellular receptors, cellular membrane fusion or endocytosis starts, allowing viral genome to get into cytoplasm. ORF1a and ORF1b are first translated to produce two large polymerase proteins: pp1a, pp1ab. These polymerase proteins are cleaved to form the RNA replicase-transcriptase complex, which serves for the viral genome replication and viral protein transcription. Then the viral mRNAs hire the host cell's translation machines to produce their own proteins. The resulting structural proteins are ready to wait for the viral genome packed by nucleocapsids to assemble new virus particles. Finally the nascent virions release from the infected cell.

Coronavirus Reservoirs and SARS-CoV-2 Reservoirs, Transmission, Clinical Symptoms and Diagnosis

Most reported coronaviruses exist in wild animals, especially in bats.

After years of investigation, the origin of SARS-CoV (Li *et al.*, 2005; Menachery *et al.*, 2015) as well as the MERS-CoV-like viruses (Drexler *et al.*, 2014) were finally tracked to bats. Since SARS-CoV-2 has not been diagnosed in humans previously and all the first infection cases showed strong correlation with the wholesale seafood market in Wuhan city, SARS-CoV-2 was more commonly considered to be originated from wild animals. Bats are possible origins again in this case, given the fact that virus isolated from patients showed 96% similarity with a bat coronavirus on the whole-genome level (Wu *et al.*, 2020; Zhou *et al.*, 2020). Although there was also evidence showing SARS-CoV-2 may come from snakes (Ji *et al.*, 2020) or pangolin (Wong *et al.*, 2020), further researches on SARS-CoV-2 are still going on. Thus, the discontinuation of zoonotic activities with the animals that are potentially carrying the viruses would be helpful to control the breakout and development of infections.

It has been evident that SARS-CoV-2 is able to transmit from person to person (Riou and Althaus, 2020). It has an incubation period of 1–4 days, and patients develop disease

symptoms within 24 days. A furin-like cleavage site was identified from the SARS-CoV-2 sequence which is absent in SARS-CoV. Cleavage by furin, which is highly expressed in lungs, has been found to be associated with pathogenicity of viruses including other clades of coronaviruses as well as influenza (Coutard *et al.*, 2020). Therefore, inhibitors for the cleavage could be one of the possible therapeutic strategies.

As for the transmission within the human society, studies show the nosocomial transmission is the predominant route of both SARS-CoV and MERS-CoV (Chowell *et al.*, 2015; Hunter *et al.*, 2016), which is possibly due to the close distance and high regional density of suspending viral particles. Nowadays, it has been clearer that SARS-CoV-2 could transmit via an airborne route as well as physical contact.

Although bats carrying the virus showed no symptoms, infected human individuals present illnesses such as coughing, fever and breathing difficulties, some developing fluid in the lungs (Huang *et al.*, 2020), which is a typically severe pneumonia symptom and causes a drop in oxygen levels, followed by organ failure and eventually death. Under chest X-rays, severe patients showed decreased brightness of lungs and large ground-glass opacity. According to the reported infection cases, there is no age or gender bias for SARS-CoV-2. However, among the fatal cases, 74% have been shown to be age-advanced males in weaker health conditions, such as cancer, renal and lung diseases as well as diabetes mellitus.

The earliest clinical diagnosis had mostly relied on Real Time-PCR (RT-PCR) for specific genetic regions in SARS-CoV-2, which could distinguish it from common pneumonia and flu viruses as well as more specific human coronavirus strains like SARS-CoV and MERS-CoV. Two of the four earliest certified products came from BGI, one of which was RT-PCR based. The other diagnostic kits are based on sequencing, another golden standard for detection. RT-PCR and sequencing sometimes would lead to false negative results due to low virus load in infected patients or samples damaged during storage. Later on, when the genomic sequencing was available, the enzyme-linked immunosorbent assay (ELISA) detection kits based on viral proteins were developed. Colloidal gold immunochromatographic signal-amplifying test strips were as well widely used as diagnostic kits, as a result of their user-friendly format, short time required (2~3 hours), stability in various storage conditions and low price. Now several companies are working with full 24-hour shifts to develop and produce enough SARS-CoV-2 diagnostic kits that could give accurate results. To date, hospitals and CDCs have also started setting CT imaging on lung as another diagnostic standard. But there are also some recent reports about individuals that showed positive results by RT-PCR diagnosis kit but have few or almost no symptoms, while capable of transmitting the virus. The difficulty in clinical diagnosis obviously impeded the controlling of virus spreading.

The Immune Responses Induced by Coronavirus and Potential Therapies

Receptors

Coronaviruses use diverse cellular surface proteins as receptors, which profoundly increases the difficulty for prophylaxis. Among three of the most significant examples that caused outbreaks, SARS-CoV uses ACE2 as receptor (Li *et al.*, 2003), MERS-CoV adopts dipeptidyl peptidase 4 as receptor (Raj *et al.*, 2013), while human coronavirus 229E utilizes zinc metalloprotease aminopeptidase N (APN, CD13) as cellular entry access (Delmas *et al.*, 1992; Tresnan and Holmes, 1998; Yeager *et al.*, 1992). There are coronaviruses that infect other animals; for example, mouse hepatitis virus (MHV) takes advantage of murine carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), members of the immunoglobulin receptor superfamily.

During the procedure of identifying receptors for these viruses, immunoprecipitation and mass spectrometry always served as detectors. Once several candidates were determined, other assays followed to confirm the hypothesis, such as syncytia formation among cells that express viral fusion proteins and receptors on the cell surface. These approaches have previously been applied to identify the receptors successfully for SARS-CoV (Imai *et al.*, 2005; Li *et al.*, 2003) and MERS-CoV (Raj *et al.*, 2013).

The pathology distribution of SARS-CoV in human body is consistent with ACE2 expression pattern, which is primarily in the bronchus, lung parenchyma and kidney. The specific tissue or organ infection sites remain to be confirmed on both clinical and molecular-biological level, so that the symptoms could be treated with more accurate medicines and therapies.

Similarly, once the receptor of SARS-CoV-2 is identified, assuming it is/they are not novel proteins, the already existing antibodies, peptides and small compounds targeting it/them could be useful in the treatment. There were evidences (Lu *et al.*, 2020; Wan *et al.*, 2020; Wrapp *et al.*, 2020) that showed binding affinity of SARS-CoV-2 to hACE2, raising the possibility that the medicines used in SARS treatment against ACE2 would be effective.

SARS-CoV-2 was first discovered in patients' lower respiratory tract (Huang *et al.*, 2020), which is consistent with the ACE2 expression pattern. ACE2 was reported to especially highly expressed on type 2 alveolar cells. Another possibility would be that free ACE2 or other chemical substances with higher affinities delivered to pulmonary alveoli would compete with host cells that could probably "trick" the virus, similar to the mechanism by which Oseltamivir and Zanamivir work on influenza virus neuraminidase (McNicholl and McNicholl, 2001).

Virus replication and innate immune response

Since the coronavirus has a huge single positive RNA, once the genome was released into the infected cell, there is a high possibility that it would activate the pattern recognition receptor (PRR) signal pathway, especially dsRNA-related pathways, for example, melanoma differentiation-associated protein 5 (MDA5) (Siu *et al.*, 2014) and retinoic acid-inducible gene I protein (RIG-I) pathway (Jensen and Thomsen, 2012) in cellular plasma, or endosomal Toll-like receptors (TLRs) TLR7 and TLR8 (de Marcken *et al.*, 2019) if the viral single-strand RNAs were sensed in endosome (Fig. 3a). This will lead to activation of transcription factor nuclear factor-κB (NF-κB) or Interferon regulatory factor 7 (IRF7) followed by production of type I IFNs. Type 1 IFN would work through IFNα/β receptor and downstream

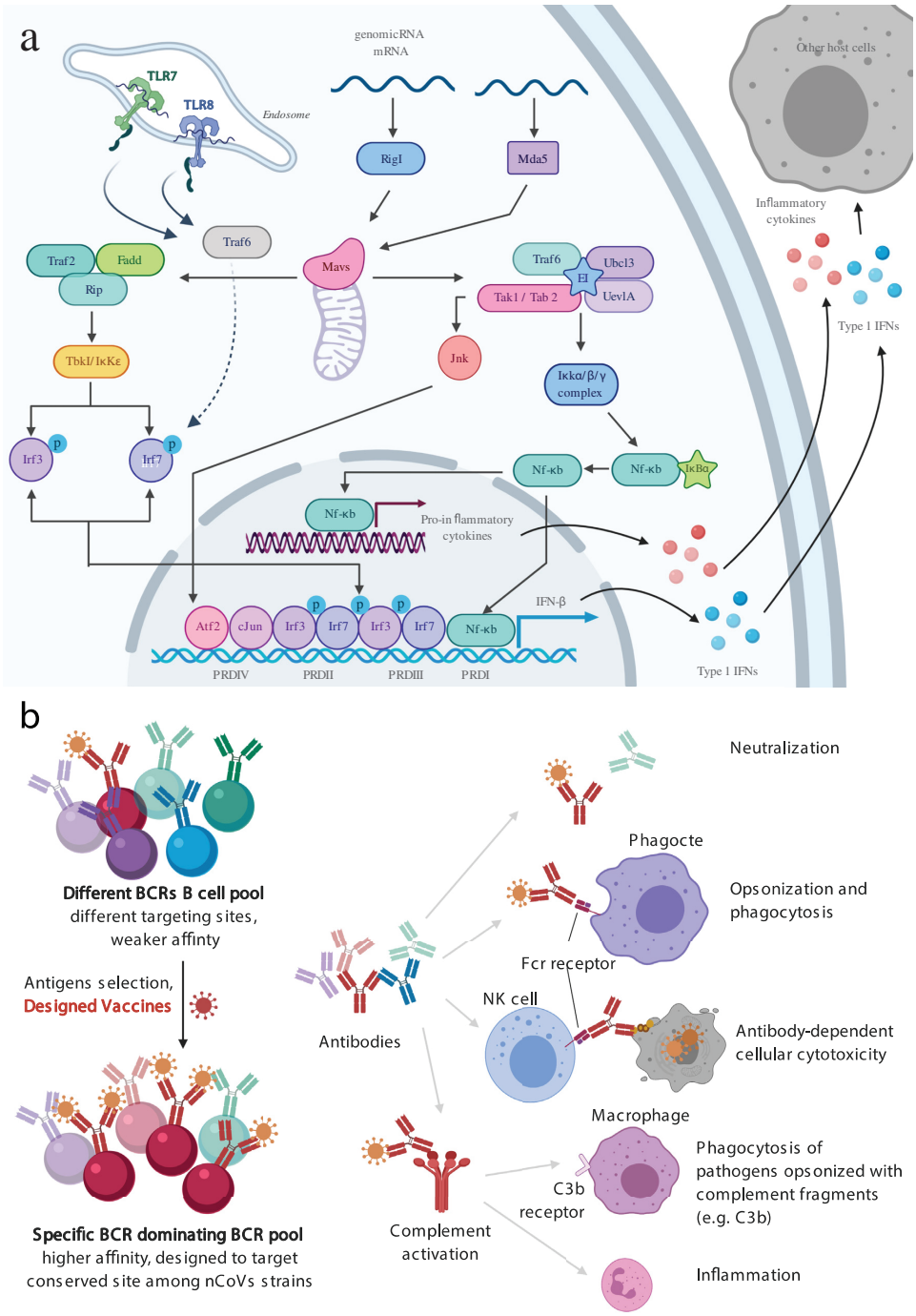

FIGURE 3. Immune response caused by betacoronavirus in human body.

cascades to generate antiviral proteins encoded by IFN-stimulated genes (ISG). NF-κB in turn induces transcription of pro-inflammatory cytokines. These two critical secreted ligands thus trigger the individual's antiviral innate system to limit viral replication in infected and neighboring cells. This innate immune response activation has been observed and proved in cases of SARS-CoV and MERS-CoV. In a mouse model infected by SARS-CoV, TNFα, IL-6, CXCL10, CCL2, CCL3, and CCL5 were largely enhanced (Chen *et al.*, 2010). However, these two viruses adopt many strategies to avoid being detected by the host cell, for example, replicating in virus-induced double-membrane vesicles that lack PRRs. On the other hand, when the chemokines and cytokines are released without constraint, they would cause organ damage even failure, which is called cytokine storm. In the 2003 SARS epidemic, as well as H5N1 and H7N9 infections, lungs were the major targeted organ.

a) Innate immune response: once the virus unloaded its genome RNA, and related transcribed RNA, they would be detected by pattern recognition receptors (PRRs), as double-stranded RNA (dsRNA) or uncapped mRNA. Retinoic acid-inducible gene I protein (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) are activated by foreign RNA, then they activated MAVS. If the single stranded RNAs are located in endosome, they would be detected by TLR7 or TLR8. The following pathways of MAVS, meaning NF-κB

get activated and translocated to nucleus, as well IRF3 and IRF7 get phosphorylated and translocated to nucleus. They boost the production of type I IFN and pro-inflammatory cytokines. These IFNs and pro-inflammatory cytokines would bind to the cell's own or other cell's IFN receptors, activate STAT1/STAT2, and induce IFN-stimulated genes (ISGs). ISGs largely boost an antiviral innate immune response, limiting viral replication and infection on nearby host cells.

b) Humoral immune response: once the antigens from the virus are presented, they would contact millions of BCRs with a wide range of affinities to antigens. If the affinity is higher, the BCR signal would be stronger, thus B cells with specific BCRs would proliferate faster and survive longer, and would more possibly develop into plasma B cells. During this development, the cytokines secreted by T cells help the B cells to proliferate and mature into antibody producing plasma cells. Designed vaccines are assumed and proved to work in most of the cases that would largely boost specific BCRs, normally aiming broadly neutralizing antibodies, to be produced or dominated during the infection, then remain active as memory B cells and ready for upcoming infection. The antibodies work through several ways. 1. Directly neutralizing the antigens, to precipitate the antigen, waiting to be eliminated by macrophages or other physical ways like sneezing. 2. Labeling the antigens and recruiting FcR cell like phagocytes for endocytosis and degradation. 3. The antigens presented by the infected cells would recruit and activate NK cell to attack infected cells for halting virus replication. 4. Followed by complementary system activation, which could either lead to lysis on pathogen itself or infected cells. 5. Complementary proteins as well would recruit NK cells or macrophages and induce inflammatory cells, like neutrophil enrichment and activation.

Soon after coronaviruses enter the host cell, translation of ORF1a 3CLpro and PLpro is necessary for viral polyprotein cleavage. Their differences from host cellular protease makes them ideal targets for drug design, which could be contributed by the conservation of 3CLpro and PLpro among different strains in betacoronavirus and the already known crystal structures. This possibility of inducing broad-spectrum anti-coronaviral activity had been explored by Rolf Hilgenfeld's group (Hilgenfeld, 2014), who found that SG85 showed good effect on betacoronavirus clade c Bt-CoV-HKU4 and clade d Bt-CoV-HKU9, but not on alphacoronavirus Bt-CoV HKU8. No Further research is coming up to a broad-spectrum Main protease (M^{pro}) and papain-like protease (PL^{pro}) largely due to sharply declined funding during 2005 to 2006.

Other chemicals, such as 6-mercaptopurine and 6-thioguanine, could inhibit both SARS-CoV and MERS-CoV in vitro (Cheng *et al.*, 2015). However, the species difference and in vivo tests always drew a big gap from screening work to clinical usage. There are teams working on screening current commercial medicines for targeting SARS-CoV-2 Mpro (Liu and Wang, 2020), trying to discover the fastest treatment available, even medicines that would be used off-label in this first early phase.

Remdesivir, which was intentionally designed and applied in Ebola treatment, is a novel nucleotide analog prodrug, and has already largely reduced severity of the first SARS-CoV-2 patient in USA on the second day after receiving it (Holshue *et al.*, 2020). However, this was a compassionate usage based on the worsening clinical status of the patient. Safety and efficacy of remdesivir for treatment of patients with SARS-CoV-2 infection needs to be more closely examined and monitored. As Reuters recently reported, a cocktail of flu and HIV drugs, ritonavir and high doses of Oseltamivir, used by Thai doctors largely reduced the load of SARS-CoV-2 virus to none-detectable level in several severe cases. The underlying mechanisms remain to be further investigated. Darunavir (prezista), used in HIV/AIDS, was as well considered in this epidemic by its antiretroviral ability (Leonis *et al.*, 2012). Arbidol, which was used in flu treatment, has also been applied in SARS-CoV-2 infected patients.

Symptoms in respiration system caused by SARS-CoV-2 are similar to those caused by SARS, H5N1 and H4N9, but with lower fatality rate. Therefore, the treatments could possibly refer to the ones used in H7N9, which is serum filtering (Hu *et al.*, 2013), as a supporting method to win the time for adapted immune system to start functioning in patients' body. Nowadays, there have been case reports about virus carriers that showed no symptoms described above.

Humoral immune response

Besides chemical drugs described above, most of the treatments in the first cured cases are supportive methods and symptom management, like acetaminophen and ibuprofen for antipyretic therapy, guaifenesin for easier coughing, saline for prevention of dehydration, and oxygen supplementation for maintaining oxygen saturation level.

Application of specific antibodies against the viruses is always considered as a better specialized therapy. Antibodies generated in individuals can achieve protection either by neutralizing viruses that entered the body, which could be eliminated by innate killer cells and complementary system, or by interrupting virus release, such as the anti-neuraminidase antibodies (Doyle *et al.*, 2013; Karunarathna *et al.*, 2019). Antibodies show high specificities and affinities, thus could stimulate the participation of individual immune responses avidly (Fig. 3b). Studies have clearly illustrated how antibodies from SARS survivors could block attachment of SARS-CoV to the host cell receptor and anti-SARS-CoV S antibody could further trigger fusogenic conformational changes by mimicking the binding interaction (Walls *et al.*, 2019). Serum transfusion protection could be a feasible and promising therapeutic strategy before further studies come up. Antibodies that can bind to invading virus particles were assumed to exist in plasma from survivors, secreted by B cells. Although no high technology is required for drawing the blood or filtering the plasma, the scale of donors and willingness of patients as acceptors, as well as the inflammation-related chemokines and cytokines in serum, are problems that need to be solved. B cells secreting the efficient antibodies could

be sorted out for monoclonal antibody production, thus could better help with the patients or potential patients.

Vaccine is another way to elicit humoral responses leading to high-affinity antibodies. However, not enough effort was made on the drug and vaccine development after SARS abated, and the vaccines for SARS-CoV did not achieve good effects as expected (Roper and Rehm, 2009). Since the majority of coronaviruses cause respiratory system inflammation, broadly neutralizing or targeting antibodies to conserved region of the viruses should be taken into consideration. Yet it is reasonable that the industry did not keep investing on development of SARS-CoV vaccines, since clinical trials often take years, and the virus had probably faded away from human society during this time period.

The concept of broadly neutralizing antibodies was raised and developed with considerable academic achievements in viruses such as HIV (Dubrovskaya *et al.*, 2019; Gorman *et al.*, 2019; Kong *et al.*, 2019; Pinto *et al.*, 2019; Wise *et al.*, 2020) and influenza (Ellebedy *et al.*, 2014; Kadam *et al.*, 2017; Raymond *et al.*, 2018; Tharakaraman *et al.*, 2014, 2015; Xiong *et al.*, 2015). The recent work by McLellan lab (Wrapp *et al.*, 2020) revealed high resolution cryo-EM structure of SARS-CoV-2 spike protein in 3.5 Å-resolution and showed no cross-binding to the already existing SARS mAbs that target the Receptor Binding Domain (RBD). Vaccines aiming at cross-strain activities would be more achievable compared to RBD binding. The stem part of the spike protein could be taken into consideration for vaccine design to elicit broadly neutralizing antibodies (Fig. 3b).

DNA vaccines are a much faster way when confronting an epidemic outbreak. Genetically engineered DNA is injected into individuals and incorperated into the host cell genome, thus can use host cells to produce antigens and continuously induce immune responses, including antibodies (Kutzler and Weiner, 2008) (Fig. 4). DNA vaccine strategies share the advantages of good biocompatibility of plasmid DNA, cost-efficient production, long shelf life, and wider range of immunoglobulin responses. DNA vaccines would also be recommended since normal protein vaccines need at least several months for manufacturing and scaling up. However, due to their targeting efficiency and safety, they are not yet popular, but its era is coming (Hobernik and Bros, 2018).

The codon or immunogenicity optimized antigen DNA was designed and assembled with plasmid carrying necessary transcriptional elements. The plasmids mostly are delivered to the skin, subcutaneum or muscle. The mixed vaccine formulation and adjuvants would help human body response to the vaccine. The plasmids could enter myocytes or keratinocytes, in which the antigen could be expressed and presented on MHC I, released extracellularly, or endocytosed by the dead host cells and presented by APCs. Plasmids could as well enter directly into APC. Then the antigen-loaded APCs would drain to the second lymph node, where CD8 T cells, CD4 T cells and B cells would be activated.

There are DNA vaccines for MERS-CoV (Modjarrad *et al.*, 2019; Yoon and Kim, 2019; Zumla *et al.*, 2019). Till Sep. 2019, one of them had been on clinical trial phase 1. Because the common carriers of MERS-CoV are dromedary camels, a vaccine used on camels was developed as well (Haagmans *et al.*, 2016). Vaccines for bats against coronaviruses are impossible, therefore, the easiest and most reasonable way to avoid exposure to unknown viruses

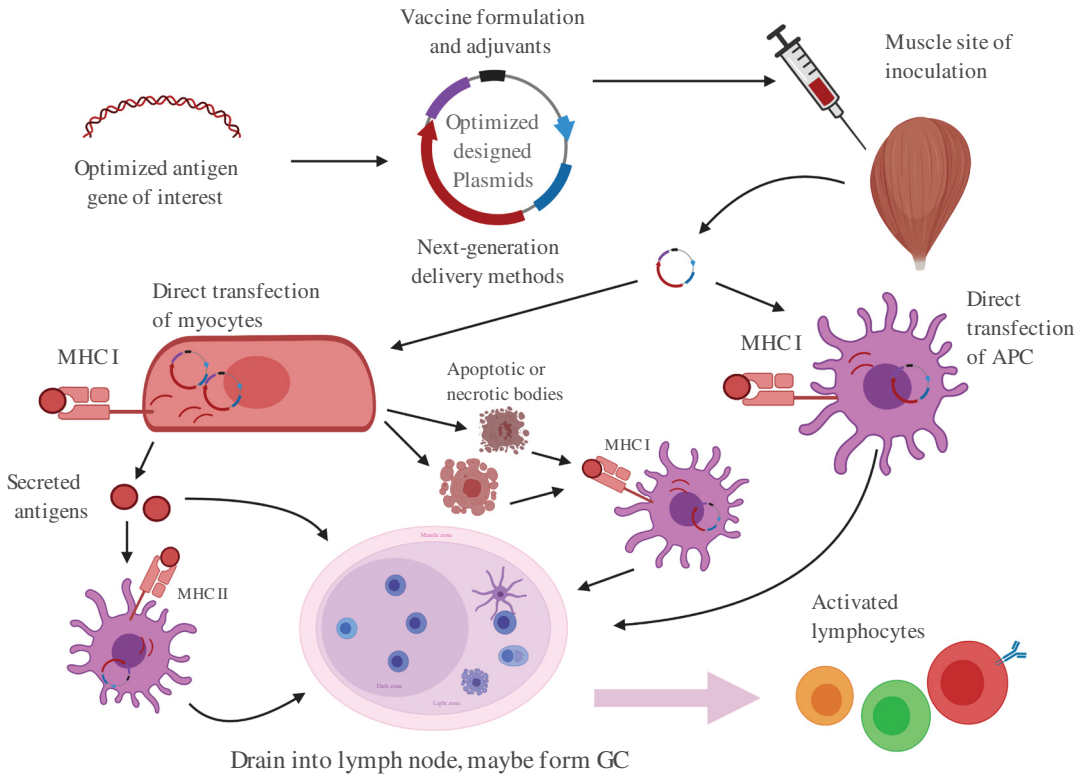

FIGURE 4. Vaccines strategies illustration. DNA vaccine procedure.

would be keeping a distance from the wild animals and develop vaccines on human. This vaccine was developed by Moderna, who also responded quickly in the SARS-CoV-2 event, by using mRNA converted from viral sequences. These mRNAs were injected into host individuals and started to produce a viral protein that could potentially trigger humoral response in DNA vaccine receivers.

Outlook

The effort of seeking more efficient, specific targeting therapies for the coronavirus is necessary, not only for the current outbreak of SARS-CoV-2, but also for the continuing coronavirus infections coming up. SARS-CoV in years 2002 to 2003 did not alert human population, MERS-CoV remains circulating in camels, longer observation and further research on the SARS-CoV-2 still need close attention.

There are several cases of individuals who are carrying SARS-CoV-2 but do not show symptoms, which means the hosts would spread the virus unconsciously. Thus best- and worst-case scenarios should be prepared for. If the coronaviruses stay endemic, which means it circulates continuously in a community, then this bomb could explode anytime or regularly, like influenza virus. More efficient broadly cross-strain drugs or vaccines against coronaviruses should be carrying on.

The huge effort of controlling this epidemic, from every aspect (governments, organizations, researchers, all the residents), especially the health-care workers, some of whom even sacrificed their precious lives, should be cherished and deeply honored. Every individual should not forget the huge price paid for disturbing the balance on this planet. This review only briefly summarized the fast-growing knowledge about SARS-CoV-2, lots of important information is still being contributed by many research groups all over the world, and would hopefully help us successfully fight this virus in the near future.

Funding Statement: The authors received no specific funding for this study.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, Xing F, Liu J, Yip CCY, Poon RWS, Tsoi HW, Lo SKF, Chan KH, Poon VKM, Chan WM, Ip JD, Cai JP, Cheng VCC, Chen H, Hui CKM, Yuen KY (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* **395**: 514–523. DOI 10.1016/S0140-6736(20)30154-9.

Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, Subbarao K (2010). 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. *Journal of Virology* **84**: 1289–1301. DOI 10.1128/JVI.01281-09.

Cheng KW, Cheng SC, Chen WY, Lin MH, Chuang SJ, Cheng IH, Sun CY, Chou CY (2015). Thiopurine analogs and mycophenolic acid synergistically inhibit the papain-like protease of Middle East respiratory syndrome coronavirus. *Antiviral Research* **115**: 9–16. DOI 10.1016/j.antiviral.2014.12.011.

Chowell G, Abdirizak F, Lee S, Lee J, Jung E, Nishiura H, Viboud C (2015). Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC Medicine* **13**: 210. DOI 10.1186/s12916-015-0450-0.

Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E (2020). The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Research* **176**: 104742. DOI 10.1016/j.antiviral.2020.104742.

de Marcken M, Dhaliwal K, Danielsen AC, Gautron AS, Dominguez-Villar M (2019). TLR7 and TLR8 activate distinct pathways in monocytes during RNA virus infection. *Science Signaling* **12**: eaaw1347. DOI 10.1126/scisignal.aaw1347.

Delmas B, Gelfi J, L'Haridon R, Vogel S, Norén H, Laude H (1992). Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. *Nature* **357**: 417–420. DOI 10.1038/357417a0.

Dong N, Yang X, Ye L, Chen K, Chan EWC, Yang M, Chen S (2020). Genomic and protein structure modelling analysis depicts the origin and infectivity of 2019-nCoV, a new coronavirus which caused a pneumonia outbreak in Wuhan, China. bioRxiv: 2020.01.20.913368.

Doyle TM, Hashem AM, Li C, Van Domselaar G, Larocque L, Wang J, Smith D, Cyr T, Farnsworth A, He R, Hurt AC, Brown EG, Li X (2013). Universal anti-neuraminidase antibody inhibiting all influenza A subtypes. *Antiviral Research* **100**: 567–574. DOI 10.1016/j.antiviral.2013.09.018.

Drexler JF, Corman VM, Drosten C (2014). Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Research* **101**: 45–56. DOI 10.1016/j.antiviral.2013.10.013.

Dubrovskaya V, Tran K, Ozorowski G, Guenaga J, Wilson R, Bale S, Cottrell CA, Turner HL, Seabright G, O'Dell S, Torres JL, Yang L, Feng Y, Leaman DP, Vazquez Bernat N, Liban T, Louder M, McKee K, Bailer RT, Movsesyan A, Doria-Rose NA, Pancera M, Karlsson Hedestam GB, Zwick MB, Crispin M, Mascola JR, Ward AB, Wyatt RT (2019). Vaccination with glycan-modified HIV NFL envelope trimer-liposomes elicits broadly neutralizing antibodies to multiple sites of vulnerability. *Immunity* **51**: 915–929.e7. DOI 10.1016/j.immuni.2019.10.008.

Ellebedy AH, Krammer F, Li GM, Miller MS, Chiu C, Wrammert J, Chang CY, Davis CW, McCausland M, Elbein R, Edupuganti S, Spearman P, Andrews SF, Wilson PC, Garcia-Sastre A, Mulligan MJ, Mehta AK, Palese P, Ahmed R (2014). Induction of broadly cross-reactive antibody responses to the influenza HA stem region following H5N1 vaccination in humans. *Proceedings of the National Academy of Sciences* **111**: 13133–13138. DOI 10.1073/pnas.1414070111.

Gorman J, Mason RD, Nettey L, Cavett N, Chuang GY, Peng D, Tsybovsky Y, Verardi R, Nguyen R, Ambrozak D, Biris K, LaBranche CC, Ramesh A, Schramm CA, Zhou J, Bailer RT, Kepler TB, Montefiori DC, Shapiro L, Douek DC, Mascola JR, Roederer M, Kwong PD (2019). Isolation and structure of an antibody that fully neutralizes isolate SIVmac239 reveals functional similarity of SIV and HIV glycan shields. *Immunity* **51**: 724–734.e4. DOI 10.1016/j.immuni.2019.09.007.

Haagmans BL, van den Brand JM, Raj VS, Volz A, Wohlsein P, Smits SL, Schipper D, Bestebroer TM, Okba N, Fux R, Bensaid A, Solanes Foz D, Kuiken T, Baumgartner W, Segales J, Sutter G, Osterhaus AD (2016). An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. *Science* **351**: 77–81. DOI 10.1126/science.aad1283.

Hilgenfeld R (2014). From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *FEBS Journal* **281**: 4085–4096. DOI 10.1111/febs.12936.

Hobernik D, Bros M (2018). DNA vaccines—how far from clinical use? *International Journal of Molecular Sciences* **19**: 3605. DOI 10.3390/ijms19113605.

Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, Diaz G, Cohn A, Fox L, Patel A, Gerber SI, Kim L, Tong S, Lu X, Lindstrom S, Pallansch MA, Weldon WC, Biggs HM, Uyeki TM, Pillai SK (2020). First case of 2019 novel coronavirus in the United States. *New England Journal of Medicine* **382**: 929–936. DOI 10.1056/NEJMoa2001191.

Hu Y, Lu S, Song Z, Wang W, Hao P, Li J, Zhang X, Yen HL, Shi B, Li T, Guan W, Xu L, Liu Y, Wang S, Zhang X, Tian D, Zhu Z, He J, Huang K, Chen H, Zheng L, Li X, Ping J, Kang B, Xi X, Zha L, Li Y, Zhang Z, Peiris M, Yuan Z (2013). Association between adverse clinical outcome in human disease caused by novel influenza A H7N9 virus and sustained viral shedding and emergence of antiviral resistance. *Lancet* **381**: 2273–2279. DOI 10.1016/S0140-6736(13)61125-3.

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**: 497–506. DOI 10.1016/S0140-6736(20)30183-5.

Hunter JC, Nguyen D, Aden B, Al Bandar Z, Al Dhaheri W, Abu Elkheir K, Khudair A, Al Mulla M, El Saleh F, Imambaccus H, Al Kaabi N, Sheikh FA, Sasse J, Turner A, Abdel Wareth L, Weber S, Al Ameri A, Abu Amer W, Alami NN, Bunga S, Haynes LM, Hall AJ, Kallen AJ, Kuhar D, Pham H, Pringle K, Tong S, Whitaker BL, Gerber SI, Al Hosani FI (2016). Transmission of middle east respiratory syndrome coronavirus infections in healthcare settings, Abu Dhabi. *Emerging Infectious Diseases* **22**: 647–656. DOI 10.3201/eid2204.151615.

Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436**: 112–116. DOI 10.1038/nature03712.

Jensen S, Thomsen AR (2012). Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion. *Journal of Virology* **86**: 2900–2910. DOI 10.1128/JVI.05738-11.

Ji W, Wang W, Zhao X, Zai J, Li X (2020). Cross-species transmission of the newly identified coronavirus 2019-nCoV. *Journal of Medical Virology* **92**: 433–440. DOI 10.1002/jmv.25682.

Kadam RU, Juraszek J, Brandenburg B, Buyck C, Schepens WBG, Kesteleyn B, Stoops B, Vreeken RJ, Vermond J, Goutier W, Tang C, Vogels R, Friesen RHE, Goudsmit J, van Dongen MJP, Wilson IA (2017). Potent peptidic fusion inhibitors of influenza virus. *Science* **358**: 496–502. DOI 10.1126/science.aan0516.

Karunarathna H, Perera R, Fang VJ, Yen HL, Cowling BJ, Peiris M (2019). Serum anti-neuraminidase antibody responses in human influenza A(H1N1)pdm09 virus infections. *Emerging Microbes & Infections* **8**: 404–412. DOI 10.1080/22221751.2019.1572433.

Kong R, Duan H, Sheng Z, Xu K, Acharya P, Chen X, Cheng C, Dingens AS, Gorman J, Sastry M, Shen CH, Zhang B, Zhou T, Chuang GY, Chao CW, Gu Y, Jafari AJ, Louder MK, O'Dell S, Rowshan AP, Viox EG, Wang Y, Choi CW, Corcoran MM, Corrigan AR, Dandey VP, Eng ET, Geng H, Foulds KE, Guo Y, Kwon YD, Lin B, Liu K, Mason RD, Nason MC, Ohr TY, Ou L, Rawi R, Sarfo EK, Schon A, Todd JP, Wang S, Wei H, Wu W, Mullikin JC, Bailer RT, Doria-Rose NA, Karlsson Hedestam GB, Scorpio DG, Overbaugh J, Bloom JD, Carragher B, Potter CS, Shapiro L, Kwong PD, Mascola JR (2019). Antibody lineages with vaccine-induced antigen-binding hotspots develop broad HIV neutralization. *Cell* **178**: 567–584.e19. DOI 10.1016/j.cell.2019.06.030.

Kutzler MA, Weiner DB (2008). DNA vaccines: ready for prime time? *Nature Reviews Genetics* **9**: 776–788. DOI 10.1038/nrg2432.

Leonis G, Czyżnikowska Ż, Megariotis G, Reis H, Papadopoulos MG (2012). Computational studies of Darunavir into HIV-1 protease and DMPC bilayer: necessary conditions for effective binding and the role of the flaps. *Journal of Chemical Information and Modeling* **52**: 1542–1558. DOI 10.1021/ci300014z.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**: 450–454. DOI 10.1038/nature02145.

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005). Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**: 676–679. DOI 10.1126/science.1118391.

Liu X, Wang XJ (2020). Potential inhibitors for 2019-nCoV coronavirus M protease from clinically approved medicines. bioRxiv: 2020.01.29.924100.

Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* **395**: 565–574. DOI 10.1016/S0140-6736(20)30251-8.

McNicholl IR, McNicholl JJ (2001). Neuraminidase inhibitors: zanamivir and oseltamivir. *Annals of Pharmacotherapy* **35**: 57–70. DOI 10.1345/aph.10118.

Menachery VD, Yount BL Jr., Debbink K, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Ge XY, Donaldson EF, Randell SH, Lanzavecchia A, Marasco WA, Shi ZL, Baric RS (2015). A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* **21**: 1508–1513. DOI 10.1038/nm.3985.

Modjarrad K, Roberts CC, Mills KT, Castellano AR, Paolino K, Muthumani K, Reuschel EL, Robb ML, Racine T, Oh MD, Lamarre C, Zaidi FI, Boyer J, Kudchodkar SB, Jeong M, Darden JM, Park YK, Scott PT, Remigio C, Parikh AP, Wise MC, Patel A, Duperret EK, Kim KY, Choi H, White

S, Bagarazzi M, May JM, Kane D, Lee H, Kobinger G, Michael NL, Weiner DB, Thomas SJ, Maslow JN (2019). Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial. *Lancet Infectious Diseases* **19**: 1013–1022. DOI 10.1016/S1473-3099(19)30266-X.

Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Tsiodras S (2020). Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. bioRxiv: 2020.01.26.920249.

Pasternak AO, Spaan WJ, Snijder EJ (2006). Nidovirus transcription: how to make sense? *Journal of General Virology* **87**: 1403–1421. DOI 10.1099/vir.0.81611-0.

Perlman S, Netland J (2009). Coronaviruses post-SARS: update on replication and pathogenesis. *Nature Reviews Microbiology* **7**: 439–450. DOI 10.1038/nrmicro2147.

Pinto D, Fenwick C, Caillat C, Silacci C, Guseva S, Dehez F, Chipot C, Barbieri S, Minola A, Jarrossay D, Tomaras GD, Shen X, Riva A, Tarkowski M, Schwartz O, Bruel T, Dufloo J, Seaman MS, Montefiori DC, Lanzavecchia A, Corti D, Pantaleo G, Weissenhorn W (2019). Structural basis for broad HIV-1 neutralization by the MPER-specific human broadly neutralizing antibody LN01. *Cell Host & Microbe* **26**: 623–637.e8. DOI 10.1016/j.chom.2019.09.016.

Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, Dijkman R, Muth D, Demmers JA, Zaki A, Fouchier RA, Thiel V, Drosten C, Rottier PJ, Osterhaus AD, Bosch BJ, Haagmans BL (2013). Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* **495**: 251–254. DOI 10.1038/nature12005.

Raymond DD, Bajic G, Ferdman J, Suphaphiphat P, Settembre EC, Moody MA, Schmidt AG, Harrison SC (2018). Conserved epitope on influenza-virus hemagglutinin head defined by a vaccine-induced antibody. *Proceedings of the National Academy of Sciences* **115**: 168–173. DOI 10.1073/pnas.1715471115.

Riou J, Althaus CL (2020). Pattern of early human-to-human transmission of Wuhan 2019-nCoV. bioRxiv: 2020.01.23.917351.

Roper RL, Rehm KE (2009). SARS vaccines: where are we? *Expert Review of Vaccines* **8**: 887–898. DOI 10.1586/erv.09.43.

Siu KL, Yeung ML, Kok KH, Yuen KS, Kew C, Lui PY, Chan CP, Tse H, Woo PC, Yuen KY, Jin DY (2014). Middle east respiratory syndrome coronavirus 4a protein is a double-stranded RNA-binding protein that suppresses PACT-induced activation of RIG-I and MDA5 in the innate antiviral response. *Journal of Virology* **88**: 4866–4876. DOI 10.1128/JVI.03649-13.

Tharakaraman K, Subramanian V, Cain D, Sasisekharan V, Sasisekharan R (2014). Broadly neutralizing influenza hemagglutinin stem-specific antibody CR8020 targets residues that are prone to escape due to host selection pressure. *Cell Host & Microbe* **15**: 644–651. DOI 10.1016/j.chom.2014.04.009.

Tharakaraman K, Subramanian V, Viswanathan K, Sloan S, Yen HL, Barnard DL, Leung YH, Szretter KJ, Koch TJ, Delaney JC, Babcock GJ, Wogan GN, Sasisekharan R, Shriver Z (2015). A broadly neutralizing human monoclonal antibody is effective against H7N9. *Proceedings of the National Academy of Sciences* **112**: 10890–10895. DOI 10.1073/pnas.1502374112.

Tresnan DB, Holmes KV (1998). Feline aminopeptidase N is a receptor for all group I coronaviruses. In: Enjuanes L, Siddell SG, Spaan W, eds. *Coronaviruses and Arteriviruses*. Boston, MA: Springer, 69–75.

Walls AC, Xiong X, Park YJ, Tortorici MA, Snijder J, Quispe J, Cameroni E, Gopal R, Dai M, Lanzavecchia A, Zambon M, Rey FA, Corti D, Veesler D (2019). Unexpected receptor functional mimicry elucidates activation of coronavirus fusion. *Cell* **176**: 1026–1039.e15. DOI 10.1016/j.cell.2018.12.028.

Wan Y, Shang J, Graham R, Baric RS, Li F (2020). Receptor recognition by the novel coronavirus from wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *Journal of Virology* **94**: e00127-20. DOI 10.1128/JVI.00127-20.

Wise MC, Xu Z, Tello-Ruiz E, Beck C, Trautz A, Patel A, Elliott ST, Chokkalingam N, Kim S, Kerkau MG, Muthumani K, Jiang J, Fisher PD, Ramos SJ, Smith TR, Mendoza J, Broderick KE, Montefiori DC, Ferrari G, Kulp DW, Humeau LM, Weiner DB (2020). *In vivo* delivery of synthetic DNA–encoded antibodies induces broad HIV-1–neutralizing activity. *Journal of Clinical Investigation* **130**: 827–837. DOI 10.1172/JCI132779.

Wong MC, Javornik Cregeen SJ, Ajami NJ, Petrosino JF (2020). Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019. bioRxiv: 2020.02.07.939207.

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. bioRxiv: 2020.02.11.944462.

Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML, Zhang YL, Dai FH, Liu Y, Wang QM, Zheng JJ, Xu L, Holmes EC, Zhang YZ (2020). A new coronavirus associated with human respiratory disease in China. *Nature* **579**: 265–269. DOI 10.1038/s41586-020-2008-3.

Xiong X, Corti D, Liu J, Pinna D, Foglierini M, Calder LJ, Martin SR, Lin YP, Walker PA, Collins PJ, Monne I, Suguitan AL Jr., Santos C, Temperton NJ, Subbarao K, Lanzavecchia A, Gamblin SJ, Skehel JJ (2015). Structures of complexes formed by H5 influenza hemagglutinin with a potent broadly neutralizing human monoclonal antibody. *Proceedings of the National Academy of Sciences* **112**: 9430–9435. DOI 10.1073/pnas.1510816112.

Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao P (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Science China Life Sciences* **63**: 457–460. DOI 10.1007/s11427-020-1637-5.

Yeager CL, Ashmun RA, Williams RK, Cardellichio CB, Shapiro LH, Look AT, Holmes KV (1992). Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature* **357**: 420–422. DOI 10.1038/357420a0.

Yoon IK, Kim JH (2019). First clinical trial of a MERS coronavirus DNA vaccine. *Lancet Infectious Diseases* **19**: 924–925. DOI 10.1016/S1473-3099(19)30397-4.

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**: 270–273. DOI 10.1038/s41586-020-2012-7.

Zumla A, Memish ZA, Hui DS, Perlman S (2019). Vaccine against Middle East respiratory syndrome coronavirus. *Lancet Infectious Diseases* **19**: 1054–1055. DOI 10.1016/S1473-3099(19)30477-3.