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MINI-REVIEW

COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics

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ABSTRACT

The novel coronavirus infection (COVID-19 or Coronavirus disease 2019) that emerged from Wuhan, Hubei province of China has spread to many countries worldwide. Efforts have been made to develop vaccines against human coronavirus (CoV) infections such as MERS and SARS in the past decades. However, to date, no licensed antiviral treatment or vaccine exists for MERS and SARS. Most of the efforts for developing CoV vaccines and drugs target the spike glycoprotein or S protein, the major inducer of neutralizing antibodies. Although a few candidates have shown efficacy in *in vitro* studies, not many have progressed to randomized animal or human trials, hence may have limited use to counter COVID-19 infection. This article highlights ongoing advances in designing vaccines and therapeutics to counter COVID-19 while also focusing on such experiences and advances as made with earlier SARS- and MERS-CoVs, which together could enable efforts to halt this emerging virus infection.

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Introduction

Coronaviruses (CoVs) are positive-sense, single-stranded RNA viruses of the family *Coronaviridae* (subfamily *Coronavirinae*) that infect a wide host range to produce diseases ranging from common cold to severe/fatal illnesses. The novel virus was initially named "2019-nCoV" which was changed to "SARS-CoV-2" by the Coronavirus Study Group (CSG) of International Committee on Taxonomy of Viruses (ICTV), since it was found to be the sister virus of severe acute respiratory syndrome coronavirus (SARS-CoV).[1] The ongoing coronavirus threat that emerged in China has rapidly spread to other countries and has been declared as a global health emergency by the World Health Organization (WHO). Many nations are diverting their best efforts for the implementation of appropriate preventive and control strategies. Neither vaccines nor direct-acting antiviral drugs are available for the treatment of human and animal coronavirus infections.[2-4]

Many efforts have been directed to develop vaccines against human CoV infections in recent decades, but a limiting factor is the degree of cross-protection rendered by these vaccines due to their extensive sequence diversity.[5] Various vaccines, immunotherapeutics, and drug options have been explored during the recent threats of Zika, Ebola, and Nipah viruses[6-8] as well as against previous CoVs including SARS- and MERS-CoVs.[3,5,9-12] These valuable options can be exploited for their potency, efficacy, and safety along with expediting other ongoing research[2,4,13-15] so as to discover valuable modalities for tackling the emerging COVID-19, but as yet there is no effective vaccine or therapeutic, for which intense efforts are ongoing.

Most of the therapeutic options that are available for managing COVID-19 are based on previous experiences in treating SARS- and MERS-CoV. A major reason for the lack of approved and commercially available vaccines or therapeutic agents against these CoVs might be the relative lack of interest among the pharmaceutical companies.[13] These are outbreak scenarios: the demand for drugs or vaccines lasts only for a period while the outbreak lasts. The number of affected people will also be a small proportion of the global drug and vaccine market. So by the time a new drug or vaccine is developed, there might not be any patients for clinical trials and also no meaningful market for newly discovered drugs. According to WHO guidelines, infected patients will receive supportive care including oxygen therapy, fluid therapy, and antibiotics for treating secondary bacterial infections. The WHO also recommends the isolation of patients suspected or confirmed for COVID-19.[16] The major therapeutic drugs that might be effective in managing COVID-19 include remdesivir, lopinavir/ritonavir alone or in combination with interferon-β, convalescent plasma, and mAbs.[17] Nevertheless, before utilizing these drugs for COVID-19 pneumonia patients, clinical efficacy, and safety studies should be conducted.

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This article describes advances in designing vaccines and therapeutics to counter COVID-19 while also discussing experiences with SARS- and MERS-CoVs, which together could pave ways in the right direction to halt this emerging virus.

Vaccines

Multiple strategies are adopted in the development of CoV vaccines; most of these target the surface-exposed spike (S) glycoprotein or S protein as the major inducer of neutralizing antibodies. Several S-protein-based strategies have been attempted for developing CoV vaccines, e.g., use of full-length S protein or S1-receptor-binding domain (RBD) and expression in virus-like particles (VLP), DNA, or viral vectors[5,9,18-21] The S protein molecule contains two subunits, S1 and S2. The S1 subunit has an RBD that interacts with its host cell receptor, angiotensin-converting enzyme 2 (ACE2), whereas the S2 subunit mediates fusion between the virus and host cell membranes for releasing viral RNA into the cytoplasm for replication.[19] Hence, S-protein-based vaccines should induce antibodies that block not only viral receptor binding but also virus genome uncoating. It has been shown that the C-terminal domain of the S1 subunit of porcine *Deltacoronavirus* constitutes the immunodominant region, and the immune response to this region shows the most potent neutralizing effect.[22] The S protein has a major role in the induction of protective immunity during infection with SARS-CoV by eliciting neutralizing-antibodies and T-cell responses.[19] Thus, full-length or appropriate parts of the S glycoprotein are believed to be the most promising candidate CoV vaccine composition. It was also reported that neither the absence nor presence of the other structural proteins affects S protein immunogenicity or its binding to the ACE2 receptor that is a critical initial step for virus to access into the host cell.[23,24] Due to the superior ability of RBD to induce neutralizing antibody, both recombinant proteins that contain RBD and the recombinant vectors that encode RBD can be used for developing the effective SARS-CoV vaccines.[18]

Recombinant adenovirus-based vaccine expressing MERS-CoV S protein induces systemic IgG, secretory IgA, and lung-resident memory T-cell responses when administered intranasally into BALB/c mice and provide long-lasting neutralizing immunity to MERS spike pseudotyped virus, thereby suggesting that the vaccine may confer protection against MERS-CoV.[24] Furthermore, rabies virus (RV) as a viral vector as well as Gram-positive enhancer matrix (GEM) as a bacterial vector has been used to express MERS-CoV S protein. The immune responses to these vaccine candidates were evaluated in BALB/c mice for cellular and humoral immune responses, which showed that RV-based vaccine stimulates significantly higher levels of cellular immunity and earlier antibody responses in comparison to the GEM particle vector.[12]

The possibility of developing a universal CoV vaccine was assessed based on the similarity in T-cell epitopes of SARS- and MERS-CoV that confirmed the potential for cross-reactivity among CoVs.[25] SARS-CoV-2 shares high genetic similarity with the SARS-CoV[26] such that vaccines developed for SARS-CoV may exhibit cross-reactivity to SARS-CoV-2. The comparative evaluation performed on full-length S protein sequences of SARS-CoV-2 and SARS-CoV identified that the most variable residues were located in the S1 subunit of S protein, the critical CoV vaccine target.[27] These findings suggest that the specific neutralizing antibodies that are effective against the SARS-CoV might not be effective against the SARS-CoV-2. Even though the S protein of SARS-CoV-2 has key mutations compared to the SARS-CoV, they will still act as a viable target for vaccine development.[28] Likewise, the close similarity of SARS-CoV-2 to the SARS-CoV suggests that the receptor of SARS-CoV-2 might be the same as that of SARS-CoV receptor (ACE2).[29]

Immuno-informatics approach can be used for the identification of epitopes for inclusion in COVID-19 vaccine candidates. Recently, immuno-informatics was used to identify significant cytotoxic T lymphocyte (CTL) and B-cell epitopes in SARS-CoV-2 S protein. The interactions between these epitopes and their corresponding MHC class I molecules were studied further by using molecular dynamics simulations and found that the CTL epitopes bind with MHC class I peptide-binding grooves *via* multiple contacts, thus indicating their potential for generating immune responses.[30] Such epitopes may possess the ideal characteristics to become part of COVID-19 vaccine candidates. The nucleocapsid (N) protein as well as the potential B cell epitopes of the E protein of MERS-CoV has been suggested as probable immunoprotective targets that induce both T-cell and neutralizing antibody responses.[31,32] Reverse genetic strategies have been successfully used in live-attenuated vaccines to inactivate the exonuclease effects of non-structural protein 14 (nsp14) or to delete the envelope protein in SARS.[5] Avian infectious bronchitis virus (IBV) is a chicken CoV. It was suggested that avian live virus IBV vaccine (strain H) might be useful for SARS[33] given that protection provided by strain H is based on neutralizing antibody production as well as other immune responses. Hence, avian IBV vaccine may be considered another option for COVID-19 after evaluating its safety in monkeys.[34]

Scientists of Rocky Mountain Laboratories are collaborating with Oxford University to develop a chimpanzee adenovirus-vectored COVID-19 vaccine candidate.[35] The Coalition for Epidemic Preparedness Innovations (CEPI) recently announced the initiation of three programs aimed to develop COVID-19 vaccines by utilizing established vaccine platforms.[36] Among the three programs, two are continuations of previously initiated partnerships. CEPI collaborated with Inovio in 2018 to developing DNA vaccine candidates for MERS ($56 M funding). The vaccine in development utilizes DNA Medicines' platform for delivering synthetic genes into cells for translation into antigenic proteins, which elicit T-cell and antibody responses. CEPI has collaborated with The University of Queensland in 2019 to develop the molecular clamp vaccine platform against multiple viral pathogens including MERS-CoV ($10 M funding). The vaccine platform functions by synthesizing viral surface proteins that get attached to the host cells and clamp them into shape. This facilitates easier recognition of antigens by the immune system.[36] Other than these ongoing programs, CEPI has announced funding to Moderna for comparing mRNA therapeutics and vaccines. They will design and manufacture an mRNA vaccine in collaboration with the Vaccine Research

Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID), a part of the National Institutes of Health (NIH).[37] NIAID-VRC scientists are developing a vaccine candidate expressing SARS-CoV-2 S protein in the mRNA vaccine platform technology. This vaccine is expected to undergo clinical testing in the coming months.[35]

Passive immunization

Direct administration of monoclonal antibodies (mAbs) may play an effective role in CoV control as an intervention in exposed individuals. It has been observed that patients recovering from SARS display potent neutralizing antibody responses.[9] A clinical trial proposed the use of a set of mAbs that functionally target specific domains in MERS-CoV S protein. These mAbs bind to six specific epitope groups interacting with the receptor binding, membrane fusion, and sialic acid-binding sites, which represent the three important entry functions of MERS-CoV S protein.[21,38] Moreover, passive immunization with poorly and potently neutralizing antibodies induces substantial protection in mice subjected to lethal MERS-CoV challenge. Thus, use of these antibodies may represent a novel approach to increase humoral protection against emerging CoVs by targeting various S protein epitopes and functions. The cross-neutralization capacity of SARS-CoV RBD-specific neutralizing mAbs greatly depends on the similarity between their RBDs. This is why SARS-CoV RBD-specific antibodies can cross-neutralize SARS-like (SL) CoVs, *i.e.*, bat-SL-CoV strain WIV1 RBD that had 8 amino acid differences to SARS-CoV, but not bat-SL-CoV strain SHC014 (24 amino acid differences).[39] Such cross-neutralizing SARS-CoV RBD-specific mAbs can be evaluated for efficacy with SARS-CoV-2. This requires a comparative analysis of SARS-CoV-2 RBD with SARS-CoV so that suitable RBD-specific mAbs can be identified and evaluated in clinical trials. Regeneron is trying to identify mAbs specific and effective for COVID-19. Combination therapy with mAbs and the drug remdesivir could be an ideal therapeutic option for COVID-19.[40] Further evaluation is required before confirming the efficacy of such combination therapy.

Technology is available for making fully human antibodies (such as human single-chain antibodies; Hu-scFvs) or humanized-nanobodies (single-domain antibodies, sdAb, VH/VHH) that can traverse across the membrane of the virus-infected cells (trans bodies) and bind to or interfere with biological activities of replicating virus proteins which consequently leads to inhibition of virus replication. Examples include trans bodies to influenza virus, hepatitis C virus, Ebola virus, and Dengue virus.[41] Thus, it is possible to generate trans bodies to CoV intracellular proteins such as the papain-like proteases (PLpro), cysteine-like protease (3CLpro) or other non-structural proteins (nsps) that are pivotal for CoV replication and transcription for safe, non-immunogenic, broadly effective passive immunization of CoV-exposed subjects and treatment of infected patients.

Animal models for vaccine evaluation

Suitable animal models for evaluating vaccines for SARS- and MERS-CoV are lacking or highly limited, making the process of vaccine development highly challenging.[42,43] Development of an efficient animal model that mimics the clinical disease can inform on pathogenesis as well as to develop vaccines and therapeutics against these CoVs. Several animal models have been evaluated for SARS- and MERS CoVs including mouse, guinea pigs, hamsters, ferrets, rabbits, rhesus macaques, marmosets, and cats.[42,44-50]

Early effort was directed in developing animal models for SARS-CoV, but the specificity of the virus to ACE2 (receptor of SARS-CoV) was a major hindrance to such efforts. Later, a SARS-CoV transgenic mouse model was developed by introducing hACE2 gene into the mouse genome.[51] The first animal model used for developing a MERS-CoV vaccine was rhesus macaques. Infected animals showed clinical symptoms such as increased body temperature, piloerection, cough, hunched posture, and reduced food intake.[52] Another frequently used animal model for MERS-CoV is the common marmoset, wherein the virus caused lethal pneumonia.[53] Humoral and cell-mediated immunity could be detected in both rhesus macaques and common marmoset following MERS-CoV immunization.[43,52,53] Roberts et al. established golden Syrian hamsters (strain LVG) as a model to assess vaccine protection to different SARS-CoV strains.[47] These hamsters are a potential model for studying CoV pathology and pathogenesis and vaccine efficacy. The attenuated NSP16 CoV vaccine was studied in mice.[54]

Attempts to develop animal models for MERS-CoV such as mice, hamsters, and ferrets face limitations due to the inability of MERS-CoV to replicate in the respiratory tracts of these species. Small animals (mice or hamsters) resisting natural infection with MERS-CoVs (which are susceptible to SARS-CoV) have been genetically modified to a more humanized structure, e.g., hDPP4 human, hDPP4-transduced, and hDPP4-Tg mice (transgenic for expressing hDPP4), and ascertained for susceptibility to MERS-CoV infection.[55] Alteration in the mouse genome using the CRISPR-Cas9 gene-editing tool could make the animals susceptible to CoV infection and virus replication.[56] Genetic engineering was used in the generation of 288–330$^{+/+}$ MERS-CoV mouse model, which is being used for the evaluation of novel MERS-CoV vaccines and drugs.[57]

Compared to the large animal models, small animals such as mice and rabbits are preferred due to lower cost, ease of manipulation, and readily available efficacy methods.[43] Further studies are needed to recognize suitable models for emerging SARS-CoV-2 by identifying receptor affinity of SARS-CoV-2 and studying disease manifestations, pathologies/viral pathogenesis associated with experimental inoculation of the virus in mice, rats, and other models, as well as examining virus-specific immune responses and protection. This would facilitate preclinical evaluations of candidate COVID-19 vaccines and drugs.

Cell culture systems

Several permissive cell lines to hCoVs including monkey epithelial cell lines (LLC-MK2 and Vero-B4) have been used in neutralization assays for assessing neutralization titers of antibody preparations. Goat lung cells, alpaca kidney cells, and dromedary umbilical cord cells have been found to be permissive for MERS-CoV.[58] SARS-CoV S protein has been found to mediate entry into hepatoma cell lines, targeted by

neutralizing antibodies in virus-infected patients.[59] Advanced *ex–vivo* 3D tracheobronchial tissue (mimicking epithelium of conductive airway) has been used for human CoVs.[60] Moreover, VLPs displaying SARS-CoV S protein were found competent for entry to permissive cells or transfected cells that overexpress virus receptors.[59,61,62] SARS-CoV-2 isolation has been attempted in Vero and the Huh-7 cells (human liver cancer cells).[63]

Pseudotyped virions/VLPs encoding reporter systems such as GFP or luciferase can be used for quantification and evaluation of the effectiveness of mAbs and drugs in inhibiting the cellular entry of CoVs.[64] Assays using pseudotyped virions/VLPs can be performed in BSL-2 facility since these do not use infectious virus. A safety concern for passive immunization with antibodies is a possible antibody-dependent enhancement (ADE) of virus replication. Antibodies with modified Fc fragments or without Fc fragment, *e.g.*, human single-chain antibodies (scFv), Fab, or $F(ab')_2$ are safe alternatives.

Several mAbs (fully human or humanized) that target both the S1-RBD and non-RBD as well as S2 domain of CoVs have been generated and tested in cell cultures for virus-neutralizing capability as well as in animal models for prophylactic and post-exposure efficacies.[65] These antibodies could be useful tools also in the development of vaccines, therapeutic drugs, and antiviral inhibitors.

Data from animal CoV vaccination suggest that systemic humoral or cell-mediated immune responses induced by parenteral administration may not be adequate to prevent respiratory tract infection.[66] Because respiratory mucosa is the initial site in CoV infection and transmission, mucosal immunization, such as using intranasal vaccine,[67] could be an effective strategy for prophylaxis by induction of mucosal and systemic immune responses. The molecular mechanisms of mucosal and systemic immunological factors are different, such that it is difficult to predict the surrogate marker for CoV efficacy. The best surrogate assays for protection as well as herd immunity toward different CoV infections warrant detailed investigations.

Therapeutics

The main measure in clinical management is focused on alleviating clinical symptoms and supportive care.[68-70] Therapeutic options that could be evaluated and used for COVID-19 include molecules binding to the virus, molecules, or inhibitors that target specific enzymes involved in viral replication and transcription, small-molecule inhibitors targeting helicase, essential proteases, or other proteins of the virus, host cell protease inhibitors, host cell endocytosis inhibitors, siRNA, anti-sense RNA and ribozyme, neutralizing antibodies, mAbs targeting host receptor or interfere with S1 RBD, antiviral peptide targeting S2, and natural products.[2,11] There is a long list of anti-CoV agents, mostly preclinical compounds yet to be evaluated as anti-COVID-19 agents. Some of these agents are in phase III trials for COVID-19, including remdesivir, oseltamivir, ASC09F (HIV protease inhibitor), lopinavir, ritonavir, darunavir, and cobicistat.[71] Many existing MERS- and/or SARS-CoVs inhibitors can be screened for efficacy. The RNA-dependent RNA polymerase (RdRp) sequence of SARS-CoV-2 has shown 96% identity to that of SARS-CoV, a critical finding since drugs developed for SARS-CoV RdRp might show similar efficacy for SARS-CoV -2 RdRp.[28]

S protein is considered the major target for designing CoV antiviral therapies such as S protein inhibitors, S cleavage inhibitors, neutralizing antibodies, RBD–ACE2 blockers, siRNAs, fusion core blockers, and protease inhibitors.[19] All such therapeutic strategies have shown potential *in vitro* and/or *in vivo* anti-CoV activities. Comparatively, even though *in vitro* studies performed with these agents have shown efficacy, most of them lack sufficient support due to the lack of randomized animal or human trials, hence of limited use for COVID-19. Hence, the necessary support of extensive animal and human trials is required for such therapeutics to become useful. The binding of COVID-19 and ACE2 affects the balance of renin–angiotensin system (RAS), potentially leading to exacerbation of severe pneumonia. Thus, it is speculated that ACEI and angiotensin type-1 receptor (AT1R) inhibitors might be able to reduce pulmonary inflammatory responses, thereby reducing the mortality.[72]

The guidance to COVID-19 control might be based on existing measures for MERS and SARS, with some further precautions due to the unknown nature of this new CoV.[14,73] The main treatments such as mechanical ventilation, ICU admission, and symptomatic and supportive care are recommended for severe cases. Furthermore, RNA synthesis inhibitors (like 3TC, TDF), remdesivir, neuraminidase inhibitors, peptide (EK1), anti-inflammatory drugs, abidol, Chinese traditional medicine, such as Lianhuaqingwen and ShuFengJieDu Capsules, could be the promising COVID-19 treatments.[2] However, further clinical trials are required for confirming safety and efficacy for COVID-19. A major limiting factor in the quest for identifying an ideal vaccine or therapeutic agent is time. It may take months to even several years for researchers to develop, produce, standardize, evaluate, approve, and commercialize therapeutic agents for COVID-19. Hence, current efforts should be directed toward identifying and evaluating drugs and immunotherapeutics that have proven efficacy against viruses similar to COVID-19.

The time required for drug discovery programs to develop, evaluate, and obtain approval for a new potent anti-COVID-19 agent could take more than 10 years.[4] In the present scenario, the development of a new therapeutic agent for COVID-19 is not a feasible option with regard to available time. Another option is to repurpose broadly acting antiviral drugs used for other viral infections. Such drugs have the advantage of easy availability, known pharmacokinetic and pharmacodynamic properties, solubility, stability, side effects, and also well-established dosing regimens.[4] Repurposed drugs are potential therapeutic options managing CoV infections. Repurposed drugs such as lopinavir/ritonavir and interferon-1β possess *in vitro* anti-MERS-CoV activity. The *in vivo* study conducted in common marmosets (non-human primate model) showed that animals treated with lopinavir/ritonavir and interferon-1β had better outcomes than untreated animals.[74] The combination of lopinavir-ritonavir and interferon-1β is being evaluated for MERS in the MIRACLE trial.[75] The same two protease inhibitors lopinavir and ritonavir, when combined with ribavirin, were found to be associated with favorable clinical responses in SARS patients indicating therapeutic efficacy.[10] As an early attempt to evaluate these

repurposed drugs in COVID-19, a controlled trial of ritonavir-boosted lopinavir and interferon-α 2b therapy has been registered for hospitalized patients in China (ChiCTR2000029308).[76]

Oral administration of neuraminidase inhibitors such as oseltamivir has been used as an empirical drug for COVID-19 suspected cases in China hospitals even though there is no evidence of its efficacy.[2] Recently, the *in vitro* antiviral efficacy of approved drugs such as ribavirin, penciclovir, nitazoxanide, nafamostat, and chloroquine was compared with that of the two broad-spectrum antiviral drugs remdesivir and favipiravir for COVID-19. Among the evaluated drugs, both remdesivir and chloroquine were found to be highly effective in controlling COVID-19 *in vitro*.[63] The study also pointed out that the three nucleoside analogs such as ribavirin, penciclovir, and favipiravir may not have significant *in vivo* antiviral effects against COVID-19 since higher concentrations were required to reduce the viral infection *in vitro*. Both remdesivir and chloroquine are being used for the treatment of other diseases and have a well-defined safety profile. Hence, such drugs can be used for evaluating their efficacy in patients of novel CoV infections.

Achievements in the development of vaccines and therapeutic agents for SARS- and MERS-CoV as well as recent ongoing progress for COVID-19 will facilitate the development of effective vaccines and therapeutics against this emerging virus. However, the present scenario of COVID-19 warrants the need for implementing robust preventive and control measures due to the potential for nosocomial infections.[77] We need to rely exclusively on preventive measures since considerable time is required before efforts to develop a new vaccine or antiviral agent becomes fruitful.

Conclusion and future prospects

Researchers are searching for effective and suitable vaccine candidates and therapeutics for controlling the deadly COVID-19. There are no effective vaccines or specific antiviral drugs for COVID-19. Hence, we have to rely exclusively on enforcing strict preventive and control measures that minimize the risk of possible disease transmission. Results obtained from the recently conducted *in vitro* study against COVID-19 are promising since the drugs remdesivir and chloroquine were found to be highly effective in controlling the infection. Direct clinical trials can be conducted among the patients infected with COVID-19 since these drugs are being used for treating other diseases and have well-established safety profiles, making the further evaluation of these drugs much easier. S protein is considered a key viral antigen for developing CoV vaccines, as shown in several preclinical studies. Although research is in progress to improve prevention, treatment, and control of COVID-19, the documented clinical data on different therapeutic approaches for CoVs are scarce. Further research should be directed toward the study of SARS-CoV-2 in suitable animal models for analyzing replication, transmission, and pathogenesis.

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Author contributions

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