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An Overview of Immunological Response, Anti-Viral Therapy and Vaccine Development Against Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)-2

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ABSTRACT

SARS-CoV-2, the causative agent of COVID-19 disease belongs to the group beta-coronavirus, which have infected around 120 million people so far with a 2.65 million death toll worldwide. Coronaviruses are enveloped viruses having positive sense, single-stranded RNA genome (26-32 kb size). This paper mainly reviews/focuses on the existing development on the vaccination and drugs developed against severe acute respiratory syndrome coronavirus (SARS-CoV). SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and the novel SARS-CoV-2 have come out as highly fatal strains of coronavirus for human beings. Novel SARS-CoV-2 requires angiotensin-converting enzyme 2 (ACE2) as its foothold receptor to gain entry into the host cells. Infected patients develop symptoms of fever, nonproductive cough, fatigue, dyspnea, sometimes diarrhoea and radiographic evidence of pneumonia. The immune system reacts violently against the virus spread inside the body which gives rise to a condition known as a cytokine storm. The potential anti-viral medicines suggested for COVID-19 treatment were hydroxychloroquine, Lopinavir/Ritonavir etc. which showed some combined effects, positive and negative. Many vaccines candidates are under different phases of trials all over the world against SARS-CoV-2. The main cause which enabled the quick development of vaccine technologies against SARS-CoV-2 was that an already established knowledge of the structure and function of its close associates namely SARS and MESRS was present. Molecular platforms used in vaccine development against SARS-CoV-2 are live attenuated vaccines, DNA based vaccines, RNA based vaccines, protein subunits and replicating viral vector vaccines.

Key Words: Coronavirus, COVID-19, Anti-viral drug, Hydroxychloroquine

INTRODUCTION

Coronaviruses (CoVs) form a family of pleomorphic, enveloped, positive sense, and single-stranded RNA viruses infecting vertebrates. They are named for crown-like spikes that occur on their outer surface. Coronaviruses are the members of the family *Coronaviridae*, sub-family *Orthocoronavirinae* and order *Nidovirales*.^{1,2} Coronaviruses infecting humans were initially identified in the mid of 1960. As of today, seven human coronaviruses are known which are designated as CoV-229E (alpha coronavirus), CoV-NL63 (alpha coronavirus), CoV-OC43 (beta coronavirus), CoV-HKU1 (beta coronavirus), SARS-CoV (beta coronavirus), MERS-CoV (beta coronavirus), and current novel virus SARS-CoV-2 (beta coronavirus).¹⁻⁴ Among these, three highly pathogenic strains (i.e. SARS-CoV; MERS-CoV; and SARS-COV2)

are reported and emerged in the years 2002, 2012 and 2019 respectively. The causative agent of the COVID-19 disease is this novel virus named SARS-CoV-2 by the international virus classification commission (IVCC). It was initially isolated from three persons that had developed the symptoms of acute respiratory illness, reported in China (Wuhan, December 2019). Coronaviruses are zoonotic pathogens that present a wide range of clinical features ranging from asymptomatic course to need of hospitalization in ICUs, producing infections in respiratory, hepatic, and gastrointestinal system.²⁻⁴ Initially, they were considered slightly pathogenic for humans until they appear to cause severe acute respiratory syndromes (SARS) in the year 2002 and 2003. Such strains were named SARS-CoV. Approximately ten years after the SARS-CoV outbreak, another highly pathogenic strain emerged in the Middle East which was named MERS-

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CoV. Recently in December 2019, another highly pathogenic strain named SARS-CoV-2 emerged as a global pandemic and named COVID-19 on 11th February 2020 by World Health Organization (WHO). SARS-CoV-2 emergence regarded as a highly virulent coronavirus in the human population and considered as the seventh member of the coronavirus family infecting humans.³⁻⁶

STRUCTURE & HOST INVASION

Coronaviruses are enveloped viruses in nature (50-200 nanometers in diameter) having a single-stranded RNA genome (i.e. 26-32 kb).⁷ One of the highly pathogenic strains (i.e. MERS-CoV, SARS-CoV, and SARS-CoV-2) have been identified and belongs to the members of beta-coronavirus genera. The genome of the newly isolated strain of virus showed 88% identity with the genome sequences of SARS-like Coronaviruses (arise from the bat) and also its identity is almost fifty per cent with the MERS-CoV genome sequence.^{8,9} The SARS-CoV-2 genome is found to be identical to typical coronavirus which possesses open reading frames (approximately ten in number).¹⁰⁻¹² About two-thirds viral RNA, first ORFs (ORF1a/b) are translated into polyproteins (two in number; large size) which are subsequently processed to give rise to at least 16 nonstructural proteins (nsp1-nsp16). These nonstructural proteins collectively generate the viral replicase transcriptase system.¹³⁻¹⁵ All these nonstructural proteins are rearranged in RER into vesicles of double membranes and are considered as sites where viral transcription takes place. On the rest of one-third of the genome, other ORFs are housed which are different from the former one's in that they code for structural proteins.¹²⁻¹⁵ There are four main such structural proteins [spike(S); envelope (E); nucleocapsid (N); and membrane (M) proteins} which are coded by them. It is also found that apart from encoding these four structural proteins, they also code for several accessory proteins whose function is not known yet but it is known that they do not participate in the replication process of the virus. After a deep insight into physiological interactions, several groups of scientists all over the world have reported that COVs strains require the angiotensin-converting enzyme 2 (ACE2) as its foothold or keystone receptor to gain entry into the host cells.^{16,17} It seems that SARS-CoV derived from bats and then evolved as a non-bat ACE2 variant is called a spillover infection. In general, spike protein in envelope binds to its complementary cellular receptor ACE2 for SRAS-CoV-2, ACE2 as well as CD209L (a C-type lectin receptor also called as L-SIGN) for SARS-COV and DPP4 receptor for MERS-CoV.¹⁶⁻¹⁸ Spike protein domain and host receptor interaction are key for host selection and disease propagation. Penetration of SARS-CoV into the host cells was initially discovered which is to be accomplished by a direct fusion between Virus and plasma membrane of target cell but subsequently

it was found that a prerequisite proteolytic cleavage event occurs on Spike (peplomer) protein at S2 position that possibly mediates the initial membrane fusion and viral infectivity. ACE2 receptors are most abundant in type II alveolar cells of the lungs and this explains that why the lungs most affected organs in this disease are. SARS-CoV-2 may also use Basigin (CD147) receptor to get assistance in host cell entry.^{11,12,19} Initial priming of Spike protein by transmembrane protease, Serine2 (TMPRSS2) is essential to gain entry. After the virion attaches to the target cell in the host, the cells protease cuts open the Spike protein of the virus. In this step, a fusion peptide in the S2 subunit of the spike is exposed to the ACE2 receptor.²⁰⁻²³ Apart from membrane fusion events, the clathrin-dependent and independent endocytosis also strengthens the foothold and entry. After fusion occurs, the virion is surrounded by an endosome, keeping it aside from the rest of the cell. When the pH of the endosome is lowered or when cysteine protease Cathepsin cleaves it, virion escapes. It then releases its single-stranded RNA into cell cytoplasm and subsequently translated to two polyproteins, giving rise to all structural and non-structural proteins.²⁰⁻²³ The newly generated envelope glycoproteins are directly inserted into the ER or Golgi, finally forming nucleocapsid by the combination of the Genomic RNA and nucleocapsid proteins. Finally, viral particles develop into the ER -GOLGI intermediate compartment (ERGIC) which are now ready to fuse up with cell plasma membrane for their release as progeny virus to infect nearby cells.

IMMUNOLOGICAL INTERVENTIONS

Infected patients develop symptoms of fever, nonproductive cough, fatigue, dyspnea, sometimes diarrhoea and radiographic evidence of pneumonia. All these symptoms are similar to those developed by SARS-CoV and MERS-CoV infections. The immune system reacts violently against the virus spread inside the body which gives rise to a condition known as a cytokine storm, which is defined as a deadly uncontrolled inflammatory response. All this result from the quick release of the massive release of pro-inflammatory cytokines (IFN-gamma, IL-6, TNF α etc.) and chemokines (i.e. CCL3/CCL5, CXCL8/CXCL10 etc.) by immune system cells after detection of infection.²⁴⁻²⁷ Cytokine storm, in turn, triggers a violent attack to even normal cells of the body, causing ARDS (adult respiratory distress syndrome) and multiple organ failure which may prove fatal. This all has occurred in patients with severe infection with SARS-CoV-2 which reveals that ARDS due to cytokine poisoning, may be considered as one of the main cause of death in COVID-19 disease. Such an exaggerated immune response against coronaviruses marks them as super-antigens. After the virus gains entry into the body cells, its antigenic peptides are processed by antigen-presenting cells (APC'S) and upload

to the Major histocompatibility complex (MHC) on their surface. On the other hand, infected body cells start losing the expression of MHC1 on their surface and are quickly targeted by patrolling Natural killer cells. Infected cells that successfully upload the antigenic peptide to MHC-I molecules on their surface are conceded through virus-specific CTLs. In SARS-CoV2, antigen presentation is totally dependent on MHC-I and MHC-II molecules and subsequently inducing both cellular and humoral immune arms.^{24,25} In literature, IgM antibodies have a short half-life and start disappearing (week 12) while IgG antibodies retain for a longer time duration which means that IgG antibodies mainly play a protective role against various pathogenic micro-organisms.²⁶

For surviving in the host cell, SARS-CoV along with or without MERS-CoV incorporate multiple strategies about avoid immune attack. The evolutionarily conserved motifs existing on microbial surfaces known as MAMPs (microbe-associated molecular patterns) which cognize through PRRs (pattern recognition receptors) present on host APCs. However, SARS-CoV and MERS-CoV induce the generation of vesicles having double membranes that lack MAMPs and successfully replicate in them. This avoids the host PRRs to recognize the antigen.²⁷ Thus, the development of SARS-CoV-2 disease depends on the interaction between the immune system and the virus. If the immune system dominates initially, the patient is recovered quickly but if the virus got success in immune evasion, it can prove fatal.

ANTIVIRAL THERAPY

Medicines that could show antiviral effect against the SARS-CoV-2 novel virus are under investigation. No medication has yet been shown to inhibit the virus or its replication process. Drug and vaccine development is a complex process that requires more than five years of assuring the efficiency and safety of the new agent. In Feb 2020, WHO said that it is not possible to develop a safe and efficient vaccine in less than 18 months. By August 2020, several potential antiviral therapies including Remdesivir, Favipiravir, and Lopinavir involved in international solidarity trial, were in the last stage of human testing.^{25,28} Other potential therapeutic agents that were brought under clinical trials to evaluate their efficiency against COVID-19 disease were corticosteroids, vasodilators, anti-parasitic agents, immune therapies, bevacizumab, recombinant angiotensin-converting enzyme -2, among others.^{25,29} WHO in March 2020 launched an integrated 'Solidarity trail' in almost ten countries to evaluate in thousands of infected people the potential efficiency and safety of already available antiviral agents. The potential medicines that undergo initial studies were Remdesivir, Lopinavir, Ritonavir, Lopinavir-Ritonavir combined, Lopinavir-Ritonavir combined with

interferon- beta, Hydroxychloroquine. In April 2020, the British recovery (Randomized evaluation of COVID-9 Therapy) trial was started as one of the largest COVID-19 clinical studies which evaluated another potential therapy against COVID -19 diseases. Agents under investigation were Lopinavir/Ritonavir, low dose Dexamethasone (an anti-inflammatory corticosteroid) and Azithromycin.³⁰

Remdesivir is a nucleotide analogue, an antiviral medication originally developed to treat Ebola virus disease. This specific adenosine analogue gets inserted into viral RNA chains, causing the premature breaking of the chains. Remdesivir is a broad-spectrum anti-viral medication whose emergency use authorization (EUA) was approved on 1 May 2020 for people with severe COVID-19 infection.^{31,32} It has been shown to shorten the period it needs to recover from the infection. As of April 2020, the most promising treatment for COVID-19 as reported in various trials was Remdesivir as its use in *Rhesus macaque* monkeys suffering from COVID-19 infection shows that it reduced damage and progression of the disease, but don't affect viral shedding. Side effects that were reported by the use of Remdesivir are low blood pressure, low count (red blood cells), nausea, vomiting and elevation in the levels of liver enzymes and bilirubin.³³

Chloroquine, an anti-malarial medication was initially used in India, China, South Korea and Italy for the treatment of COVID-19. Hydroxychloroquine is more commonly available in many countries than chloroquine. Through solidarity clinical trials and recovery trials, it was evaluated that these medications do not show any promising results and thus their use was withdrawn. It was also reported that both these drugs have potentially serious side effects such as Retinopathy, Hypoglycemia, Cardiomyopathy, and life-threatening Arrhythmia.^{29,32}

In March 2020, '3CLpro' protease in SARS-CoV-2 was spotted as a target for drugs that can be used after infection. The service of this enzyme is essential in processing the polyprotein that is related to replication. Protease inhibitors like lopinavir and ritonavir that are approved for treating HIV have evidence of activity against coronaviruses.^{33,34} New protease inhibitors that can specifically target protease 3LCpro are being researched in many countries. The main among them are Rupintrivir, Cipro-1, and GC373.

As of August 2020, various categories of early-stage clinical research for evaluating anti-COVID-19 therapeutic agents included 81 candidates of antibodies, 31 candidates of anti-viral, 34 candidates of cell-based compounds, 6 candidates of RNA based candidates along with other therapies including interferon, protein-based, Receptor modulating compounds, and antibiotics.³⁵ Some drug candidates are listed in Table-1.

Table 1: Selected Drug Candidates for Covid-19 Treatment

Drug name	Mechanism of action	Previous indications	Trial Sponsors	Results
Remdesivir	Anti-viral action, an Adenosine nucleotide analogue, RNA synthesis inhibitor.	Originally developed for the treatment of Hepatitis C and Ebola virus disease	Inserm WHO, Gilead, NIAID, USA	After evaluation in several clinical trials, both promising and negative effects reported
Hydroxychloroquine/chloroquine	Anti-parasitic and anti-rheumatic agents	Malaria, Rheumatoid arthritis	CPI, WHO, Inserm, France	Withdrawn in June 2020 from solidarity and RECOVERY trials because of no beneficial effects on COVID-19 disease and potential side effects
Favipiravir	Anti-viral agent	Used against influenza virus	Coalition for Epidemic Preparedness Innovations (CEPI), U.K.	Reduces the period needed for recovery but may be less effective in severe cases.
Ritonavir/lopinavir with and without interferon beta-1a.	Antiviral agents, Protease inhibitors	Originally developed for HIV and other retroviruses.	WHO, CEPI, INSERM, Oxford University, U.K.	No clinical benefit on COVID-19 disease
Dexamethasone	Anti-inflammatory corticosteroid	Used for treatment of multiple conditions such as rheumatic disorders, asthma, skin diseases, allergy and inflammation.	Oxford University, U.K.	Preliminary results reveal that this drug could reduce mortality by about a third in patients on ventilators and by about a fifth in patients on concentrated oxygen therapy, but do not benefit patients who are not on respiratory support.
Remdesivir with Baricitnib	An antiviral agent with an anti-Rheumatic agent of a class inhibitor of Janus Kinase (JAK2)	Baricitnib, a drug used Rheumatoid arthritis	National Institute of Allergy and Infectious Diseases, USA	After evaluation in several clinical trials, both promising and negative effects reported.
Remdesivir with interferon beta-1a	Interferon-beta-1a is a cytokine.	Interferon-beta-1a used in the management of multiple sclerosis (MS)	National Institute of Allergy and Infectious Diseases, USA	After evaluation in several clinical trials, both promising and negative effects reported
CD24Fc	Immunomodulator against inflammatory response	New drug candidate.	OncImmune, Inc., USA	Under investigation as of August 2020.

VACCINATION AGAINST COVID-19 DISEASE

A COVID-19 vaccine is a biological preparation from the SARS-CoV-2 virus intended to induce acquired immunity against COVID-19 disease. Vaccine development is a complex process that requires more than five years to assure its efficiency and safety for human use. The main cause which enabled the quick development of vaccine technologies against SARS-CoV-2 was that an already established knowledge of the structure and function of its close associates namely SARS and MESRS was present.³⁶Molecular platforms used in vaccine development against SARS-CoV-2 are live attenuated

vaccines, DNA based vaccines, RNA based vaccines, protein subunits and replicating viral vector vaccines.³⁷ As of March 2021, more than 300 vaccine candidates were in different phases of development. Among them, almost 73 were in clinical research, in which 24 were in phase I trials, 33 in phase II trials and 16 in phase III trials. Several vaccines in phase III trials have shown more than 90% efficiency in preventing COVID-19 infections in Figure 1. As of March 2021, more than 10 vaccines were authorized for use in the general public. These vaccines included two RNA vaccines (Modern vaccine and Pfizer-Biotech vaccine), four viral vector vaccines (Convidicea, Sputnik V, oxford- Astrazeneca vaccine, Johnson &

Johnson vaccine), two protein sub-unit vaccines (RBD-Dimer and EpiVacCorona) and four inactivated vaccines (CoviVac, coronaVac, Covaxin and EpiVacCorona).^{38,39}

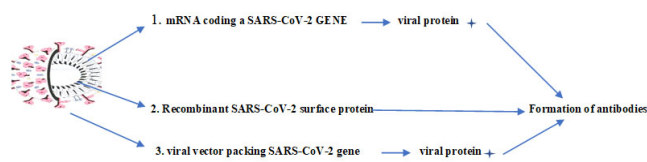


Figure 1: Antibody formation of Coronavirus

VACCINE TYPES AND THEIR TECHNOLOGY PLATFORMS

As of January 2021, nine different technology platforms were under research trials to develop an effective COVID-19 vaccine. Most of the vaccine candidates under clinical trials are mainly focused on the spike protein of SARS-CoV-2 and its variant as the primary antigen of infection. Different platforms developed include nucleic acids (nucleoside-modified m-RNA and DNA), live attenuated viruses, recombinant proteins, non-replicating viral vectors, protein subunits, peptides and inactivated viruses.^{40,41} Vaccine platforms in development are intended to enhance flexibility for antigen administration and effectiveness in immune response even in susceptible population subgroups like immunocompromised ones. Some of the different technology platforms incorporated in different vaccines against COVID-19 is as below.

RNA VACCINES

An RNA vaccine possesses RNA which after introducing into specific tissue, functions as messenger RNA (mRNA) to make cells able to produce a foreign protein which in turn triggers an adaptive immune response. In this way, the immune system is taught to identify and destroy the corresponding antigen.⁴² Most RNA vaccines use nucleoside-modified messenger RNA (Moderna) in which some nucleosides are replaced by their naturally modified or synthetic nucleoside analogues. Delivery of such mRNA into specific tissue is done by co-formulating the molecule in lipid nanoparticles which aid the absorption of mRNA into the cells. RNA vaccines were the first vaccine candidates against SARS-CoV-2 authorized in the US. Vaccines of this type include Moderna COVID-19 vaccine, Pfizer-BioNTech COVID-19 vaccine and currently awaiting CVnCoV RNA vaccine from curve ACC.⁴³

ADENOVIRUS VECTOR VACCINES: These vaccines incorporate adenovirus shell possessing DNA that codes for SARS-CoV-2 protein. These are non-replicating viral vectors which means that new virions are not made but only pro-

duce specific antigen which prompts a systemic immune response. Authorized vaccines of this type as of January 2021 are Sputnik V, Convidicea, Oxford-AstraZeneca COVID-19 vaccine and Johnson & Johnson vaccine. Among these only Convidicea and Johnson & Johnson vaccine are one shot vaccine.⁴⁴

SUB-UNIT VACCINES: sub-unit vaccines contain one or few antigens like protein sub-units or any particle fragmented from the disease-causing pathogen that specifically induces an immune response. Vaccines of this type authorized for COVID-19 as of January 2021 are peptide vaccine EpiVac-Corona, Novavax COVID-19 vaccine and RBD-Dimer.^{45,46}

INACTIVATED VIRUS VACCINES: Inactivated vaccines possess virions that have been cultured and then inactivated by heat or formaldehyde treatment to lose their disease-causing capacity but still trigger an immune response. Vaccines of this type authorized for use against COVID-19 as on January 2021 are CoronaVac from China, Indian Covaxin and CoviVac, BBIBP-CorV and Valneva COVID-19 vaccine.⁴⁷

In addition, some other types of vaccines that are currently in clinical trials include DNA plasmid vaccines, conjugate vaccine, lentivirus vector vaccine.⁴⁸

CONCLUSION

COVID-19 is a highly contagious disease having high mortality in elderly people, immune-compromised, diabetic and the patients with hypertension and respiratory disorders. Despite knowing the structure and mode of the pathogenesis of the SARS-CoV-2, we have not been successful in its treatment successfully. Some ant-viral and steroidal drugs have shown promising effects against it in different regions on different patients. But no universal drug is found effective so far in the treatment of COVID-19. Special attention is required for efficient vaccine production or drug development against SARS-CoV-2. Recently, different vaccines have hit the clinics due to untiring efforts of the scientific community working day and night on the trials in different stages in different countries. As of January 2021, nine different technology platforms were under research trials to develop an effective COVID-19 vaccine. Most of the vaccine candidates under clinical trials are mainly focused on the spike protein of SARS-CoV-2 and its variant as the primary antigen of infection. Different platforms developed include nucleic acids (nucleoside-modified m-RNA and DNA), live attenuated viruses, recombinant proteins, non-replicating viral vectors, protein subunits, peptides and inactivated viruses.

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