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Essentials of COVID-19 and treatment approaches

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1. Introduction

Coronavirus disease 2019 (COVID-19) pandemic is one of the greatest disasters witnessed by the world. It has not only devastated the economy but also caused a long-lasting effect on people. COVID-19 is caused among people of all age groups and its causative agent is the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus belongs to the family Coronaviridae that has been into existence since the 1980s and is also the family of SARS and Middle East respiratory syndrome (MERS) virus. The name corona refers to the virus' physical appearance under the microscope—crownlike projections called spikes. Researchers around the world are trying their best to come up with a treatment regimen that could beat the clinical trials in the market. Currently, there is no treatment available for this virus. However, there are proposed theories of treatment that could be made available. The main focus lies more toward prophylaxis rather than treatment.

Rigorous trials have been conducted around the world to find a solution to this deadly pandemic. Researchers have been ground toward finding more similarities between the two other diseases spread in the past two decades, i.e., SARS and MERS. There are many similarities in the SARS-CoV and SARS-CoV-2, including their mechanism of host cell interaction, that is, their spike proteins. There lies a huge responsibility on the WHO for selecting the right and efficacious agents to combat the alarming rate of infection among countries. Some drugs and combinations have been approved by the WHO to use as an emergency measure, while their trials are still in progress, including remdesivir (RDV), interferon beta, chloroquine/hydroxychloroquine, and lopinavir/ritonavir.

New vaccine-related trials have also begun, and it is estimated that the vaccine will be fully available within a year. This chapter aims to cover the structure of SARS-CoV-2, its method of infection, its life cycle, and treatment approaches with developmental measures.

2. History and epidemiology

The history of coronavirus (CoV) in existence is traced back to the late 1960s when a virologist, Tyrrel, worked with his colleagues on human stains and a number of other human viruses. They inspected the bronchitis virus, mouse hepatitis virus, and gastro-enteritis virus of transmissible origin. These appeared morphologically similar through an electron microscope. This new group of the virus was later termed as *corona* virus because of its crownlike surface projections. *Corona* was later determined as the genus of these viruses [2]. The virus belongs to the Coronaviridae family, the subfamily is Orthocoronavirinae, and the order is *Nidovirales.* This subfamily is classified into four distinct genera: Alpha Coronavirus, Beta Coronavirus, Delta Coronavirus, and Gamma Coronavirus. Beta CoV is further divided into four lineages; A, B, C, and D [10]. They were first imaged by Scottish virologists, June Almeida et al. at St. Thomas Hospital in London [1]. Before 2003, only two human CoVs were known; HCov-229E and HCoV-OC43. These were reported to cause upper respiratory tract infections in humans. In 2004, another group of previously recognized CoVs was reported and named as HCoV-NL63. It was followed by HCoV-HKU1 in the year 2005, and it is a betaCoV of A lineage [3]. All these viruses were known to cause mild upper respiratory tract infections. Other human CoV's, namely, SARS-CoV, SARS-CoV-2, and MERS-CoV, are also of beta origin but B and C lineage, respectively. MERS-CoV was reported in the year 2012. By early June 2013, about 55 cases of MERS were confirmed, with about 31 deaths (56%) in Jordan, Saudi Arabia, Qatar, and the United Arab Emirates. Reports concluded about 2500 cases and 800 deaths by the infection. SARS originated in the Guangdong province of China in 2002 and then spread to five continents through air and infected 8098 people and caused 774 deaths [1−3].

SARS-CoV-2, which is known to cause COVID-19, is now among the top three most severe of seven CoVs that humans have encountered in the past 20 years. These three viruses are said to cross special barriers and cause deadly pneumonia in humans. They are fast-evolving viruses [1,3]. CoVs are capable of combining with different strains of coexisting viruses and then produce novel strains; they are capable of crossing barriers. According to the latest research findings on the COVID-19 genome, the novel CoV has a longer genome than the flu virus, which means that there are fewer mutations. COVID-19 has shown to mutate rather slowly. It has the proofreading mechanism that minimizes the error rate and in turn slows down the speed of mutation. This is one "saccharin pill" to the developers of vaccines and medicines because if the virus showed mutation like other flu viruses, which is 24 times in a year or two times in a month, then it will be highly required to upgrade the vaccine time to time [1].

In late December 2019, an outbreak of pneumonia of an unknown cause was reported in Wuhan, China. It was considered the first case of an unknown cause. After the disease started spreading, the WHO declared it to be because of CoV and named the disease as COVID-19 [3,4,13]. It was renamed SARS-CoV-2 by the Coronaviridae Study Group (CSG) on an International Committee on Taxonomy of Viruses (ICTV), while in the interim it

was renamed HCoV-19 (common name) by a group of virologists in China. By February 24, 2020, approximately 73,331 cases were reported positive, including 2618 deaths in China. It was the same time when 27 other countries started showing their first symptoms of the disease [13]. It is seen that the mutations in CoVs do not interfere with their potency; they are small and can be neglected for a while [1]. The report presented by the WHO shows there are 4,494,873 confirmed cases of COVID-19 around the world, including 305,976 reported deaths (Table 20.1A,B).
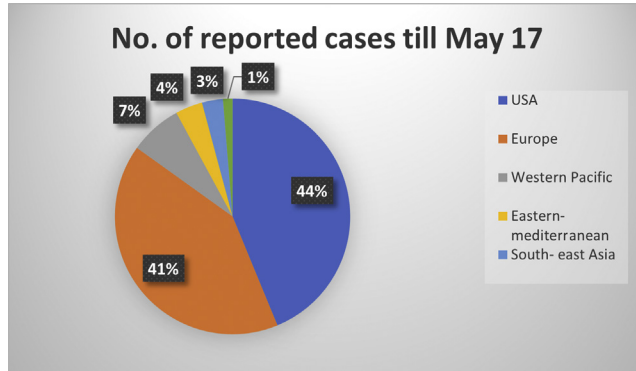

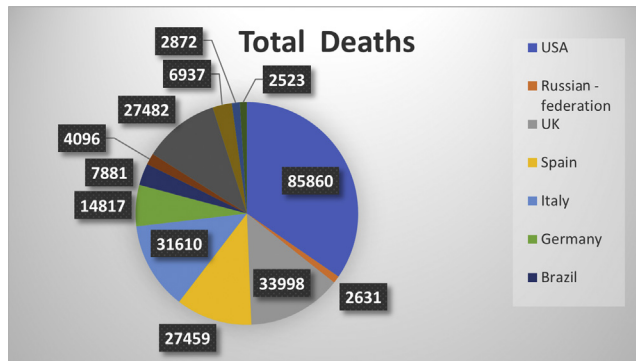**CHART 20.1** The region-wise data generated by the WHO till May 17, 2020.

CHART 20.2 The total deaths reported.

Table 20.1A   The present status of the pandemic around the world [79].

| Daily cases region-wise by the WHO	No. of reported cases	The daily increase in cases as of May 17, 2020
The United States	1,966,932	12,434
Europe	1,848,445	3275
Western Pacific	323,055	39
Eastern Mediterranean	167,546	130
South-East Asia	129,520	311
Africa	58,663	298

The region-wise data generated by the WHO till May 17, 2020, is shown.

Table 20.1B   The highest cases by country/territory/area, generated by the WHO till May 17, 2020.

Highest cases by country/territory/area	No. of reported cases	Deaths reported
The United States	1,409,452	85,860
Russian Federation	272,043	2631
The United Kingdom	236,715	33,998
Spain	230,183	27,459
Italy	223,885	31,610
Brazil	218,223	14,817
Germany	173,772	7881
Turkey	146,457	4096
France	139,646	27,482
Iran	118,392	6937
India	85,940	2872
Peru	84,495	2523

3. Structure of SARS-CoV-2

SARS-CoV2 is an enveloped, positive, single-stranded RNA virus with nucleocapsid sized 65−125 mm in diameter [3,4]. In CoVs, the genomic structure is organized in a ++single-stranded RNA of approximately 21−32 kb in length; they are the largest known RNA viruses (Fig. 20.1). They have a 5′- cap structure and 3′- poly-A tail [4]. Furthermore, the genomic characterization reveals that bats and rodents are the carriers of alpha CoVs (α-CoVs) and beta CoVs (β-CoVs), whereas the avian species showcase the gamma CoVs and delta CoVs [10]. Only α-CoVs and β-CoVs are known to affect human beings [6,9,10]. Studies have shown that SARS-CoV-2 has a similar genomic structure as the beta CoVs, comprising of a 5′-untranslated region (UTR), a replicase complex encoding for nonstructural proteins (NSPs), a spike protein gene(S), an envelope protein gene (E), a
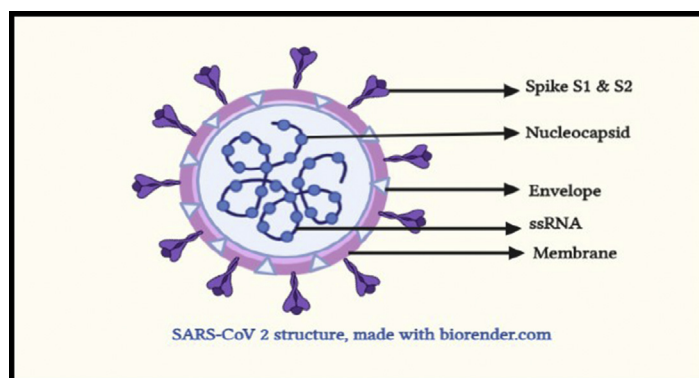
FIGURE 20.1 Structure of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *ssRNA*, single-stranded RNA.

membrane protein gene (M), a nucleocapsid protein gene (N), 3′-UTR, and various unidentified nonstructural open reading frames [9]. S glycoprotein of SARS-CoV-2 shares approximately 80% identity with the bat SARS-CoV ZXC21 S and ZC45 S glycoprotein. The two species are found in *Rhinolophus sinicus* (Chinese horseshoe bats) [6,7,9]. SARS-CoV-2 and SARS-CoV share 89.8% sequence identity in their spike (S) proteins. Spike proteins belong to the class of glycoproteins containing two subunits: S1 subunit and S2 subunit. The S2 subunit mediates the membrane fusion process, and the S1 subunit utilizes angiotensin-converting enzyme 2 (ACE2) as a receptor to infect the host cell [13]. Spikes can be seen under the electron microscope as a clear, 20-nm-long, bulbous surface projections [11]. The structure of CoV plays an important role in understanding the available treatment options that have been developed and those which are yet to be developed.

Spike proteins are clove-shaped projections on the surface of CoVs. They are divided into three distinct regions, namely, ectodomain (ED) region, transmembrane (TM) region, and intracellular domain. Both S1 and S2 subunits on the C-terminal compose the ED region. N-terminal domain and C-terminal domain are the main components of the S1 subunit. It also has a receptor-binding domain. On the contrary the S2 subunit includes two regions: two heptad repeat regions (HR1 and HR2) and a hydrophobic fusion peptide [39].

The E proteins are the smallest TM structural protein of CoV, ranging 8.4−12 kDa in size [39,40]. E protein comprises of two domains: the hydrophobic domain and the charged cytoplasmic tail. The structure may vary with different CoVs. There is no clarity yet on the function of E protein ion channels in CoVs; however, they play a role in CoV assembly and budding [39].

The M protein has the function of maintaining the shape of the virus, interacting with other proteins, and stabilizing the nucleocapsid protein [39].

Nucleocapsid protein, on the other hand, helps in dimerization and RNA binding.

CoVs contain several proteases in their genetic material, comprising a replicase gene that codes for 16NSPs in the form of two large polypeptides (PP1a and PP1b). For these PPs to release NSPs, two enzymes act on them: the C-terminal end is cleaved by chymotrypsin-like cysteine protease (3CLpro) and the N terminal is processed by papain-like proteases (PL^{pro}) [39,41].

4. Pathogenesis

SARS-CoV (S) and SARS-CoV-2 (S) are known to bind to ACE2 receptors [6]. They can bind to ACE2 receptors of different animal species; this is how the virus jumps from animals to humans [8]. ACE2 is a membrane-associated aminopeptidase expressed in vascular endothelial, renal, cardiovascular tissues, and epithelia of small intestine and testes. The extracellular portion of this receptor contains alpha-helix and lysine 353 and proximal residue of N terminus of beta-sheets 5, which interacts with
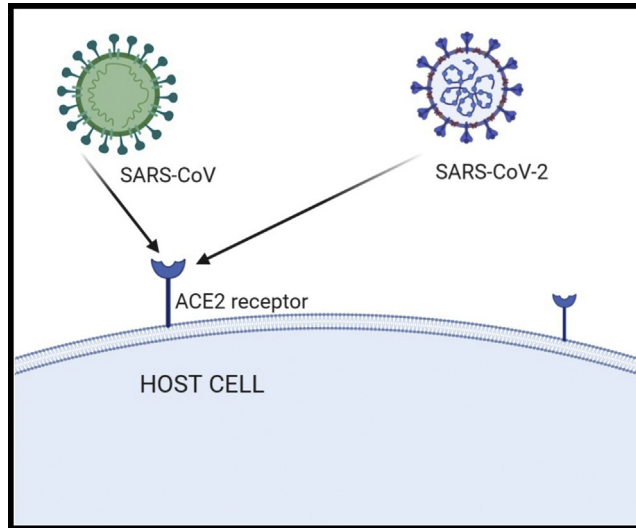
FIGURE 20.2 Severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 bind to the same angiotensin-converting enzyme 2 (ACE2) receptor.

the receptor-binding domain of the virus: the affinity of interaction is high. A study reports that the SARS-CoV infection of human airway epithelia depends upon the state of epithelial differentiation and ACE2 messenger RNA and protein expression. The virus infects the well-differentiated ciliated epithelial cells expressing ACE2 [7].

There are structural similarities and sequence conservation among the two viruses, which pose a way in which both recognize their entry into a cell through human ACE2 (Fig. 20.2).

Walls et al. conclude that most pathogenic bacteria exhibit S glycoprotein trimers in open and closed conformations to infect. As in the case of CoVs, the highly pathogenic virus has shown a partially *open* state, while the ones associated with common cold exist in closed states [6]. The S glycoprotein is exposed on the surface and directs the entry of the virus into a host cell. It can serve as a potential target of neutralizing antibodies (Abs) upon infection and also as the focus of therapeutic and vaccine design [6].

Apart from ACE2 receptors on the host cell surface, there are other facilitators of SARS-CoV-2 entry, including enzymes like furin, trypsin and other proproteins, cathepsin, TM proteases like TMPRSS and elastase. Proteases like TMPRSS2 and TMPRSS11a exist within the respiratory tract: TMPRSS2 is known to produce a complex reaction with the ACE2 receptors, which aids an efficient penetration of the virus into the cell. The enzyme activates the spike proteins, which then splits itself into S1 and S2 and S2′ subunits, promoting an endosome-independent cell entry [11,12]. They belong to the family of TM serine proteases type II and are capable of cleaving influenza virus hemagglutinin protein epithelial cells [15,16]. In their findings, Kim et al. reported that TMPRSS2 is indispensable for development and homeostasis, which makes an attractive drug target.

To understand the exact alignment of receptors and virus entry and its replication, we must understand the life cycle of SARS-CoV-2.

4.1   The life cycle of SARS-CoV-2

4.1.1   *Virus entry*

The invasion of the virus occurs in the cell via two ways: by endosome formation or plasma membrane fusion. The spike proteins S1 and S2 attach to the ACE2 receptors of the host cell. These receptors are exposed on the outer surface of the cell membrane and are responsible for the cleavage of S1 and S2 subunits. This cleavage then permits the entry of the virus into the cell.

During the uptake of the virus by the endosomes, cathepsin L activates the spike protein; spike proteins can also be activated by TMPRSS2 (cellular serine protease), and this initiates fusion of the viral membrane with the plasma membrane [17]. Studies have shown that certain receptor-induced conformational changes are essential to either expose the protease cleavage site or promote membrane fusion. This process occurs before proteolysis and at low temperatures [18].

It is thought that SARS-CoV-2 may enter the cell through pH- and receptor-dependent endocytosis [19].

4.1.2   *Translation (virion replication machinery) and replication*

After the viral RNA is released into the host cell, polyproteins (pp1a and pp1b) are translated. The genomic RNA encodes for structural proteins and NSPs that have an important role in virion assembly and viral RNA synthesis, respectively [68].

It starts with polyprotein translation: the cleavage is aided by papain-like protease (Pl^{pro}) and 3C-like protease (3CL-pro). These form functional NSPs such as helicase or the RNA replicase-transcriptase complex. It can function as one of the major inhibitory pathways [69,70].

### 4.1.3   *Translation (viral structural protein) and virion assembly*

RNA-dependent RNA polymerase (RdRp) helps in the replication of structural protein-RNA. Ribosomes that are bound to the endoplasmic reticulum (ER) aid in the translation of S proteins, envelope proteins, and membrane proteins [5]. The nucleocapsid is formed from genomic RNA. It is studied that the replicase transcriptase machinery of the CoV is found at the host ER and viral structural proteins assemble within the host ER, which makes it a potential drug target to block both viral genome replication and capsid assembly in the formation of new virus particles during infection [5,14].

4.1.4   *Release of virus*

Viruses are released out of the cell by exocytosis. There are drugs available that can inhibit the virus release out of the cell, such as oseltamivir [71].

Fig. 20.3 shows a flowchart of the life cycle of SARS-CoV-2.
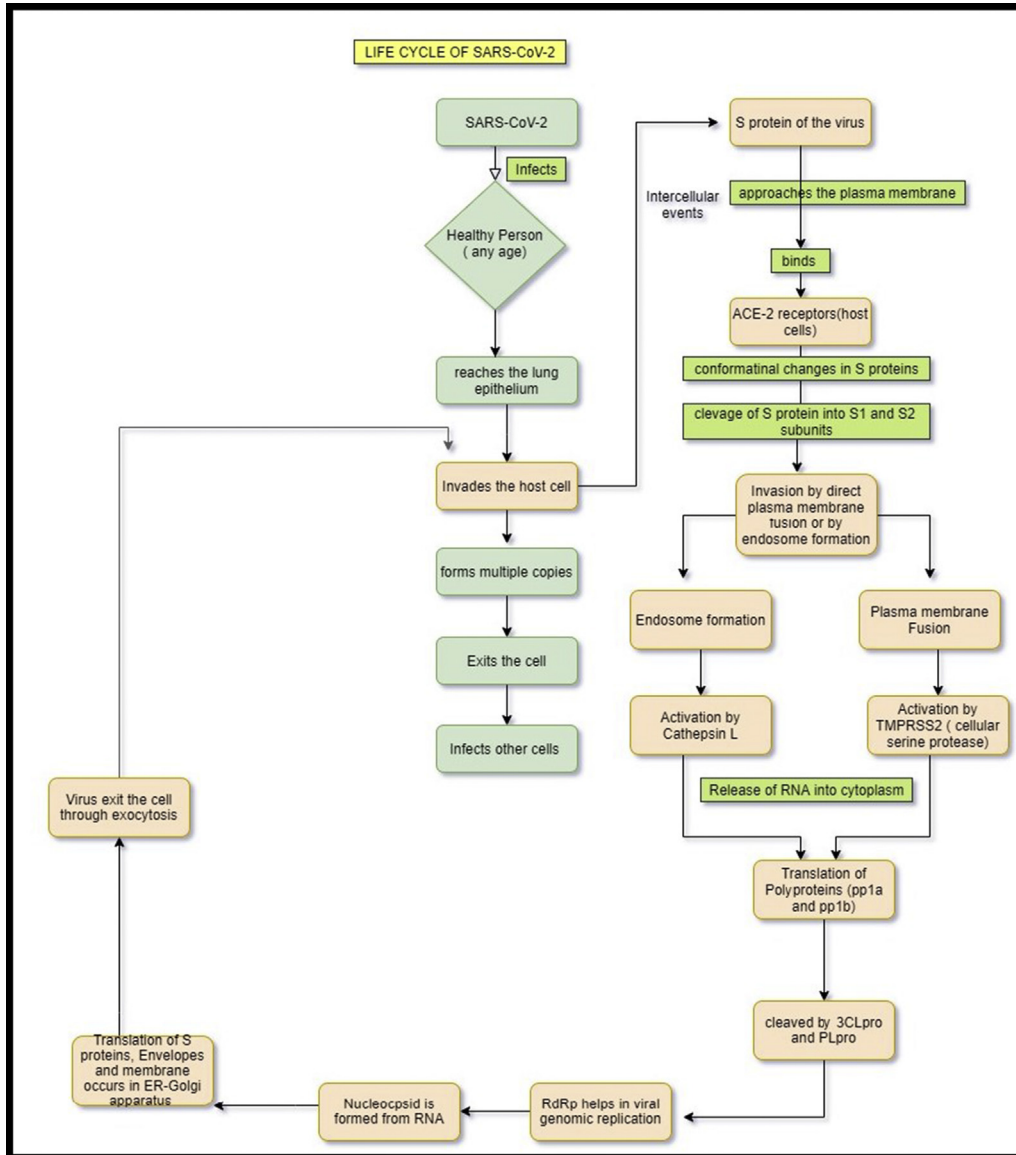
FIGURE 20.3 Flowchart of the life cycle of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *RdRp,* RNA-dependent RNA polymerase.

5. Treatment approaches

At present, there are many theories proposed about the treatment, but none of them are available for treatment. The entire treatment and prophylaxis lie on the detailed structure description we know about the virus. The first step of infection is the entry of the virus into the host cell. As discussed earlier the S protein's subunits, namely, S1 and S2,

attach themselves directly to ACE2 receptors or enter the cell via endosome formation. In both cases the S protein has a major role to play, so if we can interfere with this S protein activation, we might achieve an effective strategy for treatment. In this category, the drugs chloroquine and hydroxychloroquine have been tested extensively in patients with MERS, SARS, and SARS-CoV-2 infection.

5.1   Chloroquine and hydroxychloroquine

5.1.1   *Pharmacology of chloroquine and hydroxychloroquine*

Chloroquine is an antimalarial drug with the chemical name N4-(7-chloro-4-quinolinyl)-N1,N1-diethyl-1,4-pentane diamine, and hydroxychloroquine is its hydroxyl derivative [20–23] (Fig. 20.4). It exerts antimalarial action by integrating with the DNA and inhibiting the polymerization of heme [23]. It also raises the endosomal pH, which is required by the virus to invade into the cytosol. The availability of this medicine is in the tablet form and under the name chloroquine phosphate 500 mg and hydroxycholorquine sulfate 200 mg: both the drugs have long half-lives (20–60 days, respectively).

It can be detected up to 3 months in urine after administration. Hydroxychloroquine reaches peak plasma concentration in about 3–4 h ,and chloroquine reaches its Cmax in 30 min. The major adverse effects are related to gastric upset, nausea, vomiting, and diarrhea [21].
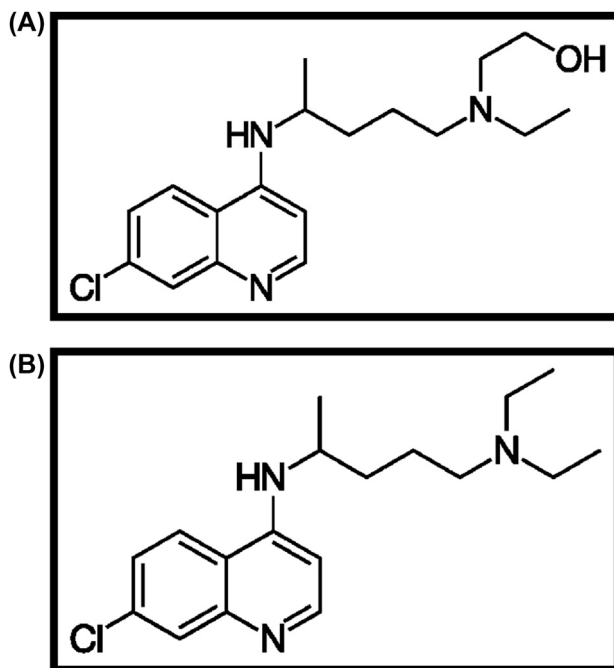

FIGURE 20.4 Chemical structures of (A) hydroxychloroquine and (B) chloroquine.

On long-term use, severe side effects are reported, one of them is retinopathy, which is nonreversible. Several drug interactions are also reported with antiepileptics, antacids, cyclosporine, amiodarone, tamoxifen, etc. [21−23].

5.1.2   Mechanism of action

In the inhibition of SARS-CoV-2, chloroquine has played an important role. It is known to inhibit the terminal glycosylation, which aids in ACE2 binding to the viral S protein. Several pieces of research have proposed that hydroxychloroquine may prevent SARS-CoV-2 binding to available ganglionic sides, which may inhibit viral contact with ACE2. Both the drugs are known to incorporate into lysosome and endosomes, raising the pH of intercellular compartments. However, the mechanism of action for the drugs is still disputable and new studies are constantly trying to come up with more accurate explanations. All the steps of binding, endocytosis, translation, replication, and exocytosis are hence misconfigured [23]. Both the drugs carry side effects that cannot be ignored. The most common side effect is the prolongation of QT interval leading to malignant arrhythmias [24].

In studies, the drug showed inhibition of the virus in vitro but was ineffective in most animal models [20]. Chloroquine was not approved by the European Medicines Agency, and it restricted its use to clinical trials and through emergency use authorization programs. It is still used in combination with azithromycin in many countries for prophylaxis and treatment; however, there are no positive clinical trial shreds of evidence to support such therapy [23]. The United States has also not promoted treatment with the drugs, as they are showing normal than usual adverse effects in patients. It has been studied that prolonged use of chloroquine may lead to blindness.

Other options include the suppression of TMPRSS2 enzyme present in the host cell that aids in the inactivation of spike protein, which allows endosome-independent cell entry.

5.2   Serine protease inhibitors (camostat mesylate, nafamostat, and bromhexine)

Studies have reported the use of TMPRSS2 inhibitors in the treatment of COVID-19 [12]. In their findings, Kim et al. reported that TMPRSS2 is considered important for development and homeostasis and thus marks as an attractive drug target [69]. Since the enzyme is extensively responsible for the activation, it required the researchers to work for the development of TMPRSS2 inhibitors. Camostat mesylate is one of the highly researched drugs in this category. It is a nonselective serine protease inhibitor and was approved first in Japan for the treatment of chronic pancreatitis and postoperative reflux esophagitis [26]. It is supplied in the form of crystalline solid and marketed under the name Foipan [27], which is manufactured by Nichi-lko Pharmaceutical co. Ltd. and Ono pharmaceuticals, Japan [26].

Camostat is well known to block the entry of the virus into the lung cell [27]. A study group created the three-dimensional structure of TMPRSS2 using molecular docking and the three drugs (camostat, nafamostat, and bromhexidine HCL) were made to interact with it. The homology model showed proper folding patterns with cysteine-rich and serine protease domains. The results of docking showed that active site residue His296 and Ser441 of TMPRSS2 interacted with the inhibitor camostat mesylate and nafamostat by hydrogen bonding interactions (Fig. 20.5), while bromhexine showed hydrophobic contacts due to its small structure [25]. Camostat is widely being evaluated in clinical trials for its effects against blocking TMPRSS2 and if sufficient concentrations can be attained in the lungs for treatment.

This led to the testing of other protease inhibitors like nafamostat mesylate, gabexate mesylate, and bromhexine. Nafamostat was found to be 10 times more potent than camostat [26−29]. It is shown to block the activation of SARS-CoV-2 aggressively whereas, gabexate mesylate was shown to inhibit the activation but very slightly and was not effective enough to stop the infection completely [28]. All three drugs are approved in Japan for clinical use but with few restrictions.

The structures of these drugs are given in Fig. 20.6.
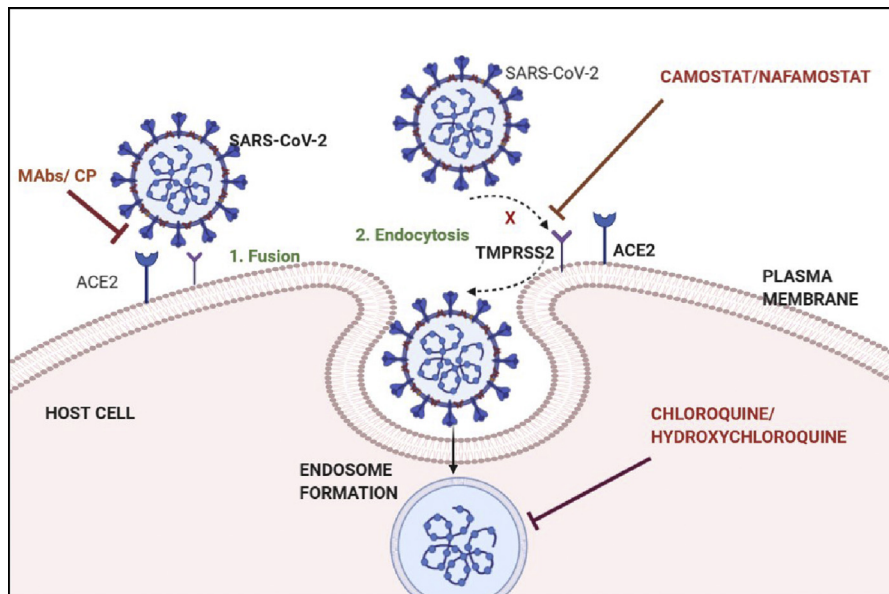
FIGURE 20.5 Mechanism of action for camostat/nafamostat and chloroquine/hydroxychloroquine. *ACE2*, angiotensin-converting enzyme 2; *CP*, convalescent plasma; *MAbs*, monoclonal antibodies; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2.
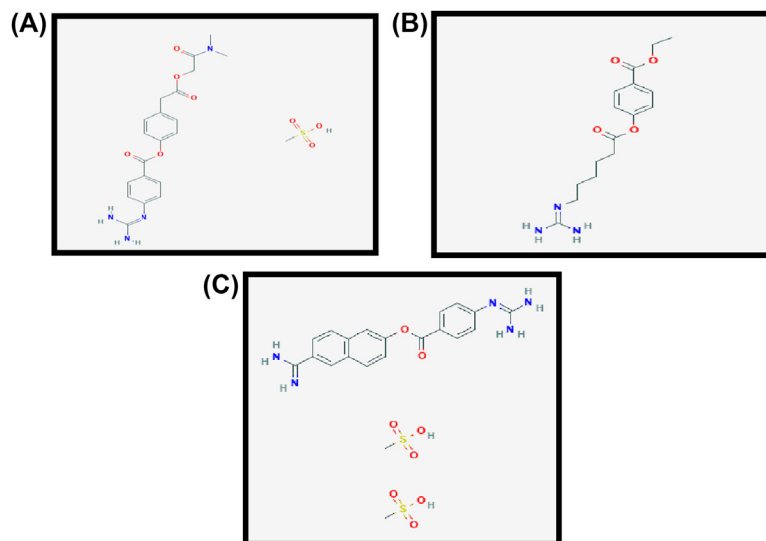
FIGURE 20.6 (A) Camostat mesylate, (B) nafamostat mesylate, and (C) gabexate mesylate.
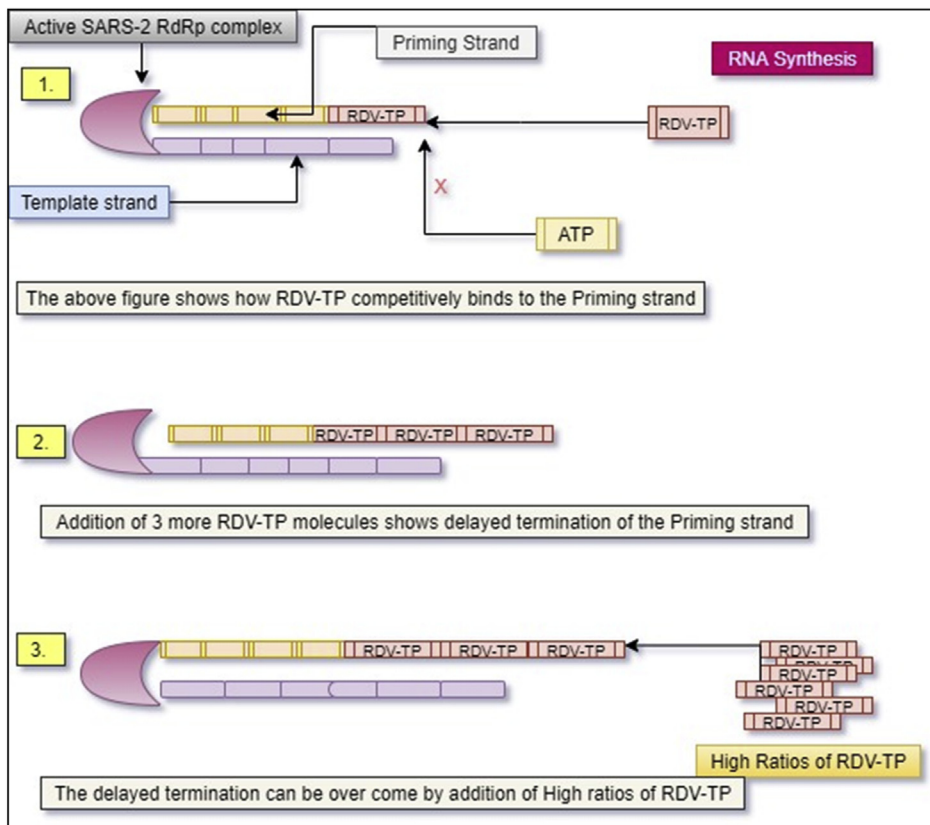
5.3 Remdesivir

SARS-CoV and SARS-CoV-2 have only 82% RNA sequence identity but their RdRp has a 96% sequence identity [30,35]. We are well aware that the replication of the SARS-CoV-2 virus is mediated by a multistep subunit replication/transcription complex of viral NSPs. The core component of this complex is nsp12 of an RdRp. Nsp12 requires accessory factors like nsp7 and nsp8, which increases the RdRp activity in template binding. Inhibiting this can be an attractive target against COVID-19 [32–34], as it will cease the replication phase. Little light in this context is available to completely understand the working mechanism of this RdRp complex [32]. It was first evaluated and used against viruses of the Filoviridae (Ebola virus) family and Nipah virus family. Studies have shown that the triphosphate forms on RDV competes with ATP for binding to Ebola virus RdRp, composed of L protein and VP35. ATP has a much higher selectivity than RDV-triphosphate (RDV-TP) [33].

RDV is known to be a phosphoramidate prodrug of a 1′-cyano substituted nucleotide analog [33]. It is a known phenomenon of RDV to get converted into its triphosphate form in the cell (RDV-TP). It is structurally similar to adenosine and is used as a substrate for viral RdRp complexes [33,34]. Studies have also shown that ATP serves as the main substrate for nsp12. This was confirmed in a study conducted by homology modeling of COVID nsp12 with a sequence identity of 95.8% [34]. Another study revealed that the relative binding free energy of ATP was calculated to be -4.14 ± 0.89 kcal/mol in the presence of Mg^{2+}, whereas the relative binding of RDV-TP was -8.28 ± 0.65 kcal/mol, which is stronger than ATP. This 800-fold difference in Kd value is enough to block ATP out of the binding pockets with RDV-TP [34]. The RDV-TP complex shows a delayed

chain termination at position i+3; this is a favorable selectivity for the nucleoside analogs (NAs) against SARS-CoV, MERS-CoV, and SARS-CoV-2. SARS-CoV-2 RdRp is known to be a relatively stable enzyme that functions as a replicate upon binding with RNA template [32].

Other drugs like favipiravir, ribavirin, penciclovir, galidesivir, and RDV can be used in the effective treatment of CoVs [35]. SARS-CoV-2 RdRps can accommodate themselves in a variety of chemical modifications of NAs, e.g., ribose, on the base, on both sides. The CoV exonucleases (ExoN) can recognize these modifications; hence it is a potential target, which can be explored for NA drug development [30]. This ExoN has the potential to remove NA that is incorporated into RNA, destroying the therapy.

However, contraindicating this statement, a study showed that RDV cannot be removed by nsp14-ExoN due to the addition of three more nucleotides after the first addition [31,36]. It is explained in Fig. 20.7. The Wuhan Virus Research Institute conducted some in vitro experiments for RDV on COVID-19 RNA synthesis and found that it is the fastest acting and most powerful antiviral agent available to us.

FIGURE 20.7 Mechanism of RNA synthesis. *RdRp*, RNA-dependent RNA polymerase; *RDV-TP*, remdesivir triphosphate.

The mechanism can be understood from Fig. 20.7 [31].

Fig. 20.7 shows a priming strand and a template strand on an active SARS-CoV-2 RdRp complex. The triphosphate form of the drug, RDV, approaches the priming strand and competes with ATP for binding. Upon successful binding of one RDV-TP, there is an addition of three more analogues, leading to delayed chain termination of the priming strand. Furthermore, it is suggested that to overcome the delayed termination reaction here, high ratios of RDV-TP can be bombarded on the priming strand.

5.4   Interferon beta-1a

Interferons are a group of signaling proteins that are secreted by a host cell whenever it is invaded by a virus. These are being extensively researched against SARS-CoV-2. Type 1 interferon (IFN-1) groups, belonging to cytokines, comprise of alpha and beta subtypes, which are of our interest. IFN-1 is secreted by plasmacytoid dendritic cells as a response to virus detection by pattern recognition receptors [60,61]. It is usually the first cytokine produced during viral infections [67]. These are recognized by IFNAR receptors, which are present on the outer surface of plasma membranes in most cell types. Due to binding of interferon to these receptors, a series of phosphorylation of transcriptional factors such as STAT1 starts to take over, and also they are relocalized to the nucleus, where they activate interferon-stimulated gene [62].

These interfere with viral replication and spread by different mechanisms like slowing down cell mechanisms or secretion of cytokines, which promote the activation of adaptive immunity [67]. IFN-β was known to be effective in treating multiple sclerosis by downregulating the MHC (major histocompatibility complex) type II expression in antigen-presenting cells. It was then used in the treatment of SARS and MERS, either alone or in combination with lopinavir/ritonavir [64]. In MERS, this combination helped improve pulmonary functions but did not stop virus replication or lung pathologic severity [60].

Several pieces of the research proposed that IFN-β is more potent than IFN-α in treating the infection. Later on, it was seen that IFN-β-1a has more clinical efficacy than other variables [63]. The activation of IFN-β in the lungs of an infected person upregulates cluster of differentiation 73 (CD73) in pulmonary endothelial cells. This results in the secretion of anti-inflammatory adenosine and also maintains the endothelial barrier functions [60,64].

It is suggested to use the drug for prophylaxis rather than for treatment [65,66]. The drug is now being used in China in combination with ribavirin. The efficacy of IFN-β has already been established in SARS; hence it should be used to combat the pandemic (COVID-19).

5.5   Favipiravir

It is an antiviral prodrug (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) that was earlier used in the treatment of Ebola and influenza virus. It is a known inhibitor of RdRp [81]. Current studies have reported the treatment time of COVID-19 with favipiravir was

found to more than that of treating influenza. However, the doses were well tolerated and showed very little adverse effects [80,81]. This makes this antiviral drug another effective drug for the treatment of COVID-19 [81]. It acts as a pseudo purine nucleic acid in virus-infected cells: like other NAs that inhibit viral RNA polymerase. It is licensed in Japan for treating influenza virus infections. Although the precise mechanism of action is lacking for the drug, it is still the drug of choice for clinical trials around the work due to its little adverse effects [82]. The drug is now facing its phase 3 clinical trial in India, initiated by Glenmark. If it gets approved then the drug will be available in India under the name "FaviFlu." The study is estimated to be complete by July or August this year [83].

5.6   Targeting the fusion machinery

Although much data is not available on the targeting of a fusion machinery, it is yet another effective approach that researchers have been working upon. A study revealed that SARS-CoV-2 exhibited a much higher capacity of plasma membrane fusion than SARS-CoV, suggesting that the fusion machinery of SARS-CoV-2 can be an important target for the development of CoV fusion inhibitors [37]. It further suggested that the Pan-CoV fusion inhibitors are point inhibitors against all CoVs. A solved X-ray crystal structure of six helical bundle (6HB) core of HR1 and HR2 domains in the SARS-CoV-2 S protein, S2 subunit, showed that many of the mutated amino acid residues in HR1 domain may be linked to enhanced interaction with the HR2 domain. S2-mediated fusion inhibitors can be effective in the treatment of SARS-CoV-2 [37,38].

EK1 is a Pan fusion inhibitor developed earlier and was used in this study. Many lipopeptides were then created from EK1; among which, EK1C4 was reported as the most potent fusion inhibitor against SARS-CoV-2 S protein-mediated membrane fusion. It was later proven effective against most of the known human CoVs such as SARS-CoV and MERS-CoV. Intranasal application of EK1C4 in mice, before and after being infected by the HCoV-OC43, protected mice from infection, showing signs of a potential area of development [13,38].

Studies have also revealed that CoV spike protein can be classified as a class I viral fusion protein. An important characteristic of this class is the cleavage of the precursor by host cell proteases into membrane distal subunits and a membrane-anchored sub-unit, an event that is essential for membrane fusion. The cleavage of S protein into S1 and S2 is known to enhance fusogenicity [37,38]. Hence this comprises another area of opportunity to develop treatment alternatives.

5.7   Convalescent blood product therapy

Convalescent blood products (CBPs) have been used in the treatment of various infectious viral diseases since the 1900s. They have been the most trusted resource for the treatment of two great pandemics the world has witnessed. Among the CBPs, convalescent plasma (CP) is the most exploited for treatment, as it has neutralizing Abs.

CBPs can be manufactured by sampling the whole blood or apheresis plasma from the convalescent donor. This therapy intends to clear viremia, which is developed 10−14 days after infection [50]. In the following sections, we will discuss more about the therapy. SARS-CoV and SARS-CoV-2 have the same receptor for binding to host cells, and hence results obtained on SARS-CoV were also extrapolated to yield important information on SARS-CoV-2 [42,48].

Passive Ab therapy is among the most researched treatment against SARS-CoV-2. In this therapy, Ab against a given agent is administered to an individual to prevent or treat an infectious disease. It is thought to provide immediate immunity to susceptible persons. The origin of passive Ab therapy can be traced back to the 1890s. Since then, it has been used in SARS, MERS, Ebola, and other viral diseases. For SARS-CoV infection, viral neutralization is necessary to avoid further damage. There are two available sources of Ab for SARS-CoV-2: human convalescent sera from individuals who showed recovery and monoclonal antibodies (MAbs) or preparations that are developed in certain animal hosts, such as genetically engineered cows that can produce human Abs. Abs for human use are usually only found Inhuman convalescent sera.

Researchers have focused more on using passive Ab therapy for prophylaxis than for treatment. This is because it is seen that the Abs aim to neutralize initial inoculums, which is smaller than the developmental stage of the disease [42−44] (Fig. 20.8).

Abs are developed at the onset of symptoms; that is, approximately 5 days after symptoms appear in an infected individual [42,44,51]. The major challenge faced by the researchers has been the requirement of high titers of neutralizing Abs needed to show responses. A study conducted on 99 samples of convalescent sera (SARS) showed that 87 had neutralizing Abs with a geometric mean titer of 1:61. There were two conclusions made on this basis: the Abs decline with time in recovered survivors and only a few patients produce high titers [42,51].

Earlier, the convalescent sera were used to treat diseases like poliomyelitis, measles, mumps, and influenza. Abs from apheresis were used here to treat severely ill cases of an H1N1 influenza pandemic (2009−10) [42]. A study was also conducted in Hong Kong on 80 patients, which was the largest study conducted in the year 2003 that utilized convalescent sera. Patients who were treated with the sera before 14 days showed marked improvement and were discharged from the hospital before 22 days [52].

MAbs are the major class of available biotherapeutics for passive immunotherapy. However, there are areas of concern in this therapy, including the availability of donors, clinical conditions, viral kinetics, and host interactions of SARS-CoV [43]. MAbs have been the preferred class owing to their specificity, purity, low risk of blood-borne pathogen contamination, and safety compared to plasma therapy and intravenous immunoglobulin (Ig) preparation [49]. The other agents that can be employed against SARS-CoV-2 are vaccines, peptides, interferon therapeutics, and small molecule drugs. This is a tedious process, as their use largely depends on clinical trial outcomes [43].

Neutralizing Abs can bind to either receptor binding domain of S proteins or specific Abs that bind to ACE2; any approach can be used to block the entry of the virus into the host cell [43] (Fig. 20.9). A study conducted by Coughlin and Prabhakar showed a series
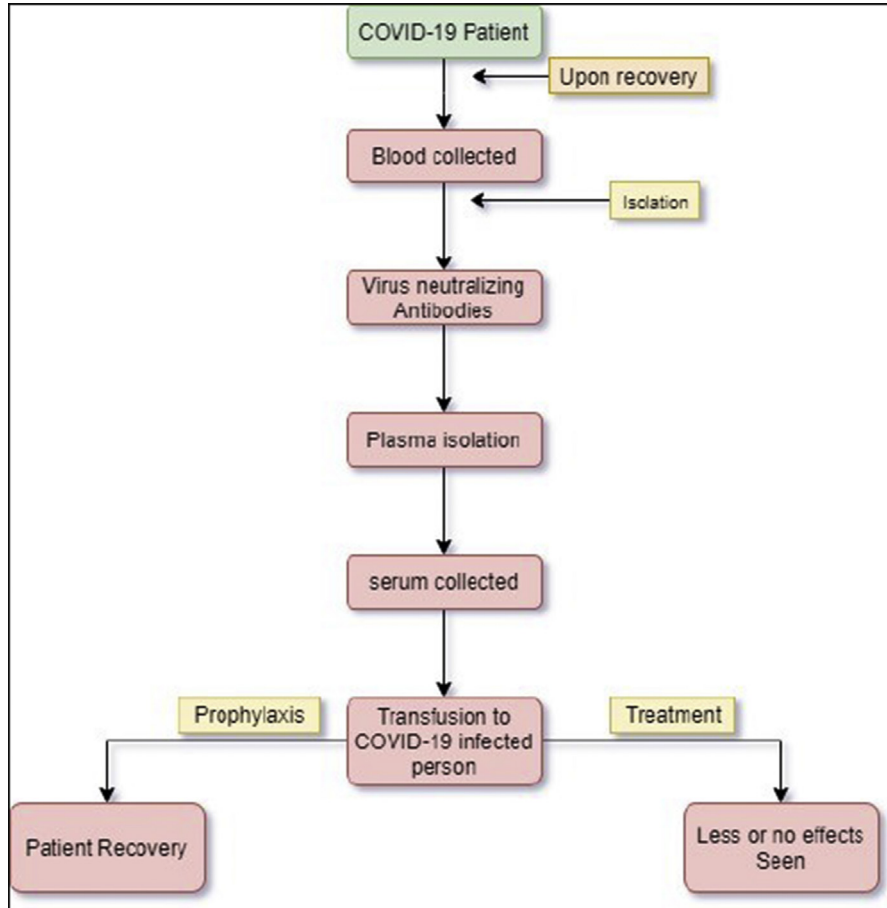
FIGURE 20.8 Neutralizing antibody treatment to coronavirus disease 2019 (COVID-19) patients shows recovery from symptoms when given as a prophylaxis therapy and not as treatment.

of human MAbs targeting the receptor-binding domain region of the S protein of SARS-CoV-2. It yielded positive results in vivo and in vitro. Few such MAbs are 80R, CR3014, M396 [53], 1A9 [54], 68 [55], 404 [56], S230 [57], and so on. These MAbs have been successful in blocking S protein from binding to ACE2 receptors. To use in treatment, we need the CP of neutralizing Ab titer greater than or equal to 1:160 to reduce mortality rates [48]. It varies from patient to patient depending on the age, weight, state of health, body response, and informed consent. A suitable donor could donate about 600 mL of plasma every 14 days for 6 months [50].

In the year 2014, CP therapy was recommended by the WHO for the treatment of Ebola virus. A study report prepared by Mair Jenkins showed that the mortality among patients reduced after receiving CP for SARS, with no adverse events [44]. During pandemics, researchers need to grab and use every opportunity that can help eradicate the
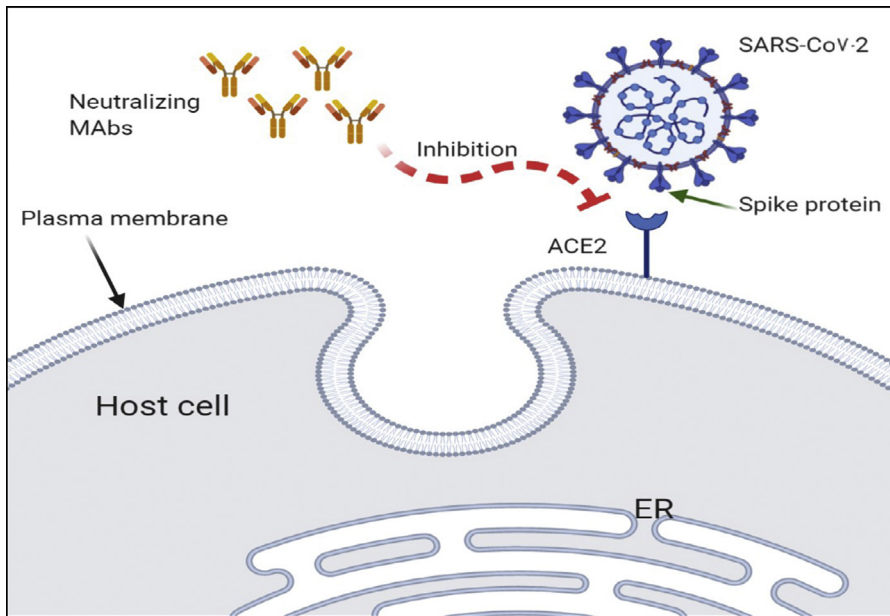
FIGURE 20.9 Binding of spike protein to angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface, inhibited by neutralizing antibodies (Abs). *ER*, endoplasmic reticulum; *MAbs*, monoclonal antibodies; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2.

disease as early as possible. CP helps suppress viremia that proceeds during the first week of infection. Another study conducted by Schools and colleagues reported that 3BNC117-mediated immunotherapy, which is a broad neutralizing Ab to HIV-1, enhanced host humoral immunity to HIV-1 [58]. Effects of this MAb not only illustrated free viral clearance and blocking of new infection but also included accelerated infected cell clearance [44].

CP has proven to be effective in SARS and MERS and to some extent even in COVID-19. A study was conducted at the Infectious Diseases department, the Shenzhen Third People's Hospital in Shenzhen, China, which included five critically ill patients who were treated using plasma from recovered individuals. All the five subjects had severe respiratory failure and three were on mechanical ventilation. The therapy was introduced around day 20, and the treatment with antiviral drugs, ritonavir and lopinavir, and interferon was continued. This therapy improved the symptoms in the patients after a week. Symptoms improved like the body temperature became normal, and improvement in sequential organ failure was seen [45,46,48]. A combination of CP with human Ig can be an efficient way to reduce symptoms in patients. Most studies prefer cocktail Ab approaches. It can be meaningful to generate neutralizing Abs targeting different epitopes on SARS-CoV-2. Computational simulations can be very effective in designing such effective therapies against the virus [50].

In reducing the respiratory viral infections, IgA Abs are the main Ig isotype on the mucosal surface. They are the key players. They are made up of two IgA molecules

(dimers), a joining protein that is also called the J chain and a secretory component [50]. IgA and IgM Abs appear after 5 days from the onset of symptoms, while IgG can be detected only after 14 days [50].

While examining the symptoms, patients also have shown an increased level of interleukin (IL)-6 and it was determined as an actionable target cytokine to treat COVID-19-related acute respiratory distress syndrome [47]. The expression of IL-6 is related to inflammatory cytokine storm verity [46]. Therefore, if we target IL-6 and its receptors using siltuximab and tocilizumab, they could reduce the cytokine-storm-related symptoms [46,49].

There are certain adverse reactions reported for CP therapy in transfusion-related events [59], involving chills, fever, anaphylactic reactions, circulatory overload, and hemolysis [48].

6. Various approaches to design vaccine

Vaccines provide the right immune responses, which confer protection from diseases. There are various challenges in developing the right type of vaccines. Also approaches involving live-attenuated or inactivated viruses, or other protein-based methodologies, viral vector-based Abs or nucleic corrosive based vaccines for developing the potential SARS-CoV-2 Abs in the pipeline-Four include nonreplicating infections or protein develops, four have a nucleic acid-based design, two contain live attenuated and one includes a viral vector [72]. However, live attenuated vaccine is not the best method for patients who are in their old age and are suffering from severe disease having higher risks [73]. Unfortunately, the knowledge of the immune responses is not enough for us to accurately predict the safety and efficacy of the vaccine. What we need to do now is test various methodologies. With the help of more results of good research, we will be able to conclude the best methodologies to develop a vaccine.

6.1   Strategies of vaccine development

6.1.1   Whole virus vaccine

Live attenuated or whole inactive virus type represents a classical strategy. As indicated by an industry bulletin, Johnson and Johnson is the only global organization setting out for COVID-19 vaccine development [74]. Besides, scientists at the University of Hong Kong have developed a live influenza Ab that can target SARS-CoV-2 proteins [74]. The major significance of the whole virus vaccine is inherent immunogenicity and its capability to stimulate toll-like receptors (TLRs, TLR3, TLR7/8, and TLR9). However, there are many tests needed to confirm safety in the case of live virus vaccines. With the abovementioned vaccination protocol, there is another issue in vaccine development, which is increased infectivity with live attenuated or killed whole virus vaccines [75].

6.1.2   Subunit vaccine

Subunit Abs for SARS-CoV and SARS-CoV-2 depend on evoking an insusceptible immune response against the spike protein, preventing its docking with the host ACE2 receptor [74]. The University of Queensland, in collaboration with the Coalition for Epidemic Preparedness Innovations, is working on incorporating viral surface proteins to present them more effectively to the immune system. Novavax has developed and delivered immunogenic viruslike nanoparticles dependent on the recombinant expression of the S-protein [76].

6.1.3   Nucleic acid vaccine

A few significant biotechs have progressed in nucleic corrosive Ab stages for COVID-19. Inovio Pharmaceuticals is developing a DNA Ab, along with others such as Moderna Therapeutics and Cuevas. The methodology of immunization is started with DNA, with promising outcomes in mice in 1993 indicating protective resistance against flu; these studies have not expressed similar findings in humans. Recently, new modifications and formulations have come up with an expectation of nucleic acid performance in humans, and this approach might eventually lead to the first incorporated human nucleic acid vaccine.

6.1.4   Problems with vaccine development

The development of vaccines without any preparations is ordinarily not a decent choice for this ongoing pandemic. An average of approximately 10 years is required to provide a safe and an effective vaccine for the prevention of disease in future recipients [77]. Vaccine advancement requires broad planning with regard to immunization development, immunization design and purification, preclinical testing in animals (to confirm safety and efficacy in humans), and numerous phases of clinical trials in humans (phase 1 for safety and phases 2 and 3 for efficacy). Approximately 13 industries are taking risks in proceeding the development of vaccines for SARS-CoV-2 [72]. This methodology has points, which are strategically and measurably complex, and designers usually stay away from preliminary trials that may generate comparative information. Also, in a high-mortality circumstance, most populations may not accept randomized controlled trials with placebo treatment; however, other approaches represent such concerns may be scientifically proven, they are ordinarily not as quick, and the outcomes can be more enthusiastic to interpret [78]. This issue can be overcome by comparing results and early immunization versus postponed vaccination, as in the "Ebola ça suffit!" trial. One potential way is to forward the test of a few vaccines in a versatile preliminary trial plan by using a single, shared control group, with the goal that more participants would get an active immunization [79].

At last, pandemics will produce a synchronous interest for vaccine demand around the globe. Clinical and serologic examinations will be expected to affirm which populations stay at most risks once vaccination is accessible and to establish an internationally reasonable immunization designation framework. Some Group of Seven nations have just called for such a worldwide framework, whose arranging must begin while immunization advancement proceeds.

7. Conclusions

COVID-19 is a pandemic that has affected over 4,819,337 people around the world (at present). There is an emergence of treatment and vaccination protocol development. SARS-CoV, MERS-CoV, and SARS-CoV-2 are all the viruses that share the same family and also have some similarities that can provide a direction to researchers and save time in return. Hence the studies conducted back then can be useful in optimizing a treatment plan for COVID-19. Till today, there is no cure available for SARS-CoV-2 infection and prevention remains the only cure. Prophylaxis should be the major aim of all the investigational drugs, as the studies suggest that most drugs are used to treat patients between 1 and 14 days of exposure and symptom development, proving clinically beneficial and reducing symptoms, showing recovery, and even patients getting discharged before 21 days of treatment. According to analysts, most of the clinical trials that are being conducted are also showing positive results. The analytics firm of GlobalData presented 16 positive data results from about 21 ongoing clinical trials. About 69% of trails are still in phase 1 and phase 2. The major investigational agents are RDV, sarilumab, and bevacizumab.

The US biotech firm Gilead has recently announced the drug RDV to have shown about 31% faster recovery time than placebo treatment. Sarilumab is undergoing clinical trials in parts of Europe, the United States, and Canada, whereas studies conducted for drugs like hydroxychloroquine and chloroquine have reported adverse events like higher mortality rates among patients and also heart rhythm problems, respectively. Although they are permitted to treat severely ill patients, their clinical benefits are not established in animal models yet. There are several RdRp inhibitors that are being preferred for treatment in different countries. As much as CP therapy sounds appealing, it will pose a challenge to the healthcare sector because of its requirements of patient consent and high titers of Abs for treatment. With the rapid spread of COVID-19, it is evident that there is an urgent need for a vaccine or treatment but also there is a need to constantly monitor the mutations of this virus.

There are new studies aligned that suggest the S and L strains are now affecting different parts of the same country, and this can be differentiated based on the mortality rate in that particular area. This briefs us about the changes that might be needed even once the vaccines and treatments are fully available for clinical use. Hence until the results of ongoing clinical trials are published, we need to follow the precautionary measures and rely on local standards of healthcare.

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