7 Emerging Technologies for the Treatment of COVID-19

Hossein Aghamollaei, Rahim Sarvestani, Hamid Bakherad, Hamed Zare, Paul C. Guest, Reza Ranjbar, and Amirhossein Sahebkar

Abstract

The new coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), turned into a pandemic affecting more than 200 countries. Due to the high rate of transmission and mortality, finding specific and effective treatment options for this infection is currently of urgent importance. Emerging technologies have created a promising platform for developing novel treatment options for various viral diseases such as the SARS-CoV-2 virus. Here, we have described potential novel therapeutic options based on the structure and pathophysiological mechanism of the SARS-CoV-2 virus, as well as the results of previous studies on similar viruses such as SARS and MERS. Many of these approaches can be used for controlling viral infection by reducing the viral damage or by increasing the potency of the host response. Owing to their high sensitivity, specificity, and reproducibility, siRNAs, aptamers, nanobodies, neutralizing antibodies, and different types of peptides can be used for interference with viral replication or for blocking internal-

H. Aghamollaei
Chemical Injuries Research Center, Systems biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

R. Sarvestani
Research and Development Department, PersisGen Par Biopharma Accelerator, Tehran, Iran

H. Bakherad
Department of Pharmaceutical Biotechnology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

H. Zare
Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

P. C. Guest
Laboratory of Neuroproteomics, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil

R. Ranjbar (✉)
Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
e-mail: ranjbarre@gmail.com

A. Sahebkar (✉)
Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Halal Research Center of IRI, FDA, Tehran, Iran

Polish Mother's Memorial Hospital Research Institute (PMMHRI), Lodz, Poland
e-mail: sahebkara@mums.ac.ir; amir_saheb2000@yahoo.com

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ization. Receptor agonists and interferon-inducing agents are also potential options to balance and enhance the innate immune response against SARS-CoV-2. Solid evidence on the efficacy and safety of such novel technologies is yet to be established although many well-designed clinical trials are underway to address these issues.

Keywords

COVID-19 · SARS-CoV-2 · Coronavirus · Treatment · Biotechnology

7.1 Introduction

The novel coronavirus, which is known as SARS-CoV-2, was first identified in the city of Wuhan, the People's Republic of China, which has spread globally. Its fast outbreak resulted in the 2019–2020 coronavirus pandemic of what has been termed COVID-19 disease. The primary symptoms of COVID-19 infection are fever, dry cough, sputum production, fatigue, and shortness of breath. In severe cases, other symptoms including persistent chest pain or pressure, confusion, anosmia, and gastrointestinal symptoms are seen.

Like other coronaviruses, such as severe acute respiratory syndrome (SARS) (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV), the new coronavirus carries a single-positive stranded RNA. The genome size for SARS-CoV-2 is 29,891 nucleotides which encodes 9860 amino acids. This genome has 82% nucleotide identity with human SARS-CoV [1]. The organization of genes in the coronaviruses shares the same order, coding for polyproteins 1a and 1b and the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins.

Of the several common drugs currently used to treat COVID-19 infection, chloroquine, remdesivir, lopinavir (LPV), and ritonavir (RTV) have gained the most attention. Despite the relative effectiveness of these medications, they also have side effects. The most hopeful antiviral to combat SARS-CoV-2 is remdesivir. Remdesivir was effective against MERS-CoV and SARS-CoV by acting as an inhibitor of RNA-dependent RNA polymerases in the RNA replication process [2–5]. LPV is an HIV-1 protease inhibitor. This drug has been used in combination with RTV to improve its half-life and found to be effective against SARS-CoV in tissue culture and in patients with HIV-1 [6]. However, the antiviral property of LPV against MERS-CoV remains ambiguous. There are some safety concerns to the use of this drug, including risk of cardiac arrhythmia, caution in patients with hepatic disease, and significant drug interactions [6]. Chloroquine is an antimalarial drug which has been shown to have in vitro activity against SARS-CoV-2. Its mechanism of action may include inhibition of viral enzymes or processes such as viral DNA and RNA polymerase. Using chloroquine may have some limitations including the risk of cardiac arrhythmia and risk of retinal injury, with cautions in patients with diabetes and those with glucose 6-phosphate dehydrogenase (G6PD) deficiency [7–10]. In addition, it has significant drug interactions.

Another possible treatment option is to use the serum from patients infected with the SARS-CoV-2. However, it is not yet clear whether a sufficient set of potential donors is possible. Studies on MERS-CoV have shown that the sera from patients recovering from infection do not appear to contain adequate antibody titers for therapeutic use [11].

Based on the recommendation of the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), there are currently no approved drugs or vaccines for the treatment or prevention of COVID-19. In recent years, with the development and integration of different scientific branches, several techniques have been proposed for the treatment of viral infections. These methods, which have emerged from a successful combination of medical sciences, biotechnology, chemistry, and bioinformatics, have shown promising results in the treatment of viral infections. Here, we have aimed to review the potency of novel and emerging techniques for the treatment of COVID-19.

7.2 Small Interfering RNA

The discovery of RNA interference (RNAi) provided a new approach for silencing the expression of specific genes in order to treat a wide range of human disorders. RNA interference as an antiviral mechanism was originally discovered in plants. Later, it was also observed in other organisms, including nematodes, Drosophila, and vertebrates [12, 13]. As a tool, siRNAs is a powerful approach specifically designed to reduce and prevent the synthesis of the target protein. This has brought the opportunity to develop a new generation of drugs for several diseases. Currently, many pharmaceutical companies are developing RNA-based therapeutics to specifically regulate various disease-causing genes. A detailed discussion of RNA therapies and their advantages, disadvantages, and challenges has been reviewed [see 14]. Several research teams have successfully used siRNA technology for developing various antiviral treatments (Table 7.1). Their effects have been evaluated on cell lines and animal models, and positive results have been reported. The great potential of siRNA for the management of serious human and animal respiratory viruses including respiratory syncytial virus (RSV), SARS-CoV, influenza, adenovirus, avian metapneumovirus, and the porcine respiratory virus has also been reviewed [see 15, 16].

Using siRNA technology to specifically target the key mRNAs for SARS-Cov-2 infection and assembly could be a valuable tool for the treatment of COVID-19. Similar to MERS and SARS, SARS-Cov-2 belongs to the coronavirus family, and its genome structure, host infection, and assembly likely share a common pattern. Therefore, reports on the application of siRNA for SARS and MERS could be informative for designing siRNA treatments for SARS-Cov-2. One of the most important proteins in the coronavirus family is the RNA-dependent RNA polymerase (RdRP), which is responsible for genome replication once the host cell is infected by the virus. One strategy to prevent the viral amplification and spread of the disease is to decrease the corresponding mRNA for this protein. Several studies have shown that targeting of RdRP using an siRNA approach is an effective strategy for controlling influenza and SARS disease in cell lines and animal models, by resulting in an 80–90% reduction of virus replication [17–21]. Other potential targets for using siRNA against SARS-CoV-2 are the structural proteins. These proteins are involved in virus assembly and binding to the host cell. Several reports have shown that siRNA against structural proteins, S, E, and M protein, could reduce the progression of coronavirus infections, including SARS-CoV, MERS-CoV, and HCoV-NL63 [19, 21–24].

Table 7.1 Reported studies on siRNA against respiratory viruses

Target disease	Target gene/genes	Reference
Respiratory syncytial virus (RSV)	Viral fusion (F) phosphoprotein (P) proteins	[105]
Influenza virus	RNA-dependent RNA polymerase (RdRP)	[17, 18, 105]
Influenza virus	Ran-binding protein 5	[106]
SARS	Spike protein	[23, 24]
SARS	RdRP protein	[19–21]
SARS	Envelop protein	[21]
SARS	Leader sequence	[107]
Human CoronavirusNL63	Spike protein	[22]
Adenoviruses	Adenoviral E1A	[108]
Influenza A1 virus	Nucleocapsid protein; polymerase acidic protein	[109–111]
SARS	Spike protein	[112]
MERS	Spike protein	[113]
SARS	Leader, TRS, 3′-UTR and spike	[114]

7.3 Neutralizing Antibodies

Since the 1980s, when the first therapeutic monoclonal antibody (mAb) was approved, dozens of monoclonal antibodies have been used in the treatment of various diseases. Today, the majority of the biotherapeutic product market is occupied by monoclonal antibodies [25].

During recent years, monoclonal antibodies are increasingly being considered as agents to fight severe viral diseases. In this section, we highlight the potential targets for neutralizing antibodies against SARS-CoV-2 inspired by those mAbs developed for combating of SARS-CoV or MERS-CoV.

Once coronavirus binds to the cell surface receptors via the spike protein, its replication begins [26]. Specific interaction between S1 subunit of the spike protein and its receptor creates a conformational change in the S2 subunit, which causes the viral envelope to fuse with the cellular membrane and release of the nucleocapsid into the cytoplasm [27]. The cell surface enzymes are used as a specific receptor for most of the human coronaviruses. For example, angiotensin-converting enzyme 2 (ACE2) works as a receptor for HCoV-NL63, SARS-CoV, and SARS-CoV-2 coronaviruses, while MERS-CoV attaches via dipeptidyl peptidase 4 (DPP4) [28] (Fig. 7.1). Thus, an effective treatment against SARS-CoV-2 might be developed based on the use of neutralizing or blocking monoclonal antibodies targeting either the viral spike protein or the host receptor [29]. Monoclonal antibodies against the spike protein in coronaviruses have shown promising results both in vitro and in vivo. Coughlin et al. generated dozens of mAbs against the SARS-CoV spike protein, and some of them were effective in in vitro studies [30]. Recently, a comprehensive review on the possibility of applying monoclonal antibody-based treatment for SARS-Cov-2 was published by Shanmugaraj et al. [29]. One potential source of mAbs for SARS-Cov-2 is via identification and isolation from either an antibody human phage library or memory B cells from infected and recovered patients.

Interleukin 6 (IL-6) acts as a pro-inflammatory cytokine by stimulating the acute phase response. Research has shown that the levels of cytokines such as IL-6 in COVID-19 cases can increase dramatically and the use of drugs that can inhibit this cytokine improves patient recovery. There are different kinds of FDA-approved antibodies that block IL-6 or IL-6 receptors such as siltuximab (Sylvant), sarilumab (Kevzara), and tocilizumab (Actemra). Studies have shown that the administration of these antibodies in COVID-19 patients with high levels of IL-6 can greatly improve the severity of this disease [31]. Currently, both intravenous and subcutaneous administration of RoActemra and subcutaneous administration of Kevzara are considered in phase 2 clinical trials as a treatment for COVID-19 [32]. The results are expected in June 2021.

7.4 Aptamer-Based Viral Treatment

Aptamers are non-coding single-stranded nucleotide sequences that specifically bind to their targets. Aptamers are synthesized by an in vitro process called systematic evolution of ligands by exponential enrichment (SELEX). Compared to mAbs, aptamers are easily synthe-

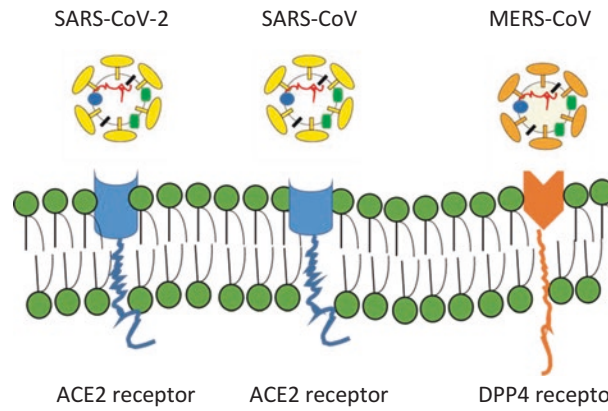

Fig. 7.1 Schematic of SARS-CoV, MERS-CoV, SARS-CoV-2, and their cellular receptors

sized, and their targets include a wide range of biomolecules. Aptamer-based therapeutics has the potential to create a revolution in the development of antiviral drugs. Nowadays, several approved aptamer-based drug for various diseases is available on the market. There are several advantages over using aptamer-based treatments including high specificity, rapid selection process, no need for the complex process of protein expression and purification, and the simple process needed for large-scale manufacturing. These advantages make aptamer technology well-suited for treatment of viral infections [33].

Because of the high specificity and affinity for their targets, aptamers are being increasingly applied in research and therapeutics. Many researchers have studied the development of aptamer-based antiviral treatment especially with respect to HIV and influenza viruses. In various studies, many proteins and enzymes from the HIV virus, including reverse transcriptase, integrase, and transactivation responsive protein, have been targeted for aptamer development [34–37]. Several studies have also focused on the hemagglutinin protein in the influenza virus structure for aptamer selection [38–41]. In addition, in two separate studies, whole influenza virus was targeted for aptamer selection, and the results showed more than 90% inhibition of receptor binding in the presence of aptamers [38, 39]. Shum et al. produced a comprehensive overview of aptamer-based therapies and their challenges in the treatment of various viruses [33].

Aptamer technology has also been used to combat the previous SARS-CoV outbreak. The SARS-CoV helicase contains a functional domain with double-stranded nucleic acid unwinding and ATPase activities. A study showed that the aptamer might bind to the nucleic acid binding site of the helicase and block the unwinding and subsequent helicase activities [42].

The number of patients infected by SARS-CoV-2 is increasing rapidly, and in these circumstances, research plans need to be pushed forward in the right direction to find effective treatments. Using aptamer technology could potentially lead to an effective treatment against COVID-19 disease in a short time and at a relatively low cost. For COVID-19, it is recommended to consider one of the strategies below. One suitable target is blocking the viral fusion with the target cell. The spike protein is responsible for cell attachment and entry, and blockade by an aptamer could be an effective way to inhibit infection. Proteins and enzymes involved in the viral replication cycle including polymerases and nucleocapsid protein are other potential targets for inhibitory aptamers. Another promising target for aptamer treatment is RNA-dependent RNA polymerase, because of its importance in virus replication. In addition, specific regions of the viral genome interact with various proteins responsible for transcription initiation, translation, and replication, or viral assembly. These proteins are also promising targets for the generation of aptamers with selective affinity to these regions [43].

Although the intrinsic physicochemical features of aptamers pose serious challenges for their transport to infected organs or cells, they may be well-suited for respiratory viruses because the upper airways and lungs are relatively accessible as target organs.

7.5 Nanobodies

Nanobodies are a new class of recombinant antibody derived from heavy-chain antibodies in camels and sharks. Unlike traditional antibodies, the variable domain of these types of antibodies is made from a single region [44]. These types of antibodies have specific properties that distinguish them from others, including smaller size, higher affinity, more solubility and resistance to denaturation, stability in intolerable condition (high and low pH, high temperature), a broad diversity of epitopes recognition, faster tissue permeability, high sequence homology with human antibodies, and cost-effective production [44–49].

Nanobodies are now used and tested in the treatment of many diseases, including viruses

such as hepatitis B, influenza, polio, rabies, HIV, RSV, FMDV, and rotavirus [50]. One of these nanobodies called ALX-0171 inhibits RSV infection by binding to the F-protein of virus and blocking uptake into the cells [51]. It is important to note that due to the specific properties of this type of antibody, ALX-0171 was used as an inhaled form. This method of administration at the site of infection has many advantages, including an increase in the efficacy of the drug and reduced dose compared to systemic injections [52].

The receptor-binding domain (RBD) of the spike protein is the priority target against the coronavirus family as this allows binding to the host cell surface receptor. In a recent study, scientists isolated nanobodies against the RBD domain of the MERS-CoV which potently neutralized MERS-CoV infection [53]. Therefore, there is some hope that a similar approach can be used against SARS-Cov-2.

7.6 Peptide Inhibitors

Peptides are short chains of amino acids that are usually composed of less than 50 amino acids. Peptides have many advantages over proteins and antibodies, such as being small in size, easy to synthesize, as well as efficient in cell and tissue penetration [54]. They can also have high affinity, specificity, and activity and do not accumulate in a particular tissue, resulting in low toxicity.

In the treatment of viral diseases, peptides have two important applications. By studying viral antigens and selecting the appropriate peptides, they can be used as vaccines, and subsequently, the immune system can detect and eliminate the virus. They can also be used competitively against viral proteins and thereby prevent viral entry into cells. Guo et al. attempted to identify the most potent peptides to stimulate the humoral immune system as a SARS vaccine [55]. They synthesized 4942 overlapping peptides from all proteins of the SARS genome and evaluated these against serum from patients recovering from the virus. Peptides recognized by antibodies in the serum samples were selected for potential use as a polyvalent immunogen [55]. In order to investigate the possibility of using these peptides against SARS-Cov-2, we conducted a blast analysis of the same peptides against SARS-Cov-2 proteins. The results of this analysis showed that among 24 peptides presented in the SARS-CoV study, 13 are highly conserved to regions SARS-Cov-2 proteins and might therefore be used as vaccine candidates against the virus (Table 7.2).

Wang et al. analyzed various peptides of the SARS-CoV spike protein using a bioinformatics approach and synthesized the most promising candidates [24]. Next, they screened these

Table 7.2 Blast of peptides recognized in SARS convalescent sera against NCBI databases which gave high sequence identities with SARS-Cov-2 proteins

Category	SARS peptide	Covid-19 peptide	Homology (%)	Antibody classes
Orf1a	NQDVNLHSSRLS	NQDVNLHSSRLS	100	IgA, IgM
Nucleocapsid (N)-protein	QLPQGTTLPKGFYA	QLPQGTTLPKGFYA	100	IgG, IgA
	TVTLLPAADMDDF	TVTLLPAADLDDF	92	IgG, IgM
	YKTFPPTEPKKD	YKTFPPTEPKKD	100	IgA
	GGSQASSRSSSR	GGSQASSRSSSR	100	IgG, IgM
	IRQGTDYKHWPQ	IRQGTDYKHWPQ	100	IgG, IgM
Spike (S)-protein	CPFGEVFNATKF	CPFGEVFNATRF	91	IgA
	PIGAGICASYHT	PIGAGICASYQT	91	IgG, IgA, IgM
	QYGSFCTQLNRA	QYGSFCTQLNRA	100	IgG, IgM
	PFAMQMAYRFNG	PFAMQMAYRFNG	100	IgM
Membrane (M)-protein	KEITVATSRTLS	KEITVATSRTLS	100	IgG, IgA, IgM
	GTITVEELKQLL	GTITVEELKKLL	91	IgG, IgA, IgM
E-protein	YVYSRVKNLNSS	YVYSRVKNLNSS	100	IgG, IgA, IgM

peptides using T cells from individuals who had recovered from the disease. They found that two peptides (FIAGLIAIV and LITGRLQSL) were immunogenic and effectively stimulated a T-cell immune response against this virus. To investigate the possibility of using these two peptides as immunogens against COVID-19, we compared the sequences against those in the SARS-CoV-2 spike protein. This revealed 100% identity, lending support to their potential use as a SARS-Cov-2 vaccine. Another study targeted MHC-I and MHC-II epitopes within the spike protein of the SARS-CoV-2 virus in an informatics-based approach to identify the most promising peptide vaccine candidates [56]. They identified 29 peptides within the MHC-I region and 8 within the MHC-II region, which they used to synthesize a single vaccine complex.

Zheng et al. synthesized 24 peptides against the SARS-COV spike protein and tested these as inhibitors of viral entry into cells. They found that SARS-CoV infection was completely inhibited by two peptides [57]. The sequence of one of these peptides (IQKEIDRLNEVAKNLNESLI) is identical to a sequence in the S2 subunit of SARS-CoV-2, suggesting that it might be a suitable candidate for the treatment of COVID-19 disease.

7.7 Fusion Inhibitors

Fusion inhibitors are a class of drugs that were first introduced in HIV infection, and their mechanism of action is to prevent and interfere with the binding, fusion, and entry of the virus into the target cells [58]. Various strategies can be proposed to produce a fusion inhibitor against SARS-CoV-2. The first is the production of small molecules that can bind to the virus target receptor and prevent its binding and entry, such as 1-thia-4- azaspiro[4.5]decan-3-one derivatives [59]. An alternative approach is the use of drugs developed for other coronaviruses such as Nafamostat, Griffithsin, and Dihydrotanshinone E-64-C and E-64-D [5, 60–64].

Xia et al. found that a peptide derived from the heptad repeat 2 (HR2) domain of human coronaviruses has a pan inhibitory function against several members of this viral family [65]. In vivo studies showed that the inhalation of this peptide had a high potency in suppressing viral infection and good safety profile [65]. Subsequently, the same research group developed lipopeptides derived from the same region and showed that one of these was more than 100-fold more potent than the original peptide in preventing infection with the SARS-CoV-2 virus [66].

The use of recombinant proteins can also be an effective way of inhibiting the virus from entering the cell. Wong et al. reported that the RBD domain of SARS-Cov S protein potently binds to ACE2 and prevents infection [67]. The main advantage of methods that disrupt the virus host interaction is that the host receptor (ACE2) does not undergo rapid mutation [68].

Li et al. demonstrated that administration of recombinant ACE2 effectively bound to the SARS-CoV virus and inhibited infection of cells in culture [69]. Recently, Monteil et al. reported that treatment of Vero E6 cells with recombinant ACE2 in the early stage of infection can reduce the SARS-CoV-2 growth rate by more than 1000-fold [70]. However, this study only examined the effects of this protein in the early stages of infection, and its effectiveness in the later stages of COVID-19 infection has yet to be determined.

7.8 Antimicrobial Peptides (AMPs)

The development of antimicrobial peptides during the late 1990s and 2000s led to first marketing approvals in 2012 for 6 peptides [68]. Peptides are an important part of the drug industry, and about 140 peptides are currently being tested in various clinical trials [71]. The use of peptides in treating infections has three advantages, including the shorter market time, inhibition of protein-protein interactions, and the availability of methods to increase the peptide half-lives. Through the creation of a pore and eliciting changes in the structure of bacterial cell membranes, peptides have broad-spectrum activity

Table 7.3 The peptide sequence of the seven selected AMPs

Peptide	Sequence
AP00225	ACYCRIGACVSGERLTGACGLNGRIYRLCCR
AP00180	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR
AP00549	GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY
AP00744	GLPQDCERRGGFCSHKSCPPGIGRIGLCSKEDFCCRSRWYS
AP00729	GLPVCGETCVGGTCNTPGCTCSWPVCTRN
AP00764	GLRSKIWLWVLLMIWQESNKFKKM
AP00223	VTCYCRSTRCGFRERLSGACGYRGRIYRLCCR

against several microorganisms [72–74], with fewer side effects compared to chemical drugs [72, 75]. Many peptides are also available which can inhibit viral activities. For example, a peptide called RVFV-6, which originates from the Rift Valley FeverVirus (RVFV) glycoprotein, is an inhibitor of viral fusion [75, 76]. Kn2-7, a new derivative of a scorpion venom peptide, has inhibitory activity against HIV-1, with a weak cytotoxic effect in mammalian cells [77]. Numerous studies have shown that AMPs are good candidates for the development of new therapeutic agents against coronaviruses [78–81]. Antiviral AMPs function in different ways, including prevention of viral entry through particular receptors, viral fusion blockage through interaction with the viral envelope and membrane, and stopping viral entry through interaction with heparansulfate [78].

Zhao and colleagues examined the antiviral activity of 11 mouse defensin-derived peptides. Among them, one peptide (NGAICWGPCPTA FRQIGNCGHFKVRCCKIR) showed strong and wide-ranging antiviral effects on several respiratory viruses including MERS-CoV, SARS-CoV, and influenza A H1N1 virus [80]. This peptide interrupts the RBD interaction [81].

In addition to the above approaches, prevention of viral replication is one of the strategies to control viral infections [82]. Mucropin-M1 (LFRLIKSLIKRLVSAFK) is a derivative from mucropin AMP (LFGLIPSLIGGLVSAFK). In this case, the proline (P) was replaced by arginine (R), and glycine (G) was changed to lysine (K). Mucropin-M1 demonstrated activity against SARS-CoV and influenza A virus H5N1, by preventing viral replication [83]. The original peptide mucropin showed no antiviral activity against any of these viruses.

In another study, Mustafa et al. developed several AMPs which bind to the MERS-CoV spike protein [84]. These peptides belong to the defensin family and may be very important in providing inhibitory activity. The results of the study showed that seven peptides had a high affinity for MERS-CoV spike protein at its active site, suggesting their potential use in the treatment of COVID-19 (Table 7.3).

Zhou et al. recognized that the glycopeptide antibiotic teicoplanin could inhibit the entrance of Ebola viruses into the cell cytoplasm [85]. This was carried out by high-throughput screening of FDA-approved drugs. Further analysis confirmed that teicoplanin was also capable of blocking the entry of SARS-CoV and MERS-CoV viruses. This antibiotic has been shown to have an inhibitory effect on viral replication and transcription.

Evaluation of the AMP rhesus theta-defensin 1 (RTD-1) showed 100% survival and a moderate decrease in lung injury in a mouse model of SARS-CoV infection [86]. The mechanism appeared via an effect on the inflammatory system as the cytokine responses in the treated animals were altered compared to the untreated group.

7.9 Interferon-Inducing Agents

Another way of modulating the body's protection system against SARS infection is through treatment with interferons or the use of agents that induce interferon production [87–89]. IFN inducers have several advantages compared to

exogenous IFN. They motivate the production of the body's own IFN, which has no antigenic properties, unlike recombinant forms of IFN [90]. IFN inducers can be mixed with IFN and other antiviral drugs, a strategy that could have both immunomodulating and etiotropic effects [91].

Bao and colleagues developed a method based on CpG oligodeoxynucleotides (ODNs) for the treatment and prevention of SARS-CoV disease [92]. They found a new CpG ODN called BW001 which could stimulate human peripheral blood mononuclear cells (PBMCs) to protect Vero cells against SARS-CoV. In addition, BW001 stimulated human dendritic cells and PBMCs to secrete high levels of IFN-α and stimulated B cell and PBMC proliferation. Additionally, BW001 can increase the secretion of IFN-γ and natural killer cell cytotoxicity. In another study, Barnard et al. used a mismatched double-stranded (ds)-RNA called Ampligen® (poly I: poly C124) as an interferon inducer and a hybrid human interferon (IFN-α B/D) against SARS-CoV infection [93]. In this study, Ampligen was injected intraperitoneally 4 h before the mice were exposed to SARS-CoV. As a result, the titers of the lung viruses decreased below the detectable level.

Kumaki and colleagues used polyriboinosinic-polyribocytidylic acid stabilized with poly-l-lysine and carboxymethyl cellulose (poly-ICLC) as an interferon inducer in SARS-CoV-infected mice [94]. Treatment with poly-ICLC (5 mg/kg) was initiated 24 h after infection with SARS- Cov and continued 2 times a day for 5 days. All treated mice were protected against lethal viral infection, and virus titers were reduced in the lungs.

7.10 Peptidomimetics

Any compound that can mimic the biological activities and structural properties of a peptide are referred to as a peptidomimetic. Changes in peptide structure in antimicrobial research include side-chain and backbone modifications with the use of unnatural amino acids (such as D-amino acids), peptoids, β-peptides, and lipidation [95]. The main protease (Mpro) is responsible for proteolytic processing of polyproteins 1a and 1ab, causing the release of 15 proteins involved in the viral replication process [96]. As Mpro is vital at the beginning of coronavirus replication, it is a promising target against infection [97, 98].

Kumar et al. designed and synthesized three peptidomimetic inhibitors that inhibit 3CLpro of SARS-CoV and MERS-CoV with IC_{50} values of 0.2–0.7 μM and 1.7–4.7 μM, respectively [98]. These agents demonstrated a desirable selectivity index and could potentially lead to the discovery of wide-spectrum antiviral drugs against newly emerging coronaviruses. In addition, Arun et al. designed and synthesized several peptidomimetic SARS-CoV protease inhibitors with good SARS-CoV 3CLpro inhibitory activity [97]. In another study, Kankanamalage et al. designed and evaluated a new compound which inhibits the 3CLpro of the MERS-CoV [99]. These compounds effectively prevented MERS-CoV replication.

Finally, Chuck et al. investigated the inhibitory effects of several numbers of nitrile-based peptidomimetic inhibitors with various peptide lengths and N-terminal protective groups, on the enzymatic activity of 3CLpro of SARS-CoV [100]. Three nitrile-based inhibitors with carboxybenzyl (Cbz), tert-butyloxycarbonyl (Boc), and 5-methylisoxazole-3-carboxyl (Mic) protective groups were synthesized containing the SARS-CoV auto-cleavage sequence AVLQ. Protease activity was measured in the presence of inhibitors, and the IC_{50} values of Cbz-AVLQ-CN, Boc-AVLQ-CN, and Mic-AVLQ-CN were 4.6 ± 0.2, 49 ± 2, and 49 ± 2 μM, respectively. Thus, the inhibitory effect of components with Cbz group was 10 times stronger than the others. This demonstrated that the nitrile cap could efficiently deactivate the 3CLpro activity. Further studies showed that Cbz-AVLQ-CN is a wide-spectrum inhibitor against several coronavirus strains (e.g., OC43, NL63, 229E, and HKU1), suggesting that this approach may have promise for treatment of COVID-19.

7.11 Toll-like Receptor Agonists

Toll-like receptors (TLRs) are a group of proteins that allow the immune system to discriminate between "self" and "non-self" [34, 35]. Consequently, TLR antagonists and agonists have been suggested as antiviral or adjuvant compounds [101, 102].

A study by Totura et al. showed that TLR signaling via the TIR-domain-containing adapter-inducing interferon-β (TRIF) protein protects mice from SARS-CoV disease lethality [102]. Their findings showed a balanced immune response that operates via both MyD88 adapter-driven and TRIF-driven pathways. Since the TLR3−/−, TLR4−/−, and TRAM−/− mice are more sensitive to SARS-CoV than normal mice, using TLR agonists can be effective in the treatment of MERS-CoV and SARS-CoV infection [101, 102].

Zhao and colleagues used intranasal poly(I·C), lipopolysaccharide, R848, or CpG (TLR3, TLR4, TLR7/8, or TLR9 agonists) in mice infected with SARS-CoV [103]. After treatment, approximately 95% survival was found for poly(I·C) against SARS-CoV. Pretreatment with poly (I·C) led to upregulation of IFN-γ, IFN-β, tumor necrosis factor alpha (TNFα), and IL-1β gene expression in the lungs. Their investigation also showed that treatment with poly(I·C) repressed viral replication in human host cells. These findings suggest that TLR adapters are crucial in producing a balanced innate immune response to COVID-19 infection.

7.12 Conclusions

Emerging techniques can be used for controlling viral infection by reducing the damage or increasing the potency of the host response. The development of siRNAs or aptamers for targeting genes coding for critical structural (i.e., S, E, and M) and nonstructural (e.g., RdRP, 3CL protease) proteins can be used to block the effects of SARS-CoV-2 infection. Also, the sensitivity, specificity, reproducibility, and ease of use make mAbs an attractive option for the treatment of COVID-19. However, this strategy might be time-consuming and costly compared to other treatments. Future studies for mAb development against SARS-CoV-2 may be focused on the identification and use of S1 epitopes as a key target for inhibition of viral entry into the cells.

Peptides are one of the most promising options for the development of anti-COVID-19 drugs as they can be used as antigens for vaccine production or as inhibitors for preventing viral infection. Due to the homology of SARS-CoV and SARS-Cov-2 protein sequences, several peptides proposed for use in the former could be applicable for the treatment of COVID-19. Based on our blast results, we propose 13 peptides with high homology for consideration as a target for vaccine development (Table 7.2). Peptidomimetics can also help to improve peptide effectiveness as antiviral agents. Unique features of nanobodies such as the small size, low immunogenicity, and capacity for conjugation with other agents make them ideal candidates for viral detection and therapy.

In addition, Toll-like receptor agonists can protect against SARS-CoV, and IFN inducers stimulate the natural production of the IFN by the host, thereby improving the host response against viral infection. Production of different inhibitors by genetic engineering and recombinant protein expression is another approach which may be promising as viral therapies. Specifically, the use of ACE2 recombinant proteins for inhibition of viral entry may also work against future coronavirus infections given that it this protein is an endogenous factor.

The approaches mentioned in this review prove that it is possible to quickly start well-designed randomized controlled studies even in the middle of a global emergency such as the COVID-19 pandemic. Table 7.4 shows the potential drugs in different phases of clinical trials for treatment of COVID-19, which highlights this capacity. However, there is a need for novel platforms for the development and

Table 7.4 Potential biological-derived drug in different phase of clinical trial for treatment of COVID-19 (clinicaltrials.gov)

Number	Drug/ Molecule	Type	Effect	Status
1	TJ003234	mAb	Anti-GM-CSF monoclonal antibody	Phase I
2	Lopinavir	Protease inhibitory	–	Phase II
3	Ritonavir	Protease inhibitory	Cytochrome P450-3A4	Phase II
4	Fludase (DAS181)	Fusion inhibitor	Preventing of viral entry by removing sialic receptor	–
5	Sarilumab	Immunosuppressive	Blocking of interleukin-6 receptor	Phase II
6	Tocilizumab	Immunosuppressive	Blocking of interleukin-6 receptor	Phase II
7	Sargramostim	Leukocyte growth factor	Recombinant granulocyte macrophage colony-stimulating factor	Phase IIII
8	Mavrilimumab	mAb	Granulocyte macrophage colony-stimulating factor receptor inhibitor	Phase II
9	bacTRL-Spike1	Vaccine	Stimulation of antibody production against SARS-CoV-2 Spike protein	Phase I
10	Ad5-nCoV	Vaccine	Stimulation of antibody production against SARS-CoV-2 Spike protein	Phase II
11	Emapalumab	mAb	Anti-interferon-gamma (IFNγ)	Phase II
12	IFN-α2β	Interferon	Recombinant human interferon α1β	Early Phase I
13	rhIFNα	Interferon	Recombinant human interferon Alpha-1b	Phase III
14	INO-4800	DNA vaccine	Stimulation of antibody production against SARS-CoV-2 Spike protein	Phase III
15	mRNA-1273	Vaccine	Stimulation of antibody production against SARS-CoV-2 Spike protein	Phase I

manufacturing of therapeutic agents and vaccines that can be readily adapted to new viral agents in line with the National Institute of Allergy and Infectious Diseases initiative [104]. Such a platform would facilitate the development of therapeutic agents and vaccines to enter clinical trials in less than 16 weeks and fast-track large-scale manufacturing if a given drug proves to be effective [115]. Such approaches are now essential given that the continuance of the current pandemic and the likely eruption of future coronavirus outbreaks. Finally, the authors of this article believe that both traditional and emerging approaches are essential for the prevention and treatment of COVID-19 [116, 117].

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