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Previous and potential mutations of Sars-cov-2 receptors and their interaction with known inhibitors

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Abstract

SARS-CoV-2, the seventh coronavirus known to infect humans after 229E, NL63, 0C43, HKU1, MERS-CoV, and the original SARS-CoV, has a large positive-stranded RNA genome of 29,891 nucleotides and 9,860 amino acids. It is a strain of coronavirus that causes COVID 19. It was first discovered in the Chinese city of Wuhan. It undergoes several mutations hence posing a great difficulty in finding a suitable inhibitor. This studies previous and potential mutations of Sars-cov-2 receptors and their interaction with known inhibitors. There are four measures to take in generating SARS-CoV-2 inhibitors that might surpass mutation should it arise which includes; a. finding inhibitors with two or more mechanism of inhibition in virus target inhibition b. employing host target inhibition c. complex forming compounds that will interfere with viral-host cell fusion independent of interaction with the host or organism d. human receptor cell lookalikes with higher affinity to the virus (proteins). Employing any of these measures might produce a good inhibitor however, drug resistance may emerge quickly after treatment as a result of the numerous mutations that RNA virus experience but host cell mutations are uncommon, treatments that target the host cells may delay the emergence of drug resistance.

Keywords: SARS-CoV-2; Inhibition; Mutation; Drug resistance; Receptors

1. Introduction

A global economic catastrophe resulted from the SARS-CoV-2 outbreak has put the world's health in danger [39]. Corona viruses are enclosed, single-stranded, positive sense RNA viruses with a helical-symmetric nucleocapsid. The new virus that was first identified as the 2019 novel coronavirus (2019-nCoV) appeared in the Chinese city of Wuhan in December 2019. The symptoms were observed to include; Fever, a dry cough, dyspnea, headaches, pneumonia with a possible risk of developing respiratory failure due to alveolar injury, and perhaps death were some of the clinical symptoms it generated [56]. It spread greatly across the world with majorly respiratory symptoms along with severe acute respiratory syndrome in some victims. Previously existing drugs have been tested on this virus with various methods and while some proved to have activity against the virus others displayed however no response.

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The present pandemic is dangerous because of the transmission rate (TR), which is measured as the number of newly infected people per infected person and is between 2.5 and 3. In contrast, if no one in the community is immune, the TR of the yearly common cold is less than 1.4 [10]. After the initial phase of the viral infection, ~ 30 % of patients hospitalized with COVID- 19 develop severe disease with progressive lung damage, known as severe acute respiratory syndrome (SARS), and a severe immune response [48]. The SARS-CoV-2 Nucleocapsid protein promotes the activation of cyclooxegenase-2 leading to inflammation in the lungs [35]

It is very important to point out the mutations experienced by SARS-CoV-2. Because extremely detrimental changes are swiftly purged, most mutations found in circulating SARS-CoV-2 virions' genomes are likely to be neutral or modestly deleterious [22]. This is because, while high-effect mutations that aid virus adaptation and fitness do occur, they are rare as compared to tolerable low-effect or no-effect "neutral" amino acid alterations. In at least certain settings, a tiny proportion of mutations are likely to affect virus phenotypic in a way that offers a fitness advantage. Pathogenicity, infectivity, transmissibility, and/or antigenicity of viruses may all be affected by such alterations [4].

Several drugs have been tested on the virus, first is some known viral drugs used for other viral diseases, for example, The US Food and Drug Administration (FDA) has authorized the experimental drug remdesivir as a treatment for COVID-19 in cases of emergency use. Remdesivir was initially created for Ebola virus infections. However, this medication still needs work to increase its efficacy and safety [56]. Another group includes the drugs used to treat coronavirus pneumonia, such as interferons, ribavirin, and cyclophilin inhibitors with the antiviral medications ribavirin, valganciclovir, and thymidine [79].

Another group of observed inhibition are the antibacterial medications chloramphenicol, cefamandole, and tigecycline, the muscle relaxant medication chlorphenesin carbamate, the antitussive medication levodropropizine, and the natural products platycodin D (Platycodon grandiflorus), baicalin (Scutellaria baicalensis), sugetriol-3 (3,4-dihydroxyphenyl) -2-[[2-(3,4-dihydroxyphenyl) -3,4-dihydro-5,7-dihydroxy-2H-1-benzopyran-3-yl]oxy] -3,4-dihydro-2H-1-benzopyran-3,4,5,7-tetrol) displayed high binding affinity to PLpro protein [79] indicating the possibility of testing these drugs for affinity in other sites important for the virology of SARS-CoV-2

This inhibition process includes both viral and host factor inhibition and also a combination of two or more of this major inhibition can generate a potential treatment for SARS-CoV-2, the importance of using multiple processes of inhibition in curbing mutation is evident and should be screened for.

1.1. Measures to take in generating SARS-CoV-2

There are four measures to take in generating SARS-CoV-2 inhibitors that might surpass mutation should it arise which includes;

- Finding inhibitors with two or more mechanism of inhibition in virus target inhibition
- Employing host target inhibition
- Complex forming compounds that will interfere with viral-host cell fusion independent of interaction with the host or organism
- Human receptor cell lookalikes with higher affinity to the virus (proteins).

2. Finding inhibitors with two or more mechanism of inhibition in virus target inhibition

The therapies that target the coronavirus directly include preventing the synthesis of viral RNA by interfering with the genetic code of the virus, preventing virus replication by interfering with essential virus enzymes, and preventing the virus from binding to human cell receptors or from self-assembling by interfering with certain structural proteins [79]. an in-dept understanding of these individual inhibition mechanism can help determine the inhibition combination process and also help in screening of drugs known for activity against a particular target site for activity against other target sites.

Drugs targeting SARS-CoV-2 can be classified according to [4]

- Fusion inhibitors: inhibit the fusion process of viral entry
- protease inhibitors: target some proteases
- Transcription inhibitors: target the reverse transcription step by blocking RNA-dependent RNA polymerase and preventing viral replication,
- nucleoside reverse transcriptase inhibitors

- Some antivirals target M2 channel protein
- inhibition of organism factors involved in viral pathology and inhibition

The above can be used in finding a candidate that falls into more than one of these listed above and also in a more elaborate manner using the process of SARS-CoV-2 pathogenesis, strategic inhibition combination can be employed to achieve a definite potential outcome.

2.1. Inhibition of binding of virus to receptors

The angiotensin-converting enzyme 2 (ACE 2) is bound by SARS-CoV-2. There has been a lot of discussion on how the enhanced risk of transmission and severity of infection may be mediated by the SARS-CoV-2's high affinity binding to ACE2. Additionally, the Spike protein of SARS-CoV-2 possesses a conserved RGD motif known to bind integrin that is absent from other coronaviruses. This motif is located within the S protein's receptor binding site, close to the ACE2 receptor-binding region [57].

Following receptor binding, the virus must next gain access to the host cell cytosol. This is generally accomplished by acid- dependent proteolytic cleavage of S protein by a cathepsin, TMPRRS2 or another protease, followed by fusion of the viral and cellular membranes [7]. In low pH settings, TMPRSS2 initiates cleavage followed by activation of the spike protein, which promotes membrane fusion, viral entrance into the cells, as well as the dissemination of infection to neighboring cells [19].

The beta coronaviruses like SARS-CoV-2 mostly use hemagglutinin- esterase (HE) to link to sialic acid on the glycoprotein surface. These fusion steps could be inhibited using fusion inhibitors [4]. Also inhibition of the receptors by competitive antagonism can play a vital role in preventing SARS-CoV from entering the host cells and further prevent infection.

A study by Haddad *et al* (2022) using a combination of experimental methods (biochemical inhibition assays, surface plasmon resonance, and quartz crystal microbalance with dissipation monitoring) confirmed the potential role of Tannic acid in the prevention of SARS-CoV-2 infectivity through the inhibition of extracellular RBD/ACE2 interactions and TMPRSS2 and 3CLpro activity.

The downside of focusing on this is that the organism mechanism is a highly determining factor here in as much as human factors can prevent binding but focusing on the organism can lead to mutations that will override this inhibition mechanism. For example; It was also projected that a single N501T mutation may considerably increase the binding affinity between SARS-CoV-2 and human ACE2 and hence potentially results in much more virulent bodies based on the comprehensive modeling of the virus-human receptor interactions. Because zoonosis is caused by mutations in the S protein, not all of these mutations will result in the S protein binding to ACE2. Therefore, certain future CoVs may not be affected by medications that block the S protein-ACE2 connection. However, the spike-shaped protein on the surface of the viruses that cause SARS, MERS, and COVID-19 offers an alluring target for antibodies or other substances, which could stop CoVs from infecting human cells. The S protein of the virus appears to emerge as the consensus target antigen [10].

2.2. Inhibition of Viral Replication

After the fusion completion, the envelope is peeled off and the genome of SARS-CoV-2 along with its nucleocapsid penetrates the cytoplasm of the host cell [3, 24] Its genome contains open reading frames 1a and 1b- (ORF1a and ORF1b) genes that produce two polyproteins (pp), pp1a and pp1b, that help in hijacking host ribosomes for the viral translation process [86]

Then, papain-like protease (Ppro) and Mpro cleave these polyproteins to produce many non-structural proteins These proteases play an extremely important role in viral transcription and replication [4]

The corona virus enzyme papain-like protease (PLpro) is necessary for processing viral polyproteins to create a functional replicase complex and promote viral spread [5]. The main protease (Mpro) is an essential coronavirus enzyme that plays a crucial role in promoting viral transcription and replication, making it an alluring therapeutic target for SARS-CoV-2 [28] nucleocapsid protein is also important for RNA synthesis [59].

With the completion of the viral translation process, a non-structural protein known as nsp12 forms a transcription and replication complex known as RNA-dependent RNA polymerases (RdRp) [54]. Nsp12 is paired with its cofactor (nsp7 and nsp8) in SARS-CoV. This protein complex generates a negative-sense complementary RNA using the initial positive

RNA as a reference which the virus now uses to synthesize new positive RNA molecules to process another stage of translation and replication and synthesize the genomes of the newest viral particles [15, 42]

Another important factor in viral replication is the NF-kB, to determine the interplay between NF-kB signaling and SARS-CoV-2 infection, a test was conducted by [44]. Here, this signaling was silenced and assessed an impact on viral host dynamics, there was diminished RelA expression correlated with a significant decrease in viral nucleocapsid protein levels. This inhibition of viral replication, as measured by viral protein expression, following short interfering RNA-mediated silencing of RelA or NF-kB1 was further confirmed by quantification of cells expressing nucleocapsid protein, as measured by immunofluorescence staining, showing significant loss of infected cells following targeting of NF-kB1, RelA, or nucleocapsid directly to ensure that loss of NF-kB signaling was the molecular basis for decreased viral replication. Further expressed a chimeric transcription factor comprised of the DNA-binding domain of RelA fused to the transcriptional activator VPR (VP64-p65-Rta tripartite activator) to agnostically induce gene activation based on enhancer availability, as previously described in wild-type or RelA knockout epithelial cells constitutively overexpressing ACE2, SARS-CoV-2 infection in RelA knockout compared to wild-type cells showed a dramatic loss in viral protein production that could be rescued following reconstitution of RelA/p65 activity. This data suggest that SARS-CoV-2 replication requires the transcriptional output of the NF-kB signaling pathway.

Successful inhibition Using reverse transcription inhibitors can disrupt replication stage by budding and assembly of the enveloped virus [42]. Also, Inhibition of Papain-like protease and main protease of SARS-CoV-2 can have effect on the viral replication and further inhibit the viral infection. Also, from the study conducted by Nilsson-payant *et al* (2021) inhibition of the NF-kB pathway can inhibit viral replication which in itself has an effect on the inhibition of SARS-CoV-2 pathogenicity. [inhibitors of NF-kB pathway]

Therefore, it has recently been suggested that using antiviral medications that target. PLpro may be advantageous in preventing not just viral replication but also the dysregulation of signaling cascades in infected cells that may result in cell death in nearby uninfected cells [10].

SARS-CoV PLpro is a cysteine protease with multiple major functions, including processing of the viral polyprotein chain for viral protein maturation, dysregulating host inflammation responses through deubiquitylation, and impairing the host type I interferon antiviral immune responses by removing interferon stimulated gene 15 (ISG15) modifications [5, 9, 46], Some already existing inhibitors of Viral replication exist.

Pyrazolidinone was discovered to be a SARS-CoV 3CLpro inhibitor by high-throughput screening (HTS). Additionally discovered to inhibit 3CLpro are nitroanilides made from the medication niclosamide. These compounds, however, experience heavy metabolism and quick elimination. Towards spite of this, they represent a potential first step in future therapeutic development [10]. However, the design of protease inhibitors that inhibit these proteases are potential antivirals against SARS-CoV-2 [77].

A prominent inhibitor of SARS-CoV-2 replication is the EIDD-2801 as tested by Wahl *et al*., 2021; the SARS-CoV-2 virus as introduced exhibited viral replication in bona fide human lung tissue and showed a predominant in vivo infection of human lung epithelial cells, including type-2 pneumocytes that are present in alveoli and ciliated airway cells. Acute infection with SARS-CoV-2 was highly cytopathic and induced a robust and sustained type-I interferon and inflammatory cytokine and chemokine response, the results however show that therapeutic and prophylactic administration of EIDD-2801—an oral broad-spectrum antiviral agent that is currently in phase II/III clinical trials—markedly inhibited SARS-CoV-2 replication in vivo.

Another inhibitor found with cell based assay performed by Fu *et al*., 2021; after the screening of chemical libaries against PLpro of SARS-CoV-2 and examining available inhibitors, is the Inhibitor GRL0617 which showed a promising in vitro IC50 of 2.1 μM and an effective antiviral inhibition in cell-based assays and recognized as a non covalent inhibition resides in the ubiquitin- specific proteases (USP) domain of PLpro. NMR data indicate that GRL0617 blocks the binding of ISG15 C-terminus to PLpro. Using truncated ISG15 mutants, they discovered that the C- terminus of ISG15 plays a dominant role in binding PLpro. Structural analysis also reveals that the ISG15 C-terminus binding pocket in PLpro contributes a disproportionately large portion of binding energy making the pocket a hubspot for chemical libaries screening and also GRL0617 structure a potential prototype for PLpro inhibition and possibly SARS-CoV-2 inhibition'

Another compound is Remdesivir, a repurposed drug. remdesivir acts as an RdRp inhibitor, targeting the viral genome responsible for the replication process. So, it inhibits the protein complex of CoVs in the RdRp process. After metabolizing remdesivir by the host to its active metabolite nortriptyline (NTP), this metabolite is conjugated to ATP,

which incorporates it into the nascent RNA strand. This incorporation of the new strand results in RNA synthesis termination, halting the RNA strand growth after adding more nucleotides [4] even though mutation is still a limiting factor for this mechanism as proved by Agostini *et al*, but it is possible that the drug however may possess alternate mechanism to bypass this mutation [2].

2.3. Inhibition of viral assembly

The proteins involved in the inhibition of viral assembly form viroporins that are proteins of hydrophobic nature and small in architecture. These viroporins are essential for viral assembly, along with its release. They also mediate pathogenic processes and induce cytotoxicity [84].

They undergo translation on the polysomes bound to the membranes, fused in the ER, and are carried towards the Golgi complex where they interact with E proteins to generate virions. Out of the three TDM, the first one is capable enough to encourage self- association of M proteins, improved membrane affinity, and retention in Golgi [68]. E protein and M protein are responsible for virion formation.

In addition, it has been discovered that the SARS CoV-2 N protein opposes antiviral RNA inhibitors [85]. M protein inhibit the virion formation and to prevent inflammatory reactions in host cells, it also forms ribonucleoproteins [50].

2.4. Inhibition of virology/pathology

Recent studies suggest that the main mechanism disrupting the endothelial barrier occurs in several stages according to Libby and Lüscher (2020), Teuwen *et al* (2020), Varga *et al* (2020) and Xiao *et al* (2020);

- A direct effect on the endothelial cells that causes an immune response of the vascular endothelium (endotheliitis) and endothelial dysfunction. Second, lysis and death of the endothelial cells
- Sequestering of human angiotensin I- converting enzyme 2 (hACE2) by viral spike proteins that activate the kallikrein–bradykinin and renin–angiotensin pathways, increasing vascular permeability
- Overreaction of the immune system, during which a combination of neutrophils and immune cells producing reactive oxygen species, inflammatory cytokines (e.g., interleukin [IL]-1β, IL- 6, and tumor necrosis factor), and vasoactive molecules (e.g., thrombin, histamine, thromboxane A2, and vascular endothelial growth factor), and the deposition of hyaluronic acid lead to disruption of endothelial junctions, increased vascular permeability, and leakage and coagulation.

The E protein is essential for the assembly, budding, production of the virus's envelope, and pathogenic stages of its life cycle [43, 52] The viral replication cycle has been found to involve the E protein in a variety of functions, including viral assembly, virion release, and viral pathogenicity [85].

It is known that the Membrane protein inhibits Nuclear factor kappa light chain enhancer of activated B cells, a transcription factor that regulates inflammatory responses, cellular growth and apoptosis [69] through interactions with IKKb (I Kappa B Kinase) and lowers COX-2 levels, which promotes the spread of viral infections [50].

NSP1, NSP2, and NSP3 are released from the N-terminal region of polyproteins by this papain-like protease domain, While NSP 2 is in charge of upsetting the environment of the host cell, NSP 1 is a powerful inhibitor of host gene expression. As a result, NSP 3 indirectly contributes to SARS-CoV-2 pathogenicity [85].

The RdRp enzyme uses the machinery of the host cell to copy viral RNA and enable transcription of the viral genome into new copies of RNA. To increase its processivity, NSP13 can also boost its helicase activity by attaching to duplex RNA, which further unwinds the RNA [85].

S proteins also help in promoting adhesion of infected cells with adjacent non-infected cells that enhance the spreading of the virus [52]. The fact that these protruding spikes are the first point of contact with host receptors, therapeutic strategies can be applied to prevent its binding to target receptors and prevent viral en- try into host cells [50].

Nucleocapsid protein participates in both the host cellular response to viral infection and CoV replication, The N protein in SARS-CoV promotes the activation of Cyclooxygenase-2 (COX-2) leading to inflammation in the lungs [83] Corona virus's nucleocapsid (N) protein is a structural protein that binds to viral RNA and provides stability [85].

E protein may facilitate disruption of the epithelium of the lungs due to binding of Protein Associated with Caenorhabditis elegans Lin-7 protein 1 (PALS1) to the PDM [65].

The 3- phosphoinositide-dependent protein kinase 1 (PDK1) is a critical kinase of Protein Kinase B (PKB) that is known to slowdown the process of apoptosis. The C terminal of M protein hinders the interaction of PDK1 and PKB and leads to the release of caspases 8 and 9, ultimately causing cell death or apoptosis [69]. The SARS CoV M also leads to the activation of b interferons (IFN-b) in cell lines [78].

3. Employing host target inhibition

3.1. Inhibition of Viral Entry into the Cell

The spike (S) protein may play a part. CoVs' S proteins give their virions a corona-like appearance and mediate viral infection of target cells, so they are essential for viral replication. The initial attachment of the virion to the host cell is initiated by interactions between the S protein and its receptor [14] For the viral membrane to bind to the host membrane, the S protein of the SARS-COV-2 virus undergoes a structural change [19] Which cells can get infected is largely determined by the interaction of CoV S proteins with particular cellular receptors, and the entrance process is a desirable target for antiviral therapy [24].

Among them, Spike encourages virus cell membrane fusion and host adhesion during virus infection. Spike thus partially determines the host range [79] and the nucleocapsid (N) protein, which contains the viral DNA [71] According to [56] these structural proteins are necessary for virus-host cell interaction and virus assembly. They first bind to a host receptor and then join the viral and host membranes, this is in charge of mediating SAR-CoV-2 entry into host cells.

The S protein aids viral entrance into the host cell and causes cell-cell fusion between nearby uninfected and infected cells [50] The amino acids glutamine, asparagine, leucine, phenylalanine, and serine increase ACE2 binding in SARS-COV-2 [76] Agents that are able to block cell receptors from the CoV spike proteins are useful in preventing infection because spike protein is the protein that allows the virus to bind to and fuse with the membrane of the host cell.

It is known to bind to viral RNA to form the core of a ribonucleo protein, this aid in the entry of the virus into the host cell and its interaction with cellular functions once the virus has fused [50] The virus also uses Spike protein to neutralize antibodies, making it easier to bind to the host receptors [53]

Antibodies, proteins, peptides, small compounds, and drugs (ACE inhibitors, inhibitors of host cell proteases, such as TMPRSS 2, furin, and cathepsin) are examples of host cell parameters used to stop the entry of the virus into the cells. These substances will prevent the virus from interacting with the host receptor site. However, as previously said, SARS-CoV-2 may adopt an alternative method and mechanism to infiltrate the cells, necessitating a therapy that will also target the virus cells. Additionally, host cell targeting might entail blocking a number of receptors to highlight potential side effects from the therapy [24]

Camostat mesylate is an inhibitor of serine protease (TMPRSS) [51] and is another medication that targets the fusion of viruses Inside the targeted host cells, SARS-CoV-2 gains access to TMPRSS2 and/or ACE-2 receptors [73] This prevents the virus from entering the cell

3.2. Inhibition of host receptors

SARS-CoV-2 enters target cells using the receptor ACE2 and the cellular protease TMPRSS2 through the spike and Nucleocapsid structural proteins. The virus comes into the steward cells during two pathways, either via plasma membrane fusibility or endosomes. Through both mechanisms, it engages ACE2 as an entry sensor and the viral S protein, which mediates binding to the host cell membrane [4]

For instance, the coronavirus strain NL63 enters the host cell via the same ACE2 receptor as SARS-CoV, but the virus entry and consequence are very different, with NL63 only causing a moderate respiratory infection while SARS causes severe respiratory distress [40]

The type II trans-membrane serine protease family member cell surface protease, transmembrane protease serine type 2 (TMPRSS2) can also be used by SARS-CoV. A recent study showed that the attachment between S protein and ACE2 is activated by TMPRSS2 [24] Despite using both host cell entrance pathways, it seems that the TMPRSS2 pathway is the main method that SARS-CoV infects the lungs [25, 56] Trypsin is one of the host proteases that SARS-COV-2 may also employ to activate S proteins [45] Given that a wide variety of proteases can activate SARS-COV-2 and that different proteases are found on the cell surfaces of various cell types, SARS-COV-2 has the ability to infect a variety of cells [63].

Transmembrane serine protease 2 (TMPRSS2) is primed with the (S) protein, and SARS-CoV-2 infection in the cells of the lungs has been seen to be avoided by the TMPRSS2 inhibitor [4]

Umifenovir is a short indole derivative that provides a range of action against RNA and DNA viruses. These viruses are host targeting and directing, thus preventing the viral entry via host cell [11] repurposed from its initial used in treatment of influenza A and B infection in China and Russia [4].

3.3. Promotion of host inhibition mechanisms

Impaired immunity with compromised lung functions and pro-inflammatory cytokine levels are COVID-19 features. Adaptive immune dysfunctions include lymphopenia and activation, granulocyte and monocyte dysfunction, and elevated immunoglobulin G (IgG) and total antibody levels, these are present in blood and convalescent plasma of infected people. The control of inflammation is achieved by immune modulation [38].

Nucleocapsid protein Inhibits type I interferon causing restriction in immune response and Interacts with P42 proteasome subunit known to degrade the viral proteins, PLpro is also suspected of cleaving proteineous post-translational modifications on host proteins as a means of avoiding host antiviral immune reactions [5].

Viral proteases frequently inhibit ubiquitin and ubiquitin-like modifications, which reduce inflammation and antiviral signaling and are thus used to modulate innate immune pathways [30]

NFkB (Nuclear Factor Kappa B) activation is necessary to generate immune responses against the pathogens. The M protein is known to inhibit NFkB through interactions with IKKb (I Kappa B Kinase) and reduces levels of COX-2, thus enhancing the proliferation of the viral pathogen [13]

- Vaccine production in the form of inactive organism particle is a way to improve host inhibition mechanisms. Due to the express advantages of recombinant protein technology, their potential for rapid development and reduced side effects. A particle of the virus responsible for immune response but lacking pathologic tendencies can be introduced into the body for initiated immune response against the virus. Examples of recombinant vaccines for SARS-CoV-2 are Nucleic acid-based coronavirus vaccine, Protein-based coronavirus vaccine, Vectored vaccines against coronavirus, Virus-like particle-based vaccine and Artificially synthesized protein-microarray [81]

A study conducted by Kim *et al* (2020). Preclinical immunogenicity of MERS-CoV vaccines where microneedle array was used to deliver recombinant coronavirus vaccine in mice using a MERS-S1 subunit vaccines fused with a foldon trimerization domain to mimic the native viral structure and inserting an immune stimulant into the trimeric region, the findings showed that the vaccine elicited strong and long-lasting antigen-specific antibody responses.

Another study by Smith *et al* (2020) reported the immunogenicity of a synthetic DNA-based vaccine against SARS-CoV-2, INO-4800 in multiple animal models. The immunized animal showed specific T cell responses, and antibodies that not only neutralized SARS-CoV-2 and blocked S protein-ACE2 interaction, but also circulated through the lungs. The study emphasized on its further evaluation as a potential contender for COVID-19 vaccine.

Additionally, several other methods are available for the development of a vaccine against SARS-CoV -2, including the use of inactive or live-attenuated viruses, virus-like particles (VLPs), viral vectors, and protein-based, DNA-based, and mRNA-based vaccines. The development of inactivated vaccines requires a target virus to be initially inactivated, either chemically or by irradiation. This allows the nucleic acids of the virus to be destroyed, while keeping the viral antigens intact [87] Recently Gao *et al* (2020) developed PiCoVacc, a purified inactivated SARS-CoV-2 virus vaccine, that was found to incite SARS-CoV-2-specific neutraliz- ing antibodies in mice, rats, and non-human primates. The generated antibodies were found to neutralize 10 representative strains of SARS-CoV-2, holding up its broad-ranged applicability against the virus.

Also Spruth *et al*, 2016 developed a double-inactivated, candidate whole-virus vaccine against SARS-CoV using sequential exposure to formaldehyde and ultraviolet radiation to ensure its safe use. The immunogenicity of this vaccine was verified using a mouse model, which showed high antibody titers against the CoV S protein and enhanced neutralizing antibodies, highlighting its potential for application as a platform for the development of a SARS-CoV-2 vaccine.

However, with these findings, there is still a potential public health risk associated with incomplete inactivation, which leads to undesired immune or inflammatory responses [81].

Live-attenuated vaccines are being developed from live coronaviruses whose virulence has been reduced under laboratory conditions However, owing to its safety concerns, in particular with regard to elderly individuals (at a higher risk of COVID 19), the use of live-attenuated virus vaccines is unlikely to represent the best approach [81].

- Virus-like particles (VLPs) that is, multi-protein supra-molecular preparations with features equivalent to those of viruses can represent a resourceful platform for vaccine development owing to their flexible immunological features, including suitable size, repetitive surface geometry, and stimulation of innate and adaptive immune responses. VLP-based vaccines target B lymphocytes and induce potent antibody responses, resulting in T helper cell activation and their presentation on MHC class II molecules via antigen-presenting cells (APCs) [32, 41]
- Apart from host immune system activation, another viable method is improving the host immune system by introducing Polyclonal Anti-SARS-CoV-2 Immunoglobulins (Hyperimmune Serum) for Passive Immunization in COVID-19 as reviewed by [16] who identified 16 clinical trials employing polyclonal immunoglobulins from convalescent donors or immunized animals, they admitted that the efficacy of COVID-19 hyperimmune serum remains hard to guess against a respiratory pathogen and in a landscape with quickly evolving viral strains, but these hyperimmune serum has several advantages including a smaller reinfusion volume, an easier administration route and easier preservation and monoclonal antibodies are more diversified against emerging variants of concern, and far cheaper. Therefore further studies can be performed on this inhibition process to ascertain the possibility of its use in the population.
- Convalescent plasma from recovered COVID-19 patients contains antibodies that can neutralize viral infection [37]. However, adverse events have been reported that include fever, allergic reactions, transfusion-related lung injury, life-threatening bronchospasm and circulatory overload especially in patients with cardiorespiratory disorders [27]
- Transplantation of Mesenchymal Stem Cells (MSC) possesses self-renewal and anti-inflammatory properties resulting in pulmonary epithelial cell repair and defense against a cytokine storm and promotion of alveolar fluid clearance [8]

4. Complex forming compounds that will interfere with viral-host cell fusion independent of interaction with the host or organism

One of this compounds that can potentially form complexes thereby preventing the viral-host cell fusion is using Natural small molecules as inhibitors of coronavirus lipid- dependent attachment to host cells with the argument that Viral infectivity depends on interactions between components of the host cell plasma membrane and the virus envelope [6].

A detailed understanding of the process of viral-host cell fusion during SARS-CoV-2 pathogenesis is an important aspect towards understanding how this process of inhibition works.

The SARS-CoV-2 virus bind to the Angiotensin-Converting Enzyme 2 (ACE2) receptors on human respiratory epithelial cells. During attachment, the S glycoprotein divides into S1 and S2 subunits. S1 contains the receptor-binding domain by which the coronavirus binds to the peptidase domain of the ACE2 receptor. S2 intervenes later, during fusion of the plasma membranes [82].

Coronaviruses are a class of viruses with a long single positive RNA molecule and a lipid envelope that requires a plasma membrane fusion process mediated by endocytosis, a mechanism in which cholesterol and lipid rafts play a fundamental role in the early stage of infection of a cell [23, 33] SARS-COV-2 is a member of a virus family with a lipid envelope that fuses with the host cell through endocytosis, internalizing its components in the cell [23].

Macromolecules such as methyl-β-cyclodextrin (MβCD) and other compounds with depletive cholesterol activity have been used to inhibit this fusion [20]. These non-toxic macromolecules mimic attack sites for the enveloped virus, competing with host cell attack sites [6].

5. Human receptor cell lookalikes with higher affinity to the virus (proteins)

Recombinant soluble ACE2, which lacks the membrane anchor and can circulate in small amounts in the blood, has been shown to be effective in binding SARS-COV-2 S proteins and preventing viral entry in chemically engineered human blood vessel organoid and human kidney organoid, but it has not been fully accessed, potential side effects have not been taken into account, and a full study on its affinity difference with that of the n-type receptor has not been conducted [45].

6. Conclusion

However, drug resistance may emerge quickly after treatment as a result of the numerous mutations that RNA virus experience. In contrast, because host cell mutations are uncommon, treatments that target the host cells may delay the emergence of drug resistance [23]. For example, if a drug is known to inhibit the binding of Spike protein of coronaviruses with host receptors in the past, the N501Y mutation seen in SARS-CoV-2 spike protein could complicate this inhibition process.

Yet these drugs targeting host cells have greater potential for adverse effects to the human. Angiotensin-converting enzyme-2 (ACE2) receptor is a protein that expresses itself in different types of cells, such as esophageal cells, absorbent enterocytes, myocardial cells, alveolar cells, and proximal cells of the kidneys.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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