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Understanding the Molecular Mechanism(s) of SARS-CoV2 Infection and Propagation in Human to Discover Potential Preventive and Therapeutic Approach

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Abstract

In December 2019, outbreak of novel coronavirus (COVID-19) occurred in Wuhan, Hubei Province, China and exported across the world leading to thousands of deaths and millions of suspected cases. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection into the host undergoes a huge number of complex replicative machineries which still remains unclear. Understanding the mechanism (s) of replication and mode of infection of SARS-CoV2 to human cells will help us in the development of novel vaccines or drugs for the eradication and prevention of the disease. This review compiles the knowledge of SARS-CoV2 replicative machinery, mode of infection to the human cells and the development of drugs and vaccines which are currently under clinical trials.

Key words: Coronavirus, COVID-19, SARS, Vaccines, Infection

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) belongs to the group of Coronavirus that causes coronavirus disease 2019 (COVID-19). A pandemic outbreak of coronavirus has been emerged from the Wuhan city, in the late December 2019. Due to its alarming increase in the spread of the disease, world health organization (WHO) declared a public health emergency of international concern on 30th January 2020. The incubation period for the infection is reported to be 1-14 days. The most common symptoms of patients with novel coronavirus infection were observed fever, dry cough, myalgia, fatigue with abnormal chest CT, and less symptoms observed were sputum production, headache, hemoptysis and diarrhea. As, a few clinical symptoms reported were different from the severe acute respiratory syndrome (SARS)

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caused by SARS coronavirus (CoV) that occurred in 2002-2003, which enabled to come to a conclusion of identifying a new infectious agent having the ability to pass the infection from a human to human caused this emergent pneumonia outbreak. Scientists from China sequenced the genome with the help of techniques such as real time PCR and next-generation sequencing and identified it as the novel coronavirus, the seventh member of the coronavirus family [1]. WHO named this novel virus as novel coronavirus (COVID-19) on 11th February 2020. while, the international committee on taxonomy of viruses (ICTV) based upon the phylogenetic and taxonomic analysis suggested the name for new coronavirus as "SARS-CoV2".

Coronaviruses are positive stranded RNA viruses which under electron microscope appears to have a crown like structure. Due to the presence of spike glycoproteins on the envelope, this virus got the name coronavirus (*coronam* in Latin means crown) (**Figure-1**). The family *coronoviridae* of subfamily *orthocoronovirinae* is classified into four generas of CoVs: alpha CoV, beta CoV, delta CoV and gamma CoV. The beta CoV is further divided into five sub-genera or lineages. In general, it has been reported that 2% of the human population are healthy carriers of CoV and 5% of these viruses are responsible to cause severe respiratory infections [3]. Common human CoVs are HCoV-OC43 and HCoV-HKU1 of beta CoV genera and HCoV-229E, and HCoV-NL63 of alpha CoV genera. These viruses are reported to cause common cold restricting to the upper respiratory infections in immunocompetent individuals. Other human CoVs are SARS-CoV, SARS-CoV-2, and MERS-CoV of beta CoV genera. These viruses are reported to cause epidemics related to upper respiratory infections. The mortality rate of these viruses is 10% and 35% respectively. The novel coronavirus belongs to the beta CoVs category. It has a round or elliptical morphology and often pleomorphic form. It has a diameter of approximately 60-140 nm. Also, these viruses can be effectively inactivated using lipid solvents such as 75% ether, ethanol, chlorine-containing disinfectants, peroxyacetic acid, and chloroform. The single stranded RNA genome of CoV contains 29891 nucleotides which encodes 9860 amino acids. Although its origin is yet established, but the genome sequencing suggests that the novel coronavirus probably evolved from a strain found in bats [4].

Coronaviruses are wide spread in humans and several other vertebrates causing major infections of respiratory, enteric, hepatic and neurons. In note, in 2012 the SARS-CoV and middle east respiratory syndrome coronavirus (MERS-CoV) have caused human epidemics. Both the coronavirus infections have higher cases of fatality rates of 40% and 10% respectively. Although the current SARS-CoV-2 infection has been reported to share a 70% similarity with the SARS-CoV genome, but it appears to be much more transmissible [5]. Both SARS-CoVs enters the host cell via the angiotensin converting enzyme-2 (ACE-2) receptor [6]. The SARS-CoV-2 initially infects the lower airways and binds to ACE-2 on alveolar epithelial cells. As, the virus is a potent inducer of cytokines, the cytokine storm or cytokine cascade is the major mechanism suggested for organ damage by the viral infection. Furthermore, the virus activates the immune cells triggering the secretion of inflammatory cytokines and chemokines into pulmonary vascular endothelial cells [7]. At the beginning of the major outbreak of coronavirus infection palm cats had been reported to be the major source for SARS CoV and camels for the MERS CoV. Later more advanced solutions, reported bats to be the host for SARS CoV, spreading to other responsible intermediate hosts before infecting humans. It has been reported that most of the bat CoVs are the

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gene source for alpha-CoV and beta CoV [8]. While the gamma and delta CoVs are reported to be originated from birds. The transmission of this novel coronavirus has been reported via the close human to human contact [9]. The transmission primarily occurs through the respiratory droplets produced when an infected person sneeze. When inhaled these droplets can settle in the lungs, nasal mucosa or in the mouth of the people. Like most of the respiratory viruses CoVID-19 is considered to be most contagious when people are most symptomatic. At the beginning of the epidemic spread, the basic reproduction number (R_0) of the novel coronal virus indicated the transmissibility of the virus to be 4.71, but now the viral reproducibility has been reported to be declined to 2.08. this trend suggests that over time there should be gradual decline in the spread of the disease [10]. The current global aim is to prevent the pandemic spread and minimize the transmission wherever possible.

In the field of therapeutics, there are no such vaccines available for the cure of the pandemic disease coronavirus. A huge number of clinical trials have been registered whole over the world especially countries like china, Italy, USA, which also indicates the necessity and importance of hardcore urge to develop new therapeutics to fight against such diseases. The agents under study involve antivirals; Griffithsin, a spike protein inhibitor, nucleoside analogues such as Lopinavir/ritonavir [11]. Also, agents such as immunomodulatory, host targeted agents like interferon, chloroquine and immunoglobulins are also under study. Corticosteroids are reported to be effective at later stages of lung damage in the disease. New therapeutic approaches involving the treatment with allogenic mesenchymal stem cells are reported to enter the clinical trials involving the n-CoVID infected human patients (e.g. NCT04252118) [12]. Several measures have to be taken to prevent the spread of the novel coronavirus infection such as timely publication of the source of the infection as to eliminate the source of the infection, early diagnosis, reporting, isolation, possible treatments, avoiding unnecessary panics. Basic sanitary measures such as washing hands frequently, using disinfectant solutions, avoiding close contact with suspected patients with infection, should be taken as to minimize the transfection of the viral disease.

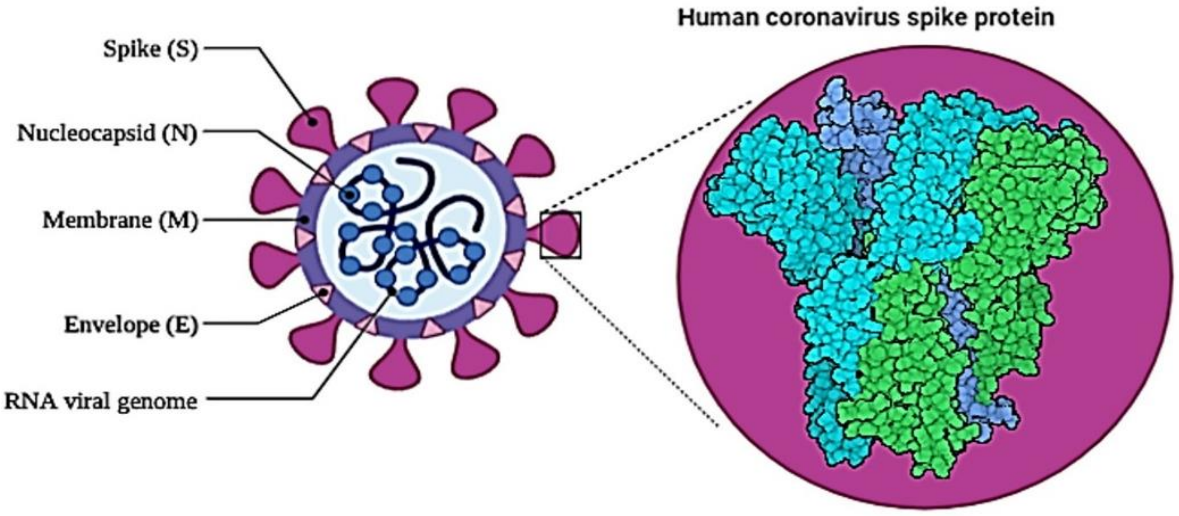

Figure-1: Figure represents the structural representation of Coronavirus, (S-spike, E-envelope, M-membrane, N-nucleocapsid) and protein visualization.

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Structure of Coronavirus (CoV)

Coronavirus (CoV) has a complex structure with incorporation of three major structural proteins glycoprotein S which represents the spike, glycoprotein M which is an unusual transmembrane and a nucleocapsid protein N which is internally phosphorylated [13]. The glycoprotein S of 200 K represents the spike that is quite bulky in nature with 15 to 20 nm ranging peplomers which is found in the viral envelope [14]. Additionally, there is also the presence of a minor transmembrane protein E in the structural region. Some species of coronaviruses include a envelope protein which has both function of hemagglutination and esterase (HE) [15]. CoVs are positive sense, single-stranded RNA viruses with the genome size of 30 kb [16]. The 5' end of the genome is capped and the 3' terminus is polyadenylated and is reported to be infectious. As it is bigger in size, the

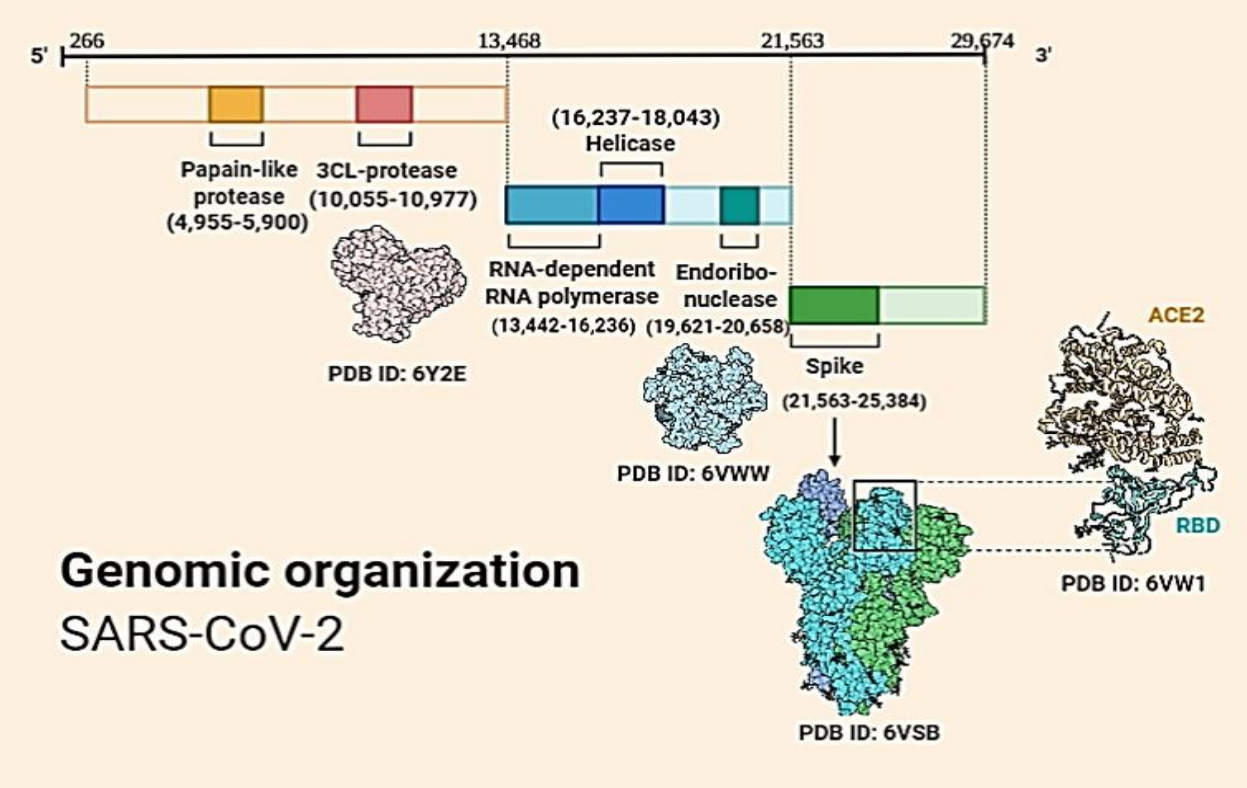

Figure-2: Figure represents the genomic organization of SARS-CoV2.

expression of individual genes takes place via a complex process where the release of sets of nested mRNAs takes place at the 5' end sequence. Heterologous RNA recombination can take place due to the extensive rearrangements. An untranslated (UTR) sequence containing 65 to 98 nucleotides which is also known as leader RNA is being occupied at 5' end of the genome and 5' end of all other subgenomic mRNAs. Another UTR region of 200 to 500 nucleotides followed by the poly A tail is being incorporated in the 3' end of the genome. The process of RNA replication and transcription process is being regulated by these two untranslated regions. There are 7 to 14 ORFs present in the genome of the coronavirus contains. The gene 1 in the beginning portion of the genome is spread across two-third of the genome and is of 20-22kb in length. This portion incorporates two ORFs (1a and 1b) which overlaps each other and collectively functions as the

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viral RNA polymerase (Pol). Four other major structural proteins are incorporated along the genome in series of 5'- S (spike)-E (envelope)-M (membrane)-N (nucleocapsid)-3' [17] (**Figure-1**). There are several other ORFs coding for non-structural proteins such as HE glycoprotein within these genes. Based upon the features of nucleotide sequence, gene order, method of expression each gene in coronavirus is marked differently, although these are conserved among the same serogroup. The SARS CoV different from other coronaviruses in the 3'region of the genome as they encode several smaller ORFs in these regions. These ORFs are under study related to the expression of 8 novel proteins marked as accessory proteins. At the N terminus both the ORFs 1a and 1b initially gets identically translated into two polyproteins whereas at the C-terminal identical polyproteins are not produced due to frame-shifting. CoVs encodes for Mpro (main protease) which is a chymotrypsin like protease and is also termed as 3CLpro due to its similarities with the 3C protease of picornaviruses [18]. This protease further processes the remaining polyprotein resulting in the production of 16 non-structural proteins. The presence of non-structural proteins is maximum in SARS-CoV species of coronavirus. Nsp3 is one such nonstructural protein which is multifunctional as it contains both ADP-ribose 1" phosphatase and protease activity [19]. A cylinder like structure is formed by two proteins nsp 7 and nsp 8 that is critical in the synthesis of RNA for CoV and to synthesize a single strand RNA binding protein (nsp 9) [20]. ORF1b encodes for the viral RNA dependent RNA polymerase and a multifunctional helicase protein (**Figure-2**). Furthermore, this protein holds the NTPase, dNTPase and 5' triphosphatase activities. The process of viral replication necessarily doesnot require the presence of all these structural protein gene products but deletion of one or more often leads to the inactivation of viral function. ORF3a also has structural protein as one of its product which is an O- glycosylated, triple-membrane spanning and has the capability to bind N, M and S glycoproteins together, suggesting its role in viral biogenesis [21].

Mechanism of Coronavirus Infection in Human

Coronaviruses mediate their pathogenic effects by cytocidal and immune related mechanisms. Several studies in the lab have reported that infection caused by CoV results in cytopathic effects such as cell lysis or apoptosis [22]. The virus form syncytia by cellular fusion. The viral replication complex formed via the replication process such as mobilization of vesicles leads to the cytopathic effects such as the disruption of the golgi complexes [23]. Cytopathic effects through the SARS-CoV infection has been reported to form the syncytia in lung tissues. The infection caused by SARS-CoV has also the potential to cause tissue fibrosis [24]. The promoter activity is induced by the N glycoprotein that induces the prothrombinase gene that correlates with fibrin deposition [25]. Next to cytocidal effects, immune mediated effects of both the innate and adaptive system has been reported to contribute to pathogenesis of SARS-CoV infections [26]. T cells and cytokines contribute a major role in development of the disease. Coronaviruses such as FIPV are reported to cause crucial infections with the help of humoral antibodies. In note of this, antibodies against spike protein were shown to induce peritonitis [27]. During the peak of the CoV infection, it has been reported an influx of cells in particular macrophages and an elevated release of cytokines (**Figure-3**).

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The spike S protein plays a major role in pathogenesis of CoV. Viral pathogenesis through this S glycoprotein is mediated through the target cell specificity mechanism. In this aspect, a single mutation in S gene can lead to significant effects on viral influence and tissue tropism [28]. Further, potentially important genes that are much needed for the viral pathogenesis are the non-essential ORFs. CoVs primarily target the respiratory epithelial cells. CoV have been reported to be seen in macrophages and many other cells and not only in respiratory tract and stool specimen. The interaction of S glycoprotein to the cellular receptors determines the CoV target cell specificity. According to the virus, receptor binding domains (RBD)sites within S1 region can be different, as some CoVs have RBD regions at the N-terminus while some have it on the C-terminus of S1 [29]. Peptidases are used by several CoVs as their cellular receptor although, the entry happens even in the absence of enzymatic domain. For the entry of CoVs into human host cells the CoV use angiotensin converting enzyme 2 (ACE 2) as their receptor and binds to ddipeptidyl-peptidase 4 (DPP4). Followed by the receptor binding the virus next gains access to the host cell cytosol with the help of cathepsin, TMPRRS2 or another protease which proteolytically cleaves the S protein, and then the procedure of viral and cellular membrane fusion takes place [30,31]. Cleavage of S protein takes place at two sites, first at the S2 portion where RBD and the fusion domains of the S protein gets separated. Second cleavage takes place at S2' to expose the fusion peptide. Fusion often takes place within the acidified endosomes, but some CoVs also fuse at plasma membranes. The fusion peptide cleaved at S' site is inserted into the membrane and forms a anti-parallel six helix bundle by joining to S2 heptad repeats. The formation of bundle helps in the

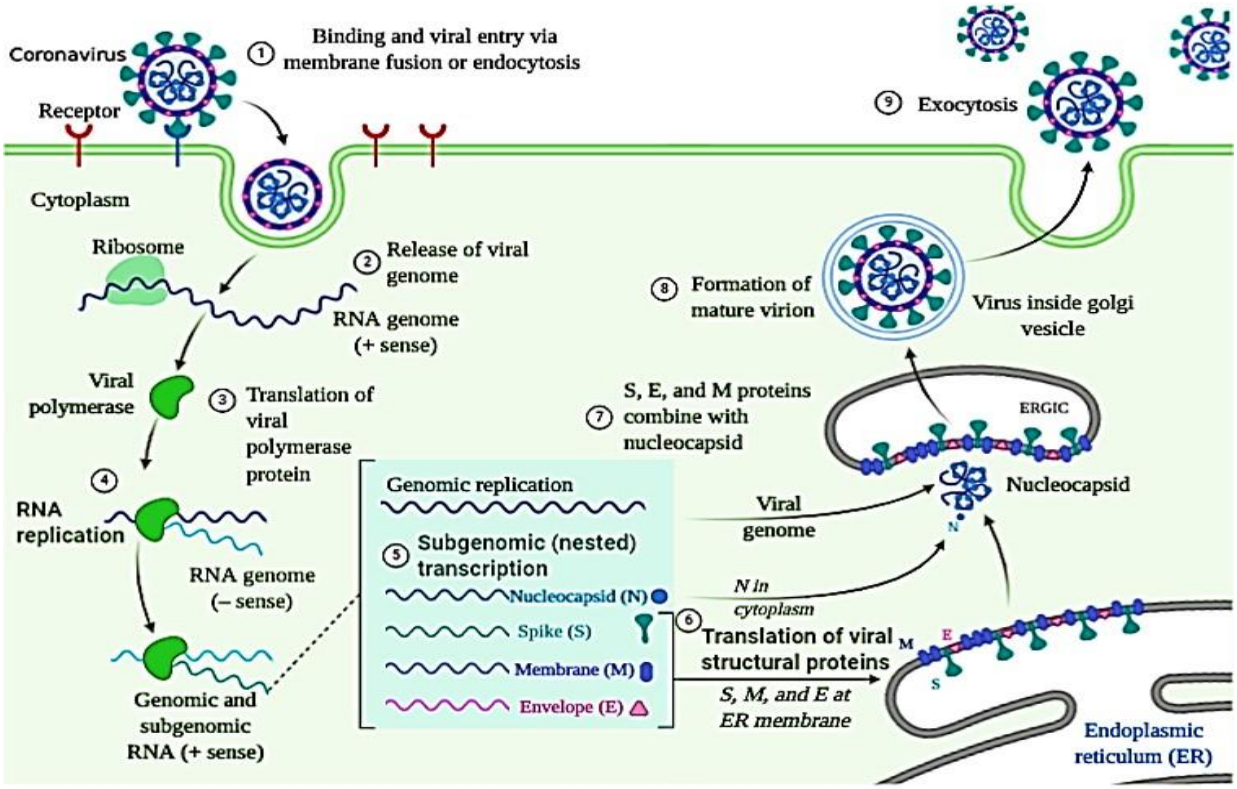

Figure-3: Figure represents the mechanisms of Coronavirus infection in host cell binding and viral entry through membrane fusion or endocytosis.

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progression of viral and cellular membrane mixing which then further releases the viral genome into the cytoplasm [32].

The translation of replicase gene from the virion genomic RNA marks the next step in CoV infection cycle. Two co-terminal polyproteins pp1a and pp1 ab and coded by two large ORFs rep 1a and rep1b. A slippery sequence (5′-UUUAAAC-3′) and an RNA pseudoknot helps in the expression of these polyproteins and leads to a ribosomal frameshift from rep1a reading frame into rep1b ORF [33]. In frequent cases the ribosome successfully unwinds the pseudoknot structure and continues the process of translation until met by the stop codon rep1a. But sometimes, ribosome gets hindered by the pseudoknot which stops the elongation and pauses itself on the slippery sequence, changing the nucleotide reading frame one shift back resulting in the translation of pp1ab [34]. The scientific explanation has not been yet found for this frameshift mechanism, but the hypothesize has been to either maintain rep1b and rep1a protein ratios or to postpone the production of rep1b products until a suitable environment for replication has been created by rep1a. contain Nsps 1-11 and 1-16 are present in pp1a and pp1b polyproteins respectively. In pp1ab, nsp11 from pp1a becomes nsp12 following extension of pp1a into pp1b [35]. These polyproteins are cleaved subsequently into individual nsps. Two-three proteases such as nsp3 encoded papain-like proteases (PLpro), and nsp5 encoded serine type protease main protease or Mpro are encoded by CoVs that play an important role in the cleavage of replicase polyproteins. PLpros are responsible for cleavage of nsp1/2, nsp2/3 and nsp3/4 boundaries and Mpro performs rest of the 11 cleavage events. Furthermore, the RNA replication and transcription of sub-genomic RNAs takes place via the replicase-transcriptase complex (RTC) formed by nsps which creates an environment suitable for RNA synthesis. There are several other enzyme domains and functions in nsps, such as nsp12 which encodes the RNA-dependent polymerase (RdRp) domain whereas nsp13 has the RNA helicase domain and RNA 5’ triphosphatase, nsp14 encodes exoribonuclease (ExonN) involved in replication fidelity and N7-methyltransferase activity; nsp16 encodes 2’-O-methyltransferase activity. Nsps have several other unknown functions as well as many other functions such as blocking innate immune responses [36]. After the translation process, synthesis of viral RNA and assembly of the viral replicase complexes takes place. At the downstream region of replicase protein, the sub-genomic RNAs serves as mRNAs for the viral structural and accessory genes. The negative strand intermediates help the development of the genomic and sub-genomic RNAs. The positive strand consists of both poly-uridylate and anti-leader sequences making the abundance of negative strand only 1% [37].

The replication of viral RNAs require several cis-acting sequences [38]. Seven stem-loop structures are present at the 5’ UTR of the genome that can extent into the replicase 1a gene [39]. The presence of a bulged stem-loop, a pseudoknot, and a hypervariable region marks he 3’ UTR regions. The overlapping of stem-loop structure and pseudoknot takes place at the 3’end and hinder their formation simultaneously [40]. These structures help in the regulation of various alternate stages of RNA synthesis. Recombination in CoVs can take place by both homologous and non-homologous recombination. Virus recombination capacity has been tied to the ability of RdRp strand switching [41].

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Next step in the viral infection is the insertion and translation of the viral structural glycoproteins S, E and M into the endoplasmic reticulum (ER). The secretory pathway helps these proteins to move towards the ER-golgi intermediate compartment (ERGIC) [42]. In this compartment the viral genomes encoding structural proteins encapsidated by N protein bud into membranes of the ERGIC and progress towards the formation of matured virions [43]. The protein-protein interaction mainly takes places through the help of M glycoprotein which is responsible for the assembly of CoVs. M protein alone is not sufficient for the formation of viral like particles. However, M protein expressed along with E protein helps in the VLP formation and is efficient by functioning together to form the envelope for CoV [44]. The fusion of encapsidated genomes into ERGIC enhances the viral development as the N glycoprotein enhances the formation of VLP. In the ERGIC compartment the S glycoprotein interacts with the M glycoprotein as it is necessary for its incorporation. As, M protein is abundant compared to E protein the various interactions of M protein can be a major source that provides the impetus for envelope maturation [45]. The E glycoprotein alters the host secretory pathway and promotes the virion assembly and release into the host. The M protein binds to the nucleocapsid at the C-terminus of the M endodomain and completely marks the completion of viral assembly [46]. Following the assembly, transportation of the virions takes place to the host cell surface in vesicles and is released by exocytosis. The S protein is responsible for the fusion of cell fusion between the infected and un-infected cells by transiting into the cell surface. This fusion forms a large multinucleated cells which allows the virus spread into the host and tackle the conditions of getting detected or neutralized by antibodies specific to virus (**Figure-3**).

Vaccines Against CoVs

Most of the patients develop strong immunity against the virus and acquire the ability to survive the infection. Some of the viral vaccine development strategies against viral infections are live attenuated vaccines, whole killed vaccines, split vaccines, recombinant subunit vaccines, Virus like particles etc. An extensively scientist community is behind this field to create effective and safe vaccine for the eruption of this disease. There are several options to develop vaccines against this disease such as live attenuated vaccines, whole killed vaccines, recombinant subunit vaccines, virus like particles etc.

Live-attenuated vaccines

Live attenuated vaccines against CoVs can be developed via the deletion in group specific genes [47]. This deletion of genes does no alter the replication properties of the virus but can provide an impact to attenuate the virus. An example of one such live attenuated vaccines to prevent CoV infection is IBV vaccines which is used in broiler chickens [48]. In animals with CoV infection live attenuated vaccines are proven to be more effective than the whole killed vaccines which suggests that a crucial defense mechanism is being played by cell-mediated immunity. However, the major drawback of this type of vaccine is that a vaccine strain can recombine with a circulating wild type strain. This remains a challenge to develop live attenuated vaccines against CoV infections.

Whole killed vaccines

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Whole killed vaccines are relatively safe and easy to be developed. These types of vaccines are majorly used in the development of vaccines like BoCV and IBV. This method has been successfully employed for the production of n inactivated canine CoV vaccine [49]. A major hit against SARS CoV using this type of vaccine was developed using the inactivated strain of SARS which was treated with F69 with formaldehyde mixed along with Al(OH)3 [50]. Although, the challenge remains here is that of inactivated vaccines might be a great challenge against different strains of CoVs till date.

Recombinant subunit vaccines

A large number of recombinant viral subunit vaccines can be developed against the pandemic causing CoVs using the molecular biology techniques, for example against the S protein. Eight recombinant single chain variable region fragments against spike protein and one single chain variable region 80R against SARS-CoV was screened from two non-immune human antibody libraries. These fragments effectively inhibited the syncytia formation between the cells expressing S protein and those expressing the ACE-2 receptor [51]. Few studies have utilized the S glycoprotein receptor binding domain aa 318-510 to boost the immunity and effectively neutralize the CoV infections in view of the variations that might occur in the genome in future outbreaks.

Another type of vaccine develop approach is virus like particles (VLPs). VLPs are multi-protein structures that possess the ability to mimic the organization and conformation of authentic native viruses but lack the viral genome, potentially yielding safer and cheaper vaccines [52]. A huge number of prophylytic based VLP vaccines are manufactured by pharma companies such as

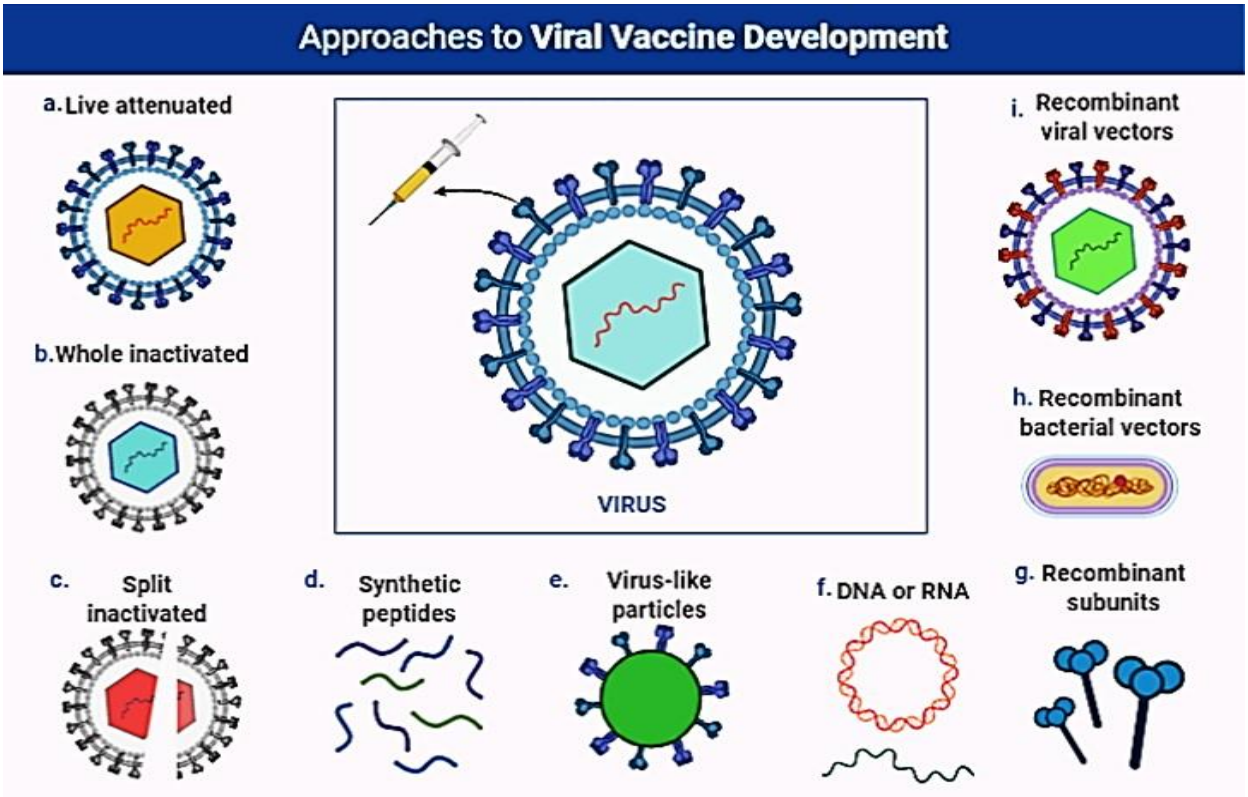

Figure-4: Schematic representation of mechanisms of vaccine development against Coronavirus.

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GlaxoSmithKline against hepatitis B virus, human papillomavirus. Some examples of such vaccines are engerix, cervarix, Gardasil, recombivax HB. Many of the VLP based vaccines are still under clinical trials against diseases such as influenza virus, parvovirus etc. (**Figure-4**).

Anti-Viral Agents against Coronavirus (CoV)

The pandemic outbreak of CoV against world-wide has created an urge to develop effective and safe anti-viral medicines to cure the disease as soon as possible. As, worst countries affected like China has successfully brought back the number of CoV positive patients via the lockdown system yet, pandemic effect to other countries urge the requirement to develop new solutions to cure the disease. This has catalyzed the development of novel coronavirus vaccines across the biotech industry, both by pharmaceutical companies and research organizations such as the national institutes of health (NIH) summarized in Table 1.

Table-1: Summary of the development of antiviral agents and vaccine development against Coronavirus (CoV).

S. No	Drug	Status	References
1.	Favilavir	Phase-III	[53]
2.	Altimmune's intranasal vaccine	stage I clinical trial	
3.	INO-4800	Pre-clinical testing	[54]
4.	NP-120 (Ifenprodil)		
5.	APN01	Phase-I pilot trial	[55]
6.	mRNA-1273	Phase-I clinical trial	[57]
7.	Avian CoV infectious Bronchitis virus vaccine	Pre- clinical trials	[54]
8.	Brilacidin	Pre-clinical stage	
9.	Clover – recombinant subunit vaccine	Pre-clinical stage	[58]
10.	Vaxart's CoV vaccine	Pre-clinical stage	
11.	CytoDyn- leronlimab	Phase-II clinical trials	[55]
12.	Linear DNA vaccine – Takis Biotech	Pre-clinical stage	
13.	Remdesivir (GS-5734)	Phase-III clinical trials	NCT04254664
14.	Chloroquine or hydroxychloroquine	clinical trial	NCT04261517
15.	Camrelizumab and thymosin	Phase II trials	NCT04268537
16.	Azvudine	Phase I	ChiCTR2000029853

Conclusion

Over the past few years' emergence of different coronavirus has taken place causing widespread infections and death all over the world. Despite the whole world's effort to resolve the SARS-CoV2 infection still many issues remain unclear. The effective option of antiviral therapy and vaccination are currently under evaluation and development. There are several druggable targets, surface glycoprotein, envelope protein, spike protein, main protease, RNA-dependent RNA polymerase etc. have been identified recently, which can be actively targeted to inhibit the infection and propagation of SARS-CoV2 in humans. Several inhibitors are under clinical trials to inhibit the endocytosis of SARS-CoV2 through modulating their pH. Though several studies are under progress to develop potential preventive and therapeutic approach for SARS-CoV2, more

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study about the viral complex mechanisms need to be studied to develop effective vaccines and drugs against the the SARS-CoV2 infection.

Conflict of Interest

Authors declare no conflict of interest

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