IJBPAS, March, 2022, 11(3): 1106-1125

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COVID-19: PATHOGENESIS AND IMMUNOLOGICAL PERSPECTIVE

GUPTA V AND BANSAL H*

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Sector 125, Noida-201313, U.P., India

***Corresponding Author: Dr. Hina Bansal: E Mail: hinabansal@gmail.com; Phone (M): +91-9811099082; ORCID: 0000-0003-1683-1581**

Received 13th May 2021; Revised 16th May 2021; Accepted 14th June 2021; Available online 1st March 2022

https://doi.org/10.31032/IJBPAS/2022/11.3.5930

ABSTRACT

Coronavirus Disease-19 (COVID-19) was first reported in Wuhan city, China in December 2019 has now spread all over the globe infecting millions of people. According to the latest update by World Health Organization (WHO), till date, more than 150 million are infected and around 3 million people have died due to COVID-19. SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) has been found as the causative agent for COVID-19. The virus is considered as zoonotic in nature and is believed to be originated from bats. The intermediate host between bats and humans has not been confirmed yet. In twenty-first century, SARS-CoV-2 is the third coronavirus to be of pandemic potential after severe acute respiratory syndrome Coronavirus and middle East respiratory syndrome coronavirus, which have caused infection in the year 2002 and 2012, respectively. SARS-CoV-2 infects the lower respiratory tract and causes lung damage manifesting as ARDS (acute respiratory distress syndrome). The current review provides brief information on SARS-CoV-2, its origin, intermediate host, routes of transmission, clinical features and prognosis of COVID-19. The study attempted to summarize the pathogenesis of COVID-19, focussing on the entry and life cycle of SARS-CoV-2 and information about the immune response initiated with the impact of dysfunctional immune response with the progression of the disease. Further it gives an insight into the possible pharmacological treatment, their mechanism of action and antiviral vaccine methods which are being employed for treatment and prevention of COVID-19.

Keywords: SARS-CoV-2, Pathogenesis, Immune response, Treatment, Vaccine

1. INTRODUCTION

In late December 2019, Wuhan city, China witnessed the outbreak of an unknown disease with an unidentified cause. Numerous cases were reported in hospitals about unexplainable pneumonia along with a past exposure to large seafood market place of Wuhan city **[1]**. The identified causative agent was beta-Coronavirus. On 12 January'20, the virus was named as 2019- novel Coronavirus by WHO. Shortly, it was assigned a new name, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by International committee on Taxonomy of Viruses. The disease caused by this pathogen was formally named as coronavirus disease-2019 (COVID-19) by WHO **[2]**. SARS-CoV-2 was not the first from the coronavirus family to cause human disease. Over the past two decades, two strains of beta-Coronaviruses i.e. SARS-CoV (severe acute respiratory syndrome Coronavirus) and MERS-CoV (Middle East respiratory syndrome coronavirus), have emerged and caused severe diseases in humans.

SARS-CoV was the first known pandemic caused by a CoV. It emerged in late 2002 having 8,098 cases with 774 deaths reported in more than 30 countries **[3]**. The epidemiological linkage studies on SARS-CoV infected individuals and other animals indicated that SARS-CoV is zoonotic in nature that means the disease was transmitted from animals to humans **[4]**. Similarly, MERS-CoV caused an outbreak of severe respiratory disease in the year 2012. It was similar to SARS-CoV in being a CoV and has originated from animal reservoirs. Both viruses have crossed the interspecies barrier to infect humans and studies showed that they cause severe lower respiratory tract infection and have high fatality rates. They were considered as a potential pandemic agent by the global health community **[5]**.

In order to outline a more precise situation of COVID-19 and predict the outbreak in near future, the reproductive number (R_0) is calculated. R_0 is defined as the average number of individuals that one infected person can spread to **[6]**. In other words, it tells how contagious infection can be. If the calculated R_0 is more than 1, the progression of transmission is high, higher the R_0, more difficult to control the disease. The calculated R_0 of SARS-CoV-2 is estimated to be around 2.2-2.6. When compared with R_0 of SARS and MERS which are less than 1 and 1.4-2.5, respectively showed that SARS-CoV-2 is more contagious **[7]**. In the current study, we have discussed about several aspects of SARS-CoV-2 virus such as the virion structure, origin, transmission, pathogenesis and immune response of the body against the virus. It provides an insight into the

potential treatments and the vaccine methods being used for prevention along with promising vaccine candidates under clinical trials of phase >2.

2. PATHOGEN

SARS-CoV-2 is the pathogen that causes COVID-19. The virus was first isolated in Broncho-alveolar lavage fluid (BALF) of COVID-19 patients. Phylogenetic study indicated that SARS-CoV-2 belongs to Coronaviridae family. It is a novel member of genus Beta-coronavirus (β-CoVs) and comes in subgenus Sarbecovirus **[8]**.

Coronaviruses (CoVs) are non-segmented, enveloped, positive sense single-stranded RNA (+ssRNA) viruses. These viruses cause enteric, neurologic, respiratory, hepatic and gastrointestinal infections in humans. They are further grouped into four genera i.e. alpha-coronavirus (α-CoV), beta-coronavirus (β-CoV), gamma-coronavirus (γ-CoV) and delta-coronavirus (δ-CoV). Studies indicated that infection in animals and humans is caused by α- and β-CoV. According to literature, there are six human CoVs that are found to be susceptible to humans. These include human coronavirus-NL63 (HCoV-NL63) and HCoV-229E of α-CoV and HCoV-OC43, HCoV-HKU1, SARS-CoV and MERS-CoVof β-CoV **[1]**. Both of α-CoVs and HCoVs-OC43 and HCoVs-HKU1 of β-CoV cause viral infection in humans by infecting the upper respiratory tract which results in respiratory symptoms such as common cold. However, the remaining two β-CoVs, i.e. SARS-CoV and MERS-CoV infects the lower respiratory tract; it causes severe and lethal respiratory infections that have a pandemic potential **[9]**. Now, SARS-CoV-2 virus is the seventh known CoVthat causes infection in the lower respiratory tract of humans. CoVs have the capacity for proofreading during replication. With the spread of SARS-CoV-2 globally, it has accumulated some mutations in the viral genome, which contain geographic signatures. These mutations have been examined to study viral characterization, transmission and epidemiology **[10]**.

2.1.Virion

SARS-CoV-2 virus possesses a genome size of approximately 29.9 kb **[11]**. Two-third of the viral RNA present in the first open reading frame i.e. ORF1a or ORF1b, codes for two polypeptide polyproteins 1a/1ab (pp1a/pp1ab), which direct for the production of sixteen non-structural proteins (NSPs). The other one-third part of the viral genome translates into four structural proteins. These include nucleocapsid or N protein, spike or S glycoprotein, membrane or M glycoprotein and envelope or E glycoprotein **[12]** as shown in **Figure 1**.

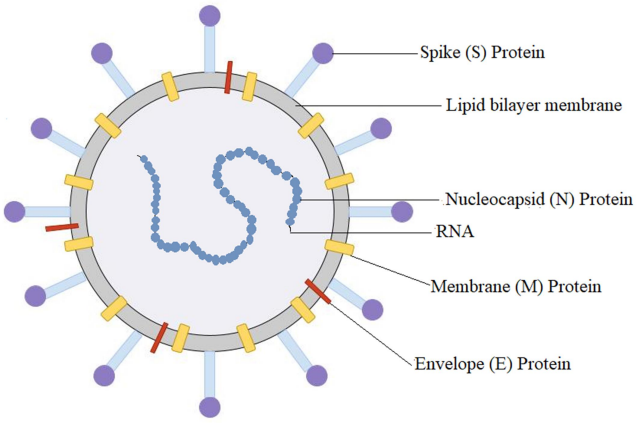

Figure 1: Diagrammatic representation of SARS-CoV-2

The virion contains a nucleocapsid which constitutes the phosphorylated N protein and genomic RNA. The N protein is present in the endoplasmic reticulum-Golgi region. Due to its association with RNA, the N protein is responsible for the cellular response of host or target cells to viral infection, viral replication cycle and pathways related to the viral genome. Nucleocapsid is enclosed within the phospholipid bilayer of the virus. This bilayer is coated with two types of transmembrane proteins i.e. the S protein. S protein forms homotrimers protruding outside the viral surface, thereby giving the characteristic "crown" look to the virus. It mediates the binding of the virus to the target cell by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, which is present in the lower respiratory tract cells. Another structural protein, M protein is responsible for determining the shape of the viral envelope. It has the capability to bind to all other structural proteins. Thus, helps to stabilize N protein-RNA complex by binding to N protein. E glycoprotein is the smallest protein and is positioned amongst the S proteins within the viral envelope. It has a crucial role in the production and maturation of SARS-CoV-2 virus **[13]**.

2.2.Origin and intermediate host

Beta-CoVs are known to be zoonotic in nature and bats are considered as the primary host for all known CoVs. Though bats are generally far away from human residential areas, they transmit SARS-like CoVs to humans via an intermediate host, most likely animals, by undergoing mutation or recombination within animal hosts. During the pandemic in 2002 by SARS-CoV, research shows that their origin is from Chinese horseshoe bats. Similarly, MERS-CoV that caused an epidemic in 2012, also has bat as its natural reservoir. As mentioned earlier these CoVs are not transmitted directly from bats to humans but involve an intermediate host.

Thus, for SARS-CoV the intermediate host is civet cats and for MERS-CoV it is camel before their transmission to humans **[14]**.

Virus genome sequencing showed approximately 96.2% sequence identity to bat-CoV RaTG13 **[15, 16]**. Thereby suggesting that bat is the possible natural host for SARS-CoV-2. Since research suggest that COVID-19 pandemic started from a seafood market in Wuhan, China, it is possible that the animal source was present at that particular location. It has been reported that SARS-CoV-2 shows high sequence identity with pangolin origin coronavirus i.e. 99%, signifying that SARS-CoV-2 might have pangolin as the intermediate host **[17]**. Moreover, various studies suggested snakes, turtles, ferrets and minks as the possible potential intermediate host reservoir for SARS-CoV-2 **[1, 15]**. However, the explanation of transmission and involvement of intermediate host has not been clarified till date.

2.3. Routes of transmission

COVID-19 can be transmitted from one person to another who intimately comes in contact with an infected person. Studies suggested that SARS-CoV-2 is transmitted through respiratory droplets and contact. A healthy person can catch the disease if he/she inhales the aerial droplets of an infected person who sneezed in close proximity. It can also spread through direct contact with an infected person or by touching a contaminated object. Moreover, various studies suggested that the virus is detected in urine and stool and can replicate in the digestive tract, thus implying the possibility of faecal–oral transmission. However, there is no such established evidence of transmission and infection of virus through the consumption of virus-contaminated foods or even during childbirth **[17]**.

3. CLINICAL FEATURES AND PROGNOSIS

Clinical findings suggest that all COVID-19 patients present with conditions of lymphopenia, leukopenia and pneumonia. High levels of certain cytokines and chemokines are found in the serum of severely infected COVID-19 patients, thereby suggesting that the damage of the host lung is caused by the dysfunctional immune response **[8]**. COVID-19 affected patients presents a broad range of symptoms from asymptomatic, moderate (such as fever and dry cough), severe (such as dyspnoea and hypoxia) to critical cases (which includes respiratory and multi organ failure). However, certain symptoms related to gastrointestinal tract have also been reported. These include diarrhoea, nausea, vomiting or abdominal pain **[18]**.

After infection with SARS-CoV-2, the incubation period of the virus before commencement of symptoms is around 4-5

days. Symptoms include dry cough, fever, difficulty in breathing, headache etc. COVID-19 reaches its peak within 5-6 days from the beginning of symptoms. The severity of COVID-19 is not only due to viral infection but also due to the dysfunctional immune response of the host. Severe COVID-19 cases have shown the progression of acute respiratory distress syndrome (ARDS) after 8-9 days of commencement of symptoms. ARDS can be characterised by low level of oxygen in blood and breathlessness **[16**]. COVID-19 patients relatively have high mortality and morbidity rates. Reports showed that older adults are more susceptible to COVID-19 mainly due to poor health which includes age related medical conditions such as diabetes, hypertension, renal, heart disorders etc. T cells are produced in thymus, a gland that undergoes quick atrophy with age, thus the production of naïve T cells diminishes as we get older. So, by the age of 40 to 50 years, production of naive T cells decreases to 1 %. As a result, the immune response to new infections such as COVID-19 are dampened, making them more susceptible to the infections **[19]**. Research studies also showed that higher percentage of men are infected by SARS-CoV-2 than women **[14]**. The difference in immunoregulatory functions of the sex hormones i.e. oestrogen and testosterone could influence immunity against COVID-19 **[16]**. Moreover, it has also been observed that COVID-19 is uncommon in children **[20]**.

SARS-CoV-2 virus enters the target cells through ACE2 receptors, present on epithelial cells of the lung, blood vessels, intestine and kidney. The distribution of ACE2 receptors in different tissues may explain the site of infection and symptoms **[10]**. Studies suggested that patients with type I and II diabetes and hypertension are considered to be more susceptible to COVID-19 infection because they are treated with ACE inhibitors and Angiotensin II receptor blockers respectively, which results in an upregulation of ACE2 receptors in such patients. Expression of ACE2 are also observed to increase by drugs like ibuprofen and thiazolidinedione **[21]**.

4. PATHOGENESIS OF COVID-19

4.1. Receptor attachment and entry of SARS-CoV-2

Virus can be transported from an infected person to a healthy person by cough or sneeze. Once the virus enters a healthy person through nose, eyes or mouth, it starts replication in mucosal epithelial layer of the upper respiratory tract and travels down to the lower respiratory tract i.e., towards alveoli in the lungs. There are two types of alveolar cells: Type I cells which are thin so that oxygen could pass through them and Type II cells which secrete a

substance known as surfactant that lines the alveolus and prevents it from collapsing. On invasion of the virus particle into an individual's lungs, it initiates the infection by binding to the host surface cellular receptor. Thus, receptor recognition is considered as an important aspect of the cell and tissue tropism of a virus **[22]**.

SARS-CoV-2 interacts with human angiotensin converting enzyme-2 (hACE2) in order to enter the cell. ACE2 enzyme is expressed by both type I and II alveolar cells of a normal human being. Though, majority of ACE2 receptors are present on type II alveolar cells **[23]**. The process of attachment and entry of the virus into the target cell is mediated by the transmembrane S protein. The S protein undergoes proteolytic cleavage by the host protease into two subunits i.e. S1 and S2 subunit. S1 subunit contain receptor-binding domain (RBD) which is responsible for receptor recognition and S2 subunit is responsible for virus cell membrane fusion. S1 subunit consist of a C-terminal domain and N-terminal domain which are considered as receptor binding entity. The RBD region of S protein bind to hACE2 receptor, which initiates the infection process. The cleavage event allows the membrane fusion and the virus injects its genomic RNA into the epithelial cells **[24]**.

4.2.Life cycle of SARS-CoV-2

After the process of binding of the RBD region of the virus to ACE2 receptor of the host cell, transmembrane protease serine 2 (TMPRSS2), a protease present besides the ACE2 receptor on the surface of the target cell helps in the entry of the virus and activates the S protein which leads to a conformational change. This facilitates the fusion between the viral envelope and the cell membrane via endocytosis. Uncoating of viral envelope occurs and this results in the release of the viral RNA into the cytoplasm of the cell. The ORF1a and ORF1b RNAs of the genomic RNA undergoes translation and forms two viral replicase polyproteins pp1a and pp1ab, respectively and structural proteins. Further, proteins pp1a and pp1ab are cleaved by viral protease to form 16 NSPs. Some of the NSP assemble to form Replicase-transcriptase complex (RNA-dependent RNA polymerase, RdRp). It is involved in the replication of the genomic RNA. Subsequently, the viral genome begins to replicate, and a large number of sub-genomic mRNAs are produced by discontinuous transcription. The mRNAs are finally translated into structural proteins (S protein, N protein, E protein and M protein). Nucleocapsid is formed by the combination of N protein and genomic RNA in Endoplasmic Reticulum or Golgi **[25]**. The virions germinate in the endoplasmic Reticulum-Golgi intermediate

compartment (ERGIC). These viruses are transported via vesicles towards the cell membrane and through the process of exocytosis the cell releases the viruses **[24]**.

5. IMMUNE RESPONSE OF THE HUMAN BODY AGAINST COVID-19

Innate immune response is considered as the first line of defence against viral infection. It is a non-specific, rapid immune response generated on the encounter with a foreign pathogen. Infection due to viruses, like SARS-CoV-2 causes pyroptosis along with vascular leakage of the virus-infected host cells as a part of their replication cycle which in turn triggers a local immune response. To mount an innate immune response, alveolar cells and macrophages uses a variety of pattern-recognition receptors (PRRs) which includes RIG-I-like receptors (RLRs), Toll like receptors (TLRs), C-type lectin like receptors, NOD-like receptors (NLRs) etc. which recognizes molecular structures of the virus known as pathogen associated molecular patterns (PAMPs), that includes viral RNA and DAMPs (Damage-associated molecular patterns) such as nucleic acid, ASC oligomers and Adenosine Triphosphate (ATP). For example, TLR-4 and TLR-7 might recognize S protein and genomic RNA of the virus, respectively **[13]**. Recognition of PAMPs by endosomal TLR-3 and TLR-7 leads to activation of a cascade of signalling pathways inside the cell which eventually leads to the activation of transcription factors such as interferon response factor 3 (IRF3), IRF7, activator protein (AP-1) and nuclear factor-κB (NF-κB), alongwith their nuclear translocation **[12]**. Recognition by cytosolic receptors such as MDA5 and RIG-I results in their interaction with mitochondrial proteins which result in the formation of NF-κB and type-I interferon (IFN). Type-I IFN goes to other cells through circulation and activates the JAK-STAT pathway. This results in the production of certain proteins and reduces the viral replication. However, in COVID-19, the virus suppresses the type-I IFN which results in an uncontrolled viral replication leading to enhanced cytopathic effects. Thereby, reducing the innate immune response to the body.

Moreover, the PAMPs are recognized by the adjacent alveolar epithelial cells, endothelial cells and macrophages; thus initiating the increased production of pro-inflammatory cytokines and chemokines such as IFN-γ, Interleukin-1 (IL), IL-6, IP-10, MCP1, microphages inflammatory protein (MIP1α), MIP1β and tumour necrosis factor- α (TFN-α). Secretion of these cytokines are an indication for T helper-1 (T_H 1) cells and results in recruiting macrophages, monocytes and T cells from blood to the infected sites. This may promote further inflammation due to the additive IFNγ generated by T-

lymphocytes and establish a pro-inflammatory feedback loop **[16]**.

Antigen presenting cells (APCs) are specialized cells such as B-lymphocytes, dendritic cells and macrophages. The virus is internalized by these cells and degraded into small peptides that forms complexes with MHC (major histocompatibility complex) or HLA (human leukocyte antigen) in humans. This complex is transported to the membrane of the cell where the antigenic peptides are displayed. Antigen presentation consequently stimulates the body's humoral and cellular immunity, which are mediated by virus-specific B and T-lymphocytes. APC presents a part of the CoV via MHC class II complex to the CD4+ T-Helper cells (T_H cells). T_H cells gets activated by binding to the complex and produces cytokines which further activates B cells, CD8+ cytotoxic T cells and other cells which helps to kill virus-infected cells as shown in **Figure 2**. Thus, initiating humoral response. Moreover, T cell-dependent B cells will produce antigen-specific (neutralizing) antibodies on activation by T_H cells. B cells generally produce antibodies such as IgM and IgG to neutralize SARS-CoV-2 virus and apoptotic cells before virus spreads. IgM antibodies lasts until 12 weeks, whereas IgG antibodies are persistent and provide long-term protection and prevents reinfection in the future **[26]**. In a healthy immune response, these antibodies can help to block the viral infection and thus the neutralized viruses and apoptotic cells are recognized and digested by the alveolar macrophages via phagocytosis. Thus, the patient experiences a minimum inflammation and lung damage **[13, 16]**. However, research is still going on for a better understanding of the immune response of body to COVID-19.

6. DYSFUNCTIONAL IMMUNE RESPONSE

The most conspicuous immune invasion processes seen in patients were: cytokine storm, antibody dependent enhancement, depletion of lymphocytes and increased levels of neutrophils. In critically ill patients, BALF has relatively high amount of $CD14^+CD16^+$ inflammatory monocytes and highly inflammatory monocyte-derived $FCN1^+$ macrophage cells in blood which triggers cytokine storm (also termed as hypercytokinemia), a fatal uncontrolled systematic inflammatory response that mediates extensive lung damage. This over-production of inflammatory cytokines and chemokines as shown in **Figure 3** can trigger violent attack by the immune system by excessive infiltration of monocytes, T cells and macrophages against the body, thus attacking endothelial cells of lungs and thereby causing acute lung injury, multiple organ failure, fibrosis and ARDS, which is the leading cause of fatality in 70% of

COVID-19 cases **[16]**. Secretion of multiple cytokines, known as Cytokine Release Syndrome (CRS), happens in people who are severely ill. Research has shown thathigh levels of granulocyte colony- stimulating factor (IL-2, IL-7, IL-10, MIP1α, IP-10 and tumour necrosis factor (TNF)) has been observed in patients with severe case of COVID-19, which recruits macrophages and neutrophils at the site of infection and stimulates IL-1β and IL-6 cytokine cascade **[26]**. IFN-γ causes chills, headaches, fever, fatigue and dizziness; TNF- α can cause flu-like symptoms, fever, fatigue, vascular leakage, lung injury, cardiomyopathy etc. IL-6 can cause vascular leakage, activation of coagulation cascade etc. **[27]**. Release of these cytokine and chemokine in the blood can cause the endothelial cells to undergo vasodilation; thus, releasing plasma fluid into the interstitial space thus causing interstitial edema. Plasma fluid starts leaking the cells and enter the alveoli. The fluid dilutes the surfactant produced by type II cells which might eventually leads to alveolar collapse. Inflammatory mediators bring neutrophils which destroys the virus by releasing excessive reactive oxygen species and proteases. This may lead to hyaline membrane formation, alveolar damage and pulmonary edema. Thereby, leading to decrease in the gas exchange and increase in work breathing, causing hypoxemia. The lung becomes more vulnerable to secondary infections. Moreover, through antibody dependent enhancement (ADE), SARS-CoV-2 can increase the infection due to non-neutralizing antibodies produced by B cells, therefore further exacerbating organ damage.

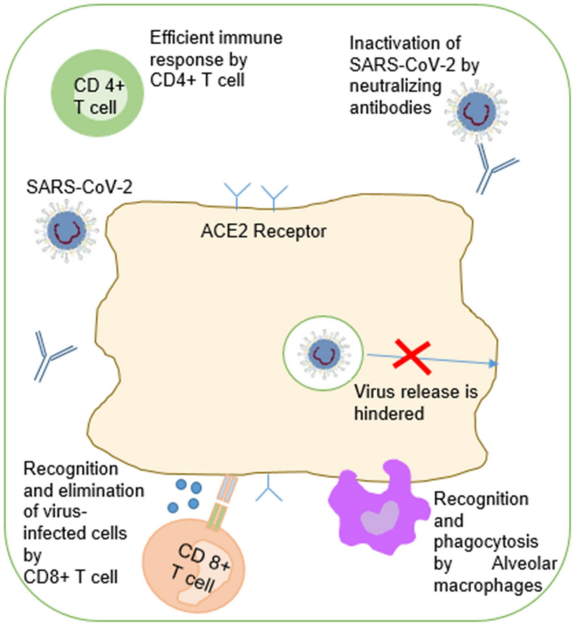

Figure 2: Diagrammatic representation of healthy immune response in lung epithelial cell

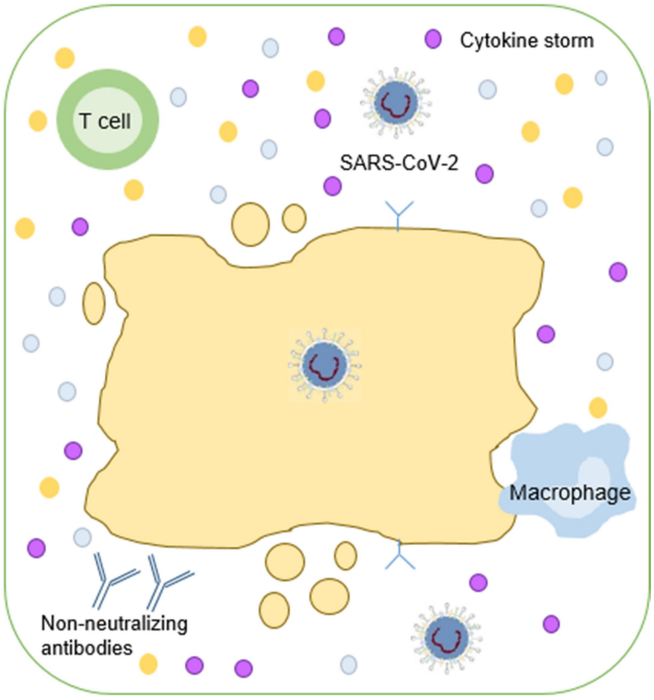

Figure 3: Diagrammatic representation of dysfunctional immune response in lung epithelial cell

7. POTENTIAL TREATMENTS

Infection with SARS-CoV-2 virus starts with the attachment of the virus's S protein to ACE2 receptor of the host cell. According to research, the effective treatment of COVID-19 can be based on use of therapeutic molecules that directly disrupts stages of lifecycle of the virus or use of broad-spectrum anti-viral drugs or by inhibiting binding with surface receptor proteins on the host cell thus blocking the attachment as well as entry of the virus. At present, there is no potential drug available to treat COVID-19. Several strategies are being employed to combat COVID-19.

7.1.Convalescent plasma therapy

Convalescent plasma therapy or the passive antibody therapy is considered as one of the most effective therapeutic approaches for the treatment of COVID-19. Convalescent plasma signifies the plasma collected from a person who has recovered from the infection and has developed required antibodies (donor). Convalescent plasma consists of neutralizing antibodies. The therapy involves transfusing antibodies from the blood of donor into the people who are sick (as therapy) or to those who can contract infection in future (as prophylaxis). Patients who have recovered from COVID-19 will produce significant IgG antibody in the serum for different antibody epitopes on the virus. And some of these will be having the ability to neutralize the virus **[28]**. For binding to the RBD on the S protein of the virus, there is

competition between the neutralizing antibody and the ACE2 receptor.

Convalescent plasma therapy faces certain challenges. Studies show that this therapy could result in decline of serum cytokine, exacerbating hyper-immune attacks etc. In addition, it has certain adverse reaction such as fever, chills, circulatory overload and transfusion-related acute lung injury **[29, 30]**. Though the preliminary trials show favourable results, yet large randomized controlled trials are required to prove the unbiased efficacy and safety of this therapy **[31]**.

7.2.Pharmacological treatments

Chloroquine Phosphate: Chloroquine is an inexpensive drug, originally used for the prevention and treatment of malaria, rheumatoid arthritis and lupus erythematosus. It also has broad-spectrum anti-viral activity. Chloroquine blocks the terminal phosphorylation of ACE2 receptor which inhibits the attachment of the virus to the receptor thus preventing its entry inside the host cell. Therefore, the anti-inflammatory and anti-viral activities of chloroquine can be used as a potential treatment for COVID-19 patients **[32]**.

Hydroxychloroquine (HCQ): Due to rapid escalation in the number of cases of COVID-19, various regimens have been employed for its prevention and treatment. According to research, efficiency of HCQ depends on the timing of its administration. HCQ raises the intracellular pH and affects the endosomal activity hence disrupting the viral fusion with the host cell. Moreover, it alters the N- terminal glycosylation of ACE2 which reduces the affinity and thus affects the binding of the S protein of SARS-CoV-2 to the receptor, hence preventing its entry into the host cell.

HCQ has potent host immunomodulatory effects. Raised endosomal pH can also alter antigen presentation by MHC class I and II, which in turn lowers both T and B cell recruitment with consequent reduction in the production of pro-inflammatory cytokines (TNF-α and IL-6). This reduces the number of rapidly proliferating T cells and inhibits cytotoxic activity of CD8 T lymphocytes. In response to viral infection, natural killer cells produce TNF-α and INF-γ. Natural Killer (NK) cells recognizes MHC class I presenting complex on the virus-infected cells and in response releases proteins. These are responsible for inducing apoptosis. HCQ reduces NK cell cytotoxicity by inhibiting perforin activation. Hence, HCQ helps in reducing the overall exuberant immune response during inflammatory phase **[33]**.

Protease Inhibitor: Camostat mesylate, is an example of protease inhibitor. As discussed in the life cycle of SARS CoV-2 section, during the entry of virus into the host cell, TMPRSS2 a clinically proven serine protease makes a complex reaction with

ACE2 receptor present on the target cell and allows the entry of SARS-CoV-2 virus. Camostat mesylate, a protease inhibitor is being studied and is known to target TMPRSS2 protein and subsequently blocks the entry of the virus in-vitro into the human lung cells. An advantage of Camostat mesylate is that it is inexpensive **[34, 35]**.

Arbidol: It is a broad-spectrum anti-viral compound, targets the interaction between S protein and ACE2 and prevents the entry of the virus into the target cell by inhibiting the fusion of the viral envelope and cell membrane of the cell, thereby preventing the infection.

Remdesivir and Favipiravir: Remdesivir, an adenosine analogue has a broad spectrum, effective action against several coronaviruses and Favipiravir is a guanine analogue. These drugs inhibit the viral RNA-dependent RNA polymerase (RdRP) formed during the viral replicative cycle thus inhibiting the replication of SARS-CoV-2 and preventing the spread of infection **[36]**.

7.3.Monoclonal antibody therapy

Antibodies can produce interference in the viral entry into the host cell by various mechanisms. One mechanism includes inhibition of viral attachment to the host cell receptors; in which antibody binds to the viral S proteins. Similarly, this effect can be achieved by blocking the receptors. Here antibodies bind to the receptors to prevent the entry of the virus. Another mechanism involves the pre-binding and creating interference due to conformational changes in cell membrane by the antibodies that target non-receptor binding region **[37]**.

Monoclonal antibodies are used by therapeutic industries and have been used as therapeutics for the treatment of previous pandemics such as SARS and MERS. In this therapy, specific neutralizing monoclonal antibodies either binds to the RBD site of the S protein or to the ACE2 receptor to effectively block the entrance of virus into the host cell **[38]**.

Tocilizumab (TCZ), is being used for the treatment of COVID-19. TCZ is a humanized monoclonal antibody, acts against interleukin-6 (IL-6). It dampens the IL-6 levels and thus reduces the cytokine storm in COVID-19 patients **[39]**.

Sarilumab, a human monoclonal antibody that blocks IL-6 receptor. Approved by FDA for the treatment of COVID -19 patients with pneumonia.

8. VACCINES FOR SARS-COV-2

Due to rapid increase in COVID-19 infection and high mortality across the globe, there is an urgent requirement of a protective vaccine. Scientific community are tirelessly working on development of different types of vaccines, based on the previous knowledge & experience with

SARS and MERS vaccine development path. The vaccines aim to expose the body to an antigen and thus provoke immune response that would help kill or block the virus if an individual gets infected. As per current update (7th May 2021) from WHO, 97 vaccines are under clinical development and 183 vaccines are under pre-clinical development. 31% of the vaccines under development are based on protein subunit platform; 10-16% are based on viral vector (replicating and non-replicating), inacti-vated virus, RNA and DNA platform; and less than 10% are based on other platforms such as virus like particle, live attenuated virus etc. Various platforms for the production of anti-viral vaccines are discussed below:

8.1.Virus vaccine/ Viral-vector vaccine

Virus vaccines include insertion of weakened or inactivated viruses inside human body **[40]**. The viruses are weakened by altering the genetic code or by passing it through animal or human cells so that it undergoes mutation and produce incompetent viral proteins that will make them less likely to cause infection. Similarly, to produce inactivated viruses they are made non-infectious with the help of heat or certain chemical such as formaldehyde. The vaccine is then injected to a person and the virus starts replicating. The macrophages ingest the viruses and project its antigen as MHC complex. This further triggers the immune response **[41]**.

In viral-vector vaccines, adenovirus or measles is genetically engineered to have CoV spike genes. Since, these viruses are weakened, therefore they cannot cause any disease. These vaccines are of two types: (1) Replicating viral vector, those who can still replicate within the cells. Vaccine having genetically altered CoV spike genes are inserted into the host cell which would replicate and produce CoV proteins; (2) Non-replicating viral vector, those that cannot replicate within cells because of inactivated key genes. In this method, booster shots could be given to induce long-lasting immunity **[42]**.

8.2.Nucleic acid vaccine

DNA and RNA based vaccines can be prepared by using the genetic material of the CoV only. They are considered harmless and easy to make. DNA vaccines are believed to be more progressive in response to the recent pathogens **[7]**. They are incorporated into the cells via a process called electroporation. Through this process, holes are created in the cell membranes which increases the chances of uptake of the vaccine containing viral DNA into the cell. The DNA inside the cell undergoes transcription and translation consecutively and produces viral proteins. Similarly, RNA vaccines consist of RNA encapsulated in a lipid coat which are

injected into the host cells. In the nucleus, RNA undergoes translation and produces viral proteins. Now, these viral proteins would be ingested by host antigen presenting cells and would initiate the immune response. RNA vaccines have competent protein translation efficacy and improved stability **[7]**.

8.3.Protein based vaccine

Several researches are ongoing to inject CoV proteins directly into the human body and to produce fragments of protein shells that would represent CoV's outer coat. (1) Protein subunits: Research is being done on vaccines with viral protein subunits such as the S protein or more precisely the RBD of the S protein. (2) Virus-like particles (VLP): In this type of vaccines, VLP mimics the outer structure of CoVs and would be non-infectious because it does not contain any viral genetic material. These vaccines would trigger a strong immune response **[43]**. In the growing state of pandemic worldwide, there is an earnest requirement of a vaccine to prevent further progression of infection in the population.

Table 1: List of vaccines against SARS-CoV-2 in clinical trials of phase >2 (Compiled from: The COVID-19 candidate vaccine landscape and Tracker, WHO [44]; *last updated, 7th May 2021)

Vaccine platform	Name of the vaccine	Developing organisation/Company	*Current Status
Protein Subunit	NVX-CoV2373	Novavax, USA	Phase 3
	CIGB-66	Center for Genetic Engineering and Biotechnology (CIGB)	Phase 3
	EpiVacCorona	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology "Vector"	Phase 3
	FINLAY-FR-2 anti-SARS-CoV-2 Vaccine	Instituto Finlay de Vacunas	Phase 3
	VAT00002: SARS-CoV-2 S protein with adjuvant	Sanofi Pasteur + GSK	Phase 3
	Recombinant SARS-CoV-2 vaccine (CHO Cell)	Anhui ZhifeiLongcom Biopharmaceutical + Institute of Microbiology, Chinese Academy of Sciences	Phase 3
	SCB-2019 + AS03 or CpG 1018 adjuvant plus Alum adjuvant	Clover Biopharmaceuticals Inc./GSK/Dynavax	Phase 2/3
	UB-612	Vaxxinity	Phase 2/3
	SCB-2019	Clover Biopharmaceuticals Inc.	Phase 2/3
Virus vaccine/ Viral Vector	ChAdOx1 nCoV-19/AZD-1222 (Covishield)	Oxford University in collaboration with AstraZeneca Inc	Phase 4
	Gam-COVID-Vac Adeno-based (Sputink V)	Gamaleya Research Institute ; Health Ministry of the Russian Federation	Phase 3
	Recombinant novel coronavirus vaccine	CanSino Biological Inc./Beijing Institute of Biotechnology	Phase 3
	Ad26.COV2.S	Janssen Pharmaceutical	Phase 3
	GRAd-COV2	ReiThera + Leukocare + Univercells	Phase 2/3
Inactivated Virus	CoronaVac	Sinovac Research and Development Co., China	Phase 4
	BBV152 (Covaxin)	Bharat Biotech International Limited	Phase 3
	BBIBP-CorV	Sinopharm + China National Biotec Group Co + Beijing Institute of Biological Products	Phase 3
	Inactivated SARS-CoV-2 vaccine (Vero cell)	Beijing Minhai Biotechnology Co	Phase 3
	SARS-CoV-2 vaccine (vero	Institute of Medical Biology + Chinese Academy	Phase 3

	cells)	of Medical Sciences	
	QazCovid-in® - COVID-19 inactivated vaccine	Research Institute for Biological Safety Problems, Rep of Kazakhstan	Phase 3
	COVID-19 inactivated vaccine	Shifa Pharmed Industrial Co	Phase 2/3
DNA based vaccines	nCov vaccine	Zydus Cadila	Phase 3
	INO-4800	Inovio Pharmaceuticals + International Vaccine Institute + Advaccine (Suzhou) Biopharmaceutical Co., Ltd	Phase 2/3
	AG0301-COVID19	AnGes + Takara Bio + Osaka University	Phase 2/3
RNA based vaccines	BNT162b2 (Comirnaty)	Pfizer/BioNTech + Fosun Pharma	Phase 4
	mRNA-1273	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	Phase 4
	CVnCoV	CureVac biopharmaceuticals	Phase 3
	SARS-CoV-2 mRNA vaccine (ARCoV)	Academy of Military Science (AMS), Walvax Biotechnology and Suzhou Abogen Biosciences	Phase 3
Virus Like Particle	Coronavirus-Like Particle COVID-19 (CoVLP)	Medicago Inc.	Phase 2/3

9. SUMMARY AND CONCLUSION

COVID-19 is caused by SARS-CoV-2 virus which belongs to CoV family and is believed to have originated from bats. The virus can be transmitted via respiratory droplets or through contact from an infected object or a person. However, the gastrointestinal symptoms due to SARS-CoV-2 infection may exhibit faecal-oral transmission which is not yet proven. There are several factors such as age, gender, comorbid diseases etc. which increases the susceptibility of humans to get COVID-19. Molecular analysis has proven that SARS-CoV-2 infects the target cells via ACE2 receptor and thereby spreads infection. Patients with healthy immune systems recover from COVID-19. However, severe cases SARS-CoV-2 has shown to suppress the innate immune response which results in an uncontrolled viral replication. Moreover, due to the dysfunctional immune response which causes cytokine storm and pulmonary edema, patients develop ARDS which results in high mortality. Currently, no specific medicine or potential drugs are available to treat COVID-19 **[26]**. Therefore, various antiviral therapeutics and treatment strategies are being tested to combat COVID-19. Several vaccines are under developmental phase for prevention of COVID-19. Therefore, more emphasis is being made on prevention strategies like wearing masks, regular washing of hands and keeping safe distance with people in public spaces.

Acknowledgments

The authors are highly grateful to founder President Dr. Ashok K Chauhan and Chancellor Dr. Atul Chauhan Amity University Uttar Pradesh, Noida, India for providing necessary support and facilities. The authors would also like to thank Dr. Ruby Gupta, Public Health Foundation of India for her guidance.

Conflicts of interest

Authors declare no conflict of interest.

Funding

No funds were received from any funding agency.

REFERENCES

[1] Wu D, Wu T, Liu Q, Yang Z. The SARS-CoV-2 outbreak: What we know. Int J Infect Dis. 2020; 94: 44-48.

[2] Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. J Med Virol. 2020; 92(6): 548-551.

[3] WHO Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). https://www.who.int/csr/sars/WHOconsensus.pdf?ua=1. Assessed on: 20th October, 2020.

[4] Cheng VC, Lau SK, Woo PC, Yuen KY. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev. 2007; 20(4): 660-694.

[5] Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev. 2015; 28(2): 465-522.

[6] Petersen, E., Koopmans, M., Go, U., Hamer, D. H., Petrosillo, N., Castelli, F., Storgaard, M., Al Khalili, S., & Simonsen, L. Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. The Lancet. Infectious diseases. 2020; 20(9), e238–e244.

[7] Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020; 38(1): 1-9.

[8] He F, Deng Y, Li W. Coronavirus disease 2019: What we know?. J Med Virol. 2020; 92(7): 719-725.

[9] Guo YR, Cao QD, Hong ZS, *et al*. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil Med Res. 2020; 7(1): 11.

[10] Cevik, M., Kuppalli, K., Kindrachuk, J., & Peiris, M. Virology, transmission, and pathogenesis of SARS-CoV-2. BMJ. 2020; 371: m3862.

[11] Naqvi AAT, Fatima K, Mohammad T, *et al*. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. BiochimBiophys Acta Mol Basis Dis. 2020; 1866(10): 165878.

[12] Rokni M, Ghasemi V, Tavakoli Z. Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: Comparison with SARS and MERS. Rev Med Virol. 2020; 30(3):e2107.

[13] Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab. Syndr. 2020; 14(4): 407-412.

[14] Yi Y, Lagniton PNP, Ye S, Li E, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci. 2020; 16(10): 1753-1766.

[15] Zhao J, Cui W, Tian BP. The Potential Intermediate Hosts for SARS-CoV-2. Front Microbiol. 2020; 11: 580137.

[16] Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. 2020; 20(6): 363-374.

[17] Jin Y, Yang H, Ji W, *et al*. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses. 2020; 12(4): 372.

[18] Smyk W, Janik MK, Portincasa P, et al. COVID-19: Focus on the lungs but do not forget the gastrointestinal tract. European Journal of Clinical Investigation. 2020; 50(9): e13276.

[19] Nikolich-Zugich J, Knox KS, Rios CT, Natt B, Bhattacharya D, Fain MJ. SARS-CoV-2 and COVID-19 in older adults: what we may expect regarding pathogenesis, immune responses, and outcomes. Geroscience. 2020; 42(2): 505-514.

[20] Lee PI, Hu YL, Chen PY, Huang YC, Hsueh PR. Are children less susceptible to COVID-19?. J Microbiol Immunol Infect. 2020; 53(3): 371-372.

[21] Fang L, Karakiulakis G, Roth M (2020) Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? The Lancet Respiratory Medicine. Lancet Publishing Group; 2020. 8:e21.

[22] Wang Q, Zhang Y, Wu L, *et al*. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell. 2020; 181(4): 894-904.e9.

[23] Shang J, Ye G, Shi K, *et al*. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020; 581(7807): 221-224.

[24] Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Journal of Advanced Research. 2020; 24: 91–98.

[25] Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020; 10(2): 102-108.

[26] Rawat K, Kumari P, Saha L. COVID-19 vaccine: A recent update in

pipeline vaccines, their design and development strategies. Eur J Pharmacol. 2021; 892: 173751.

[27] Sun X, Wang T, Cai D, *et al*. Cytokine storm intervention in the early stages of COVID-19 pneumonia. Cytokine Growth Factor Rev. 2020; 53: 38-42.

[28] Xi Y. Convalescent plasma therapy for COVID-19: a tried-and-true old strategy? Signal transduction and targeted therapy. 2020; 5(1), 203.

[29] Zhao Q, He Y. Challenges of Convalescent Plasma Therapy on COVID-19. J Clin Virol. 2020; 127: 104358.

[30] MacLennan S, Barbara JA. Risks and side effects of therapy with plasma and plasma fractions. Best Pract Res Clin Haematol. 2006; 19(1): 169-189

[31] Wooding DJ, Bach H. Treatment of COVID-19 with convalescent plasma: lessons from past coronavirus outbreaks. Clin Microbiol Infect. 2020; 26(10): 1436-1446.

[32] Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020; 14(1): 72-73.

[33] Meyerowitz EA, Vannier AGL, Friesen MGN, *et al*. Rethinking the role of hydroxychloroquine in the treatment of COVID-19. FASEB J. 2020; 34(5): 6027-6037.

[34] Uno Y. Camostatmesilate therapy for COVID-19. Intern Emerg Med. 2020; 15(8):1577-1578.

[35] Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020; 181(2): 271-280.e8.

[36] McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol Res. 2020; 157: 104859.

[37] Both L, Banyard AC, van Dolleweerd C, Wright E, Ma JK, Fooks AR. Monoclonal antibodies for prophylactic and therapeutic use against viral infections. Vaccine. 2013; 31(12): 1553-1559.

[38] Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pacific Journal of Allergy and Immunology. 2020; 38(1): 10-18.

[39] Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-

19: A single center experience. J Med Virol. 2020; 92(7): 814-818.

[40] Mulligan MJ. An Inactivated Virus Candidate Vaccine to Prevent COVID-19. JAMA. 2020; 324(10): 943-945.

[41] Xia S, Duan K, Zhang Y, *et al*. Effect of an Inactivated Vaccine Against SARS-CoV-2 on Safety and Immunogenicity Outcomes: Interim Analysis of 2 Randomized Clinical Trials. JAMA. 2020; 324(10): 951-960.

[42] Robert-Guroff, M. Replicating and non-replicating viral vectors for vaccine development. Current Opinion in Biotechnology. 2007; 18(6), 546–556.

[43] Callaway E. The race for coronavirus vaccines: a graphical guide. Nature. 2020; 580(7805):576-577.

[44] The COVID-19 candidate vaccine landscape and Tracker, WHO: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. Assessed on: 9th May 2021.