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Review Article

Pathogenesis of the SARS Coronavirus-2 and Potential Therapeutic Strategies

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A B S T R A C T

The recent outbreak of severe acute respiratory syndrome (SARS) belongs to a broad family of viruses known as Coronaviridae. *SARS-CoV-2* is an emerging global pandemic with a relatively low mortality rate. The virus has been mutated in a unique manner thus prolonging its search for its vaccine and drug therapy. *SARS-CoV-2* is an enveloped virus consisting of many spike (S) proteins, which mediates its fusion to the membrane of the host cell. Its 'crown-like' appearance under an electron microscope has led to its name. The clinical symptoms that patients experience would be due to their central immune response to the infection. Pro-inflammatory cytokines play an essential role in cell growth and regulation of the immune system. However, its abundance could contribute to pathological conditions which can cause further injury and possible death. This brief review discusses the pathogenesis of the *SARS-CoV-2* along with receptors that can be potentially targeted by therapeutic strategies, inhibiting the membrane fusion, genome replication and immune response.

Keywords: *SARS-CoV-2*, Pathogenesis, Therapeutic Strategies

Introduction

Severe acute respiratory syndrome (SARS) first emerged as an epidemic in Asia during the years 2002-2003. It rapidly spread with the etiologic agent being identified as a coronavirus. The virus has repeatedly crossed species barriers and caused epidemics with significant socioeconomic impact.[1,2] Coronaviruses primarily infect the human species in the upper respiratory and gastrointestinal tract where its transmission is predominantly via respiratory droplets. In the lung, the virus largely infects the alveolar pneumocytes (type 2) and activates the macrophages. The virus has been identified as having crossed species from animals to humans. Studies have supported the hypothesis of zoonotic origination with the host species being the Chinese horseshoe bat.[3] The virus has adopted the name due to its 'crown-like appearance under an electron microscope. The absence of antiviral therapy and vaccinations makes it challenging to regulate the spread of the coronavirus family.[4]

In December 2019, a new infectious disease emerged in Wuhan, China. This further leads to human-to-human transmission, rapidly spreading *SARS-CoV-2* within China. The virus is a member of the subgenus sarbecovirus (beta coronavirus lineage).[5] It is unique among known beta coronaviruses the incorporation of a polybasic cleavage site increases its pathogenicity and transmissibility. Human beta coronaviruses are comparable in many ways but have minor differences in their genomic and phenotypic structure

which is able to influence their pathogenesis.[6,7] Due to their resemblances, it is worth comparing the genomes of other beta coronaviruses with *SARS-CoV-2* to understand how the virus could strategically be targeted. The SARS coronavirus-2 is composed of a positive-sense single-stranded ribonucleic acid (RNA). *SARS-CoV-2* is an enveloped virus that is able to enter the host cell via membranous fusion. Four structural proteins are encoded in the genome: nucleocapsid (N), membrane glycoprotein (M), envelope (E), and spike (S). The membrane glycoprotein is the most abundant as it spans the membrane bilayer up to three times.[8,9]

Enveloped viruses are able to gain access into the host cell by either of the two possible pathways: as the cells' envelopes fuse with the plasma membrane, some transfer their genomes into the cytosol, whereas others would rely on endocytic processes to do so. In the second process, endosomes are responsible for activating the virions after they have been endocytosed.[10,11] The activation step could be a possible potential for drugs to target in order to prevent the uptake of the virus.

Angiotensin-converting enzyme 2 (ACE-2) cellular receptors and recipient cell protease enzymes that prime virus S proteins are essential for coronavirus cell entry. The N-terminal region of the S protein is known to mediate the viral infection by having a strong affinity to the cellular ACE-2 receptor.[12] It should be noted that the expression of ACE-2 initially protects the lung from injury and is downregulated by the interference of the virus. Once the S protein has attached to the ACE-2 it would need to undergo proteolytic processing by the proteases from the host cell in order to become proficient in its fusion.[12,13] The virus uncoats itself after entering the host cell, allowing its genome to undergo the replication process.

Pathogenesis of *SARS-CoV-2*

Transmission to the Lungs

The coronavirus enters the human body via the faecal or oral route. It is commonly transmitted by physical contact, droplets and fomites from another infected person. The virus travels down the respiratory airways and sets itself in the alveolar region of the lungs. *SARS-CoV-2* then invades one of the alveolar epithelial cells (type 2 pneumocytes). The S protein of the virus attaches onto the ACE-2 receptors (located on the host type 2 pneumocyte) and the uptake process is activated by the proteolytic enzyme, transmembrane protease serine 2 (TMPRSS2).[14,15]

Inside the Host Cell

The uptake of the virus can be by either direct fusion or endocytosis. The virus RNA (ssRNA) is released into the cytoplasm when the virus enters the host cell. This RNA attaches itself to the large and small subunits of host cells' ribosomes. After that, the strand will be translated into polypeptide proteins that will produce the RNA polymerase. By reading the positive strand of RNA, RNA polymerase creates a negative RNA strand. The enzyme would then form a positive RNA strand as well as other smaller positive RNA strands using the negative strand.[16] To make the structural elements of the virus, the host's ribosomes read these RNA strands in the endoplasmic reticulum. After this, the accessory and structural components would be transported into the Golgi apparatus by the endoplasmic reticulum. Together with the positive RNA strand, these structural proteins would be packaged within the Golgi apparatus. The single-stranded copy of RNA attaches onto the viral particles producing more virus particles. Newly formed progeny viruses are released from the host cell via exocytosis (through secretory vesicles).[8] Over time, the whole cascade eventually damages the type 2 lung pneumocyte, initiating an inflammatory response.

Alveolar Response

Specific inflammatory mediators (cytokines and interferons) are released due to the damaged type 2 pneumocyte. The interferons affect the surrounding cells in a paracrine manner to prepare them to fight against the virus attacks. They work primarily by causing neighbouring non-infected cells to become protected from viruses.[17] Damaged associated molecular patterns from alveolar cells are detected by alveolar macrophages. Injured type 2 pneumocytes also release cytokines that act on these cells. The alveolar macrophages are stimulated which secrete certain cytokines. These include Interleukin-1 (IL-1), interleukin-6b (IL-6b), Interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNF-α).[18,19] The inflammatory response within the lung parenchyma stimulates nerve endings that are responsible for initiating the coughing reflex (clinically expressed as dry cough).[20] The cytokines IL-6b and TNF-α travel through into the bloodstream causing the endothelial cells to undergo dilation thus increasing the expression of adhesion molecules. They are both known to be potent pro-inflammatory cytokines. IL-6 also stimulates hepatocytes to produce acute phase reactions including the production of C-reactive protein (CRP), fibrinogen and hepcidin.[21]

Immune Reaction/ Fluid Accumulation

There is the recruitment of more immune cells including neutrophils and monocytes in the capillaries. They bind to the adhesion proteins and enter the site of injury into the alveoli. The increase in vascular permeability causes leakage of the fluids into the interstitium causing interstitial oedema. The permeability of the capillary increases due to endothelial cell contraction. Fluid begins to accumulate around the alveoli.[22] Some fluid enters the alveoli (pulmonary oedema) which drowns the surfactant out leading to reduced surface tension of the alveoli Clinical signs include dyspnea and impaired oxygenation leading to

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hypoxemia. After some time, neutrophils are able to enter the alveoli and release chemicals as a by-product, damaging the surrounding tissue.[23] This results in damage of more alveolar cells thus further reducing the amount of surfactant being produced.[23] Lack of surfactant can cause the alveoli to collapse resulting in impaired oxygenation. Inflammatory mediators including arachidonic acid metabolites such as leukotrienes and prostaglandins would be released more by damaged pneumocytes. The release of leukotrienes causes bronchoconstriction and impairs ventilation, which results in hypoxia. Prostaglandins, interleukins and TNF-α are all responsible for causing fever.[24] The alveolar macrophages can initiate the adaptive and innate immune systems. They would detect the virus using its toll-like receptors (TLR which enables them to engulf the virus particles through phagocytosis). The particles would be processed and then presented onto their surface.[25] By presenting the S proteins of the virus, specific T-cells may recognise and mount an adaptive immune response (consisting of B-cells). This would allow the production of antibodies to be made against the viral S proteins.[26]

Systemic Response

A decrease in oxygen levels in the blood will stimulate chemoreceptors in the aortic arch, the carotid artery, and the brain. Consequently, the cardiopulmonary centres of the brain would be stimulated. As a result, the heart pumps faster and the lungs breathe more to increase the oxygen content of the blood, increasing the amount of oxygen in the body. [27]

Briefly, acute respiratory distress syndrome (ARDS) is characterised by an injured lung, an accumulation of fluid, a ventilation/ perfusion mismatch, and hypoxemia. In *SARS-CoV-2*, ARDS was found to be the leading cause of death. Symptomatic patients tend to get varying degrees of severity of the virus with the majority getting mild infections.[28] The incubation period is around 1-8 days. During this period, the virus is able to slowly trigger a response within the lungs. Some common clinical symptoms include fever, fatigue, dry cough, anorexia, myalgia, dyspnea and sputum production.[29]

There have been critical evaluations of several vaccines and antiviral drugs that can target *SARS-CoV-2*, halting its movement into the host cell.[30] Vaccines and antiviral therapies can be centred on the virus's S protein by the use of ACE-2 inhibitors, neutralising antibodies, protease inhibitors, S cleavage inhibitors, S protein inhibitors and small interfering RNAs.[31] Another finding imposed that the *SARS-CoV-2* domain had a higher binding affinity for ACE-2 compared to *SARS-CoV-1*, which was lower.[32] Taking this into consideration it would be a great challenge in developing a therapeutic compound that is able to interact with the S protein and ACE-2 receptor.

Potential Therapeutic Strategies against *SARS-CoV-2*

Serine Protease Inhibition

In order for SARS-CoV-2 to undergo fusion between the viral envelope and host cell membrane, the S protein would need to undergo proteolytic cleavage. Factor Xa (a membrane-bound protease) has the role in cleaving the S protein in *SARS-CoV-2* and human immunodeficiency virus (HIV) into subunits of S1 and S2.[33] When the virus is incubated with its target receptor (ACE-2), the S protein is broken down into two subunits. The amount of cleavage depends on the expression of factor Xa target cells. In conclusion, inhibition of protease factor Xa is a possible mechanism in preventing virus entry. A protease inhibitor called benzamidine hydrochloride (Ben-HCl) at a concentration of 80 mM prevents the breakdown of protein S by factor Xa and prevents it from entering the virus to the host cell, thus stopping the infection.[33] Another target of inhibition could be the main protease (Mpro) enzyme essential for processing the polyproteins that are translated from the RNA of the virus. Mpro is one of the main protease enzymes which consists of more than 11 cleavage sites. It would therefore be advantageous to inhibit the activity of the enzyme in order to block the replication of *SARS-CoV-2*.[34] Alpha-ketoamide inhibitor called 13a containing pyridone are able to inhibit the *SARS-CoV-2* protease enzyme that is used for RNA replication. Compound 13a bypasses the host's cellular membrane which is of great advantage. The pharmacokinetic and pharmacodynamic properties have been tested for the compound which showed promising results in humans.[35] Inhalation of a modified version of compound 13a, compound 13b, had been found to be well tolerated in humans as it didn't show any adverse effects.[35] This would suggest that direct administration of compound 13b into the lungs would be a possibility, providing a good framework in the development of pyridone-containing inhibitors toward anti-coronaviral drugs.

TMPRSS2 Inhibition

TMPRSS2 is a serine protease located on the membrane of the host's cell that has been found essential for S protein priming to allow the fusion of *SARS-CoV-2*.[36] Evidence provide that entry of the virus to the host cell, which is highly dependent on the ACE-2 receptor, can be inhibited by a clinical protease inhibitor of TMPRSS2.[12] The drug Camostat mesylate (serine protease inhibitor) has the ability to block the activity of TMPRSS2. This drug has been approved in Japan for human use however it was for an unrelated condition.[37] Camostat mesylate and its close analogs are fast-acting proteolytic inhibitors that can be used during hemodialysis to help stop proteolysis of fibrinogen into fibrin by inhibiting a number of serine proteases including thrombin. The compounds could be of significance as their

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antiviral activity could be considered as an unlicensed treatment for patients infected by *SARS-CoV-2*.[38] Nafamostat is another drug from the same classification however it is more advantageous in comparison. A much lower dose would be required in contrast to Camostat to achieve the equivalent response. To increase the potency of the drug they could both be given in combination with one another.[39] Nafamostat is a potent inhibitor of the Middle East Respiratory Syndrome (MERS) coronavirus which broke out in the Arabian Peninsula (2012). The results concluded that Nafamostat had inhibited the activity of the dual split protein (DSP) assay (more than 98% inhibition) by its interference with MERS-S-mediated membrane fusion. It was found that this was due to the inhibition of proteolysis of the spike protein by TMPRSS2.[40] The finding was taken further where more protease inhibitors (Gabexate and Camostat) along with selected antivirals (Semeprevir and Telaprevir) were screened for their efficacy against the virus. The drugs used however did not elicit as strong of an inhibitory response to DSP activity compared to Nafamostat. Overall, Nefamostat appears to be the most promising TMPRSS2 inhibitor.[41] However, a major downfall of this serine protease inhibitor is that it would need to be extensively reviewed in vulnerable patients with pre-existing cardiovascular comorbidities.

Suppression of Disulfide and Oxidation Reactions

Like standard cell replication, *SARS-CoV-2* along with other betacoronaviruses rely upon the functions of proteins containing key thiol/ zinc (II)-thiolate sites, for example, RNA replicase and thiol protease. The oxidation process of disulfide (caused by oxidants) may in essence turn the proteins from a primitive functional form to a targeted latent inactive form. This could possibly provide a new way to stop the life cycle of RNA viruses.[42]

It has been indicated that the activity of the drug Disulfiram had the potential of using a targeted oxidation strategy (TOS II) to reduce disulfide reaction and the oxidation quenching. Disulfiram is currently available in the United Kingdom and is licensed for use in the treatment of alcohol dependence.[43]

Thiopurine Analogue Inhibitors

Two viral proteases (Mpro and PLpro) are encoded within the host cell during the replication process. Both consist of 2 non-structural polypeptides that are then able to generate advanced proteins.[44] The drug, 6-mercaptopurine (6MP) can have an inhibitory role in targeting a viral protease. 6MP can be methylated by thiopurine S-methyltransferase (TPMT) resulting in methyl-mercaptopurine. Once methylated, it cannot be further processed by hypoxanthine phosphoribosyl transferase (HPRT) and hence any involvement with deoxyribose nucleic acid (DNA) is stopped.[45] As the methylation occurs in the thiocarbonyl group, it is this area of the compound that is promising as it can inhibit the protease enzyme in the host cell. A study carried out by Chen et al., further confirms this role of the drug as 6MP was one of the screened therapeutic compounds that were able to inhibit or reduce the viral protease PLpro.[46] 6MP showed positive results in an isopeptidase assay as it was able to reversibly hinder SARS-CoV PLpro by targeting the cysteinyl residue within the active site.[46] The thiopurine analog inhibitor, 6MP is available on the pharmaceutical market in the United Kingdom as it is licensed for use in the treatment of severe ulcerative colitis and leukaemia. It is classed as a cytotoxic drug with a narrow therapeutic index and would need regular monitoring in patients who are trialled on this.[47] To overcome this, a prodrug of 6MP could be of significance and trialled on models as it is much less cytotoxic. A significant number of patients infected with *SARS-CoV-2* would continue to develop delayed injury to the lung. This would occur when there is a drop in the viral load, supporting the notion that injury to the lungs is an immune response.[48]

Although the expression of proinflammatory cytokines in tissue injury is controlled by transcriptional mechanisms, its dysregulation could lead to chronic inflammation and an autoimmune response.[49] There are various mediators that are activated upon infection of the virus. Although the virus may have already damaged the alveoli, it would be beneficial to suppress the response from the central immune system to prevent more damage from occurring. In order to achieve this strategy, specific areas would need to be targeted so that more proinflammatory mediators are not released.[50]

JAK Inhibitors

An interesting novel therapeutic strategy that could be focused on is the intervention of the family of the tyrosine kinase enzyme, Janus kinase (JAK). In principle, JAK is able to initiate the signalling pathway which is important in the production of more inflammatory proteins, initiating the immune cascade. It would be advantageous to disrupt the central cellular reaction to the exogenous signals in the immune system. The JAK-STAT (signal transducer and activators of transcription) pathway involves the ligand (e.g. cytokine) binding onto its receptor thus recruiting JAK intracellularly and eventually producing more pro-inflammatory cytokines to be released.[51] Altogether there are two receptors on which the pro-inflammatory cytokine is able to bind onto, type 1 and type 2. Both receptors do not contain intrinsic activity and thus are heavily reliant upon the JAK-STAT mechanism.[52] JAK inhibitors (JAKi) are able to manipulate the phosphorylation and activation of JAK-STAT and hence reduce viral entry and inflammation. They are commonly used in cancer and autoimmune diseases

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such as rheumatoid arthritis. It has been founded that this therapeutic strategy efficiently diminished the p65 phosphorylation and nuclear translocation.[53]

Alongside this, it was suggested that combination therapy of a JAKi with tylophorine was of benefit as it aided with inhibition of JAK pathway.[54] AP2-2 associated protein kinase 1 (AAK-1) is a recognised regulator of endocytosis and therefore its interruption may halt the passage of the virus into the host cell. One of the possible six AAK-1 binding drugs which have been screened was a JAKi named Baricitinib. This compound has a high affinity to AAK-1 and also was able to bind to cyclin G- associated kinase (another regulator of endocytosis).[55] Baricitinib is strongly suggested to undergo trials due to its reasonable compliance. To achieve the required therapeutic concentration, 2-4 mg would need to be administered, daily to potentially inhibit AAK-1.[56] Another potential compound, Tofacitinib, inhibiting JAK activity preventing JAK-dependent phosphorylation of STAT.[57]

TNF-α Inhibition

TNF-α is generated via activated macrophages in the alveoli due to the impaired type 2 pneumocytes. The key mediator has early involvement in the regulation of immune cells, working as a natural defensive mechanism.[19] Nonetheless, overexpression of TNF-a may cause more harm than good during the viral infection as it could enhance the proliferation of fibroblasts. This would cause acute fibrotic changes to the lungs leading to pulmonary fibrosis and further inflammation. The involvement of TNF-α could have therapeutically important implications on the virus as there are inhibitors of TNF-α available in the pharmaceutical market.[58] The possible use of biological anti-TNF therapy could provide an effective way of disrupting the inflammatory cascade and therefore reducing pulmonary injury. The prevention of a TNF-α mediated immune activation could be made by an approved biological anti-TNF therapy (e.g. Infliximab). This was suggested to be for patients who are in the high-risk category of the virus.[59] As with all immunomodulating therapy, the major difficulty is the continuous suppression of the immune system which could lead to lowering the body's defence mechanism.

Conclusion

In the current review, we have shed light on *SARS-CoV-2* pathogenesis and its potential therapeutic strategies that further experiments are required to address these issues.

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