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Characteristic Features and Signalling Cascade of Novel Zoonotic Coronavirus

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Abstract

The outbreak of coronavirus disease 2019 (COVID-19) due to its highly virulent nature has created a health crisis worldwide. It has attracted biologists' attention to understand the morphology, host-pathogen interaction and signalling cascade at the core level. The whole world has come to a standstill due to the contagious nature and transmission rate of this virus. To understand the risk of COVID-19 outbreak across different parts of the globe it is crucial to provide a mechanistic framework for the interrelation of comorbidities. This review chapter focuses on the characteristic features, signalling cascade, and the interplay between pathogenesis and unfolded protein response in coronavirus. Due to the complexity of the coronavirus genome and its replication cycle, our understanding of structural, non-structural and accessory proteins in virion assembly and involvement of host machinery is significant. Thus, there is an urgent need to develop effective antiviral doses and vaccines against coronavirus. Hence, updating the detailed mechanism of cross-talk between virus and host, signal and receptor and the evasion of cellular innate immune responses is of utmost importance to develop novel therapeutic strategies.

Keywords: SARS CoV-2; COVID-19; Structural proteins; Immune response; Signalling pathway; ER-stress; Host-virus interaction.

1. Introduction

Viruses are genetically unstable organisms which are constantly mutated by genetic shift or drift. It is impossible to envisage the time as to when a cross-species jump may occur and when a seemingly harmless variant form of the virus may turn into a deadly strain. On December 31, 2019, one of the viral pandemics came into limelight when a cluster of cases with pneumonia-like symptoms was noticed first in Wuhan, China. The virus has spread with an alarming speed world over and has posed a serious threat to people and the world economy. The virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease is now called COVID-19. The World Health Organization says, 'The coronavirus pandemic is the defining global health crisis of our time.' It was called 'Novel Corona Virus' on January 12, 2020, and COVID -19 on February 11, 2020 [1]. There are myriad mechanisms where the virus has developed to invade the host cells and evade the immunity of hosts. It exclusively depends on hosts at every stage of their life cycles as how do they interact with each other via signalling pathways. Hence, the study of cell-to-cell communication is necessary to coordinate the activities of cells. The cell communication is in the form of signalling through the messenger molecules *viz.*, neurotransmitters, hormones, and interleukins, cytokines, autacoids, growth factors, catecholamine, histamine, serotonin, eicosanoids, nucleotides and extracellular vesicles from the external environment or cyclic adenosine monophosphate, cyclic guanosine monophosphate, calcium, phosphatidylinositols, nitric oxide and diacylglycerol from internal environment [2]. The signalling is the process by which cells decipher from the signal delivered from the extracellular/intracellular environment into specific cellular responses when messenger molecules interact with receptors. It has a significant role to elicit many cellular activities. Nevertheless, the interaction between virus and receptor also has a key role in viral host range, tissue tropism and pathogenesis [3]. The virus is an obligatory intracellular pathogen which has to enter the host cell to complete its' life cycle. To accomplish this, the interaction between the surface receptors of viruses and host is critical to gain entry into the host cell and take control over the cell machinery to undergo replication. It is mediated by the viral attachment protein expressed on the surface and binds to the cell surface receptor subsequently translated into signal transduction which induces a cascade of events. These cellular events are witnessed in biochemical variations and gene transcription.

2. Classification and Disease Transmission

The study by the International Committee on Taxonomy of Viruses says that coronaviruses belong to the family Coronaviridae in the order Nidovirales. The family is subdivided into four genera – the alpha, beta, gamma and delta coronaviruses – based on early serological and later genomic evidence [4]. This family of viruses mainly causes

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respiratory diseases in humans in the form of common cold or pneumonia. They can infect animals as well Esper, *et al.* [5]. Up until 2003, limited research was carried out on coronavirus (CoV). Nonetheless, after the SARS-CoV outbreak, it attracted the attention of researchers across the world [6]. A distinctive feature of these viruses is the ability to mutate in a quick time and adapt to a new host. These viruses jump from host to host owing to their zoonotic origin. In December 2019, almost seven years after the MERS 2012 outbreak, COVID-19 surfaced in Wuhan in the Hubei region of China. The pandemic grew faster than expected and spread to neighbouring countries in no time. However, due to the advancement of technology, the information dispersed worldwide in a very short period. The major part of the infection was restricted to China, and a second cluster was found on a cruise ship called the Diamond Princess docked in Japan. Experts, based on the evidence, opined that the virus, like many other respiratory viruses, found its origin in bats [5, 7]. Bats have been responsible to harbour coronaviruses for quite a while now. With the increasing selection and ecological pressure due to human activities, the virus shifted from animal to man. Combined with additional ecological pressure due to climate change, such zoonotic spillovers are now more common than ever.

3. Structural Organization of the Virus

All viruses falling under order Nidovirales are non-segmented, enveloped positive-sense RNA viruses. Most of the RNA viruses contain very large genomes, with some viruses having the largest identified RNA genomes, containing up to 33.5 kilobase (kb) genomes. Within the Nidovirales the other common attributes include:

(1) Expression of many non-structural genes by ribosomal 2 frameshifting;
(2) A highly conserved genomic organization, with a large replicase gene preceding structural and accessory genes;
(3) Expression of downstream genes by the synthesis of 3′ nested subgenomic mRNAs; and
(4) Several unique or unusual enzymatic activities encoded within the large replicase–transcriptase polyprotein [8].

The order Nidovirales (Latin word, ‘nido’ for ‘nest’) is derived from nested 3′ mRNAs as mentioned above. The major differences within the Nidovirus families are in the number, type and sizes of the structural proteins. The significant alterations in the structure and morphology of the nucleocapsids and virions are due to the following differences:

Coronaviruses are large enveloped, positive-stranded RNA viruses. Amongst all RNA viruses, their genome size is the largest, typically ranging from 27 to 32 kb [9]. The genome is packed inside a helical capsid formed by the nucleocapsid protein (N). Despite having positive-sense RNA, these viruses have helically symmetrical nucleocapsids this being one of the uncommon features. The most significant feature of coronaviruses is the club-shaped spike projection emanating from the surface of the virion. These spikes are a defining feature of the virion and give them the appearance of a solar corona or crowns, prompting the name, coronaviruses (*corona* in Latin means crown) [9].

4. Structural Protein Diversity of Virion

The viral envelope of coronavirus is encoded by four important associated structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins (Figure 1). These proteins are encoded within the 3′ end of the viral genome. Some coronaviruses also possess an envelope-associated hemagglutinin-esterase protein (HE).

4.1. Spike (S) Glycoproteins

The spike (S) transmembrane proteins (~150 kDa) form large protrusions from the virus surface amongst all structural proteins. This gives coronaviruses the appearance of having crowns. In addition to mediating virus entry, the spike is a critical determinant of viral host range, tissue tropism and a major inducer of host immune responses [9]. The spike of coronavirus has three segmented regions: a large ectodomain, a single-pass transmembrane anchor and a short intracellular tail. The ectodomain comprises a receptor-binding subunit S1 and a membrane-fusion subunit S2. The research carried out using an electron microscope revealed that the spike is a clove-shaped trimer with three S1 heads and a trimeric S2 stalk. S1 makes up the large receptor-binding domain of the S protein whereas S2 forms the stalk of the spike molecule [10].

4.2. Membrane (M) Protein

The most abundant structural protein in the virion is the M protein – a small (~25–30 kDa) protein having three transmembrane domains [11]. This protein is considered to give the virion its shape. It includes small N-terminal glycosylated ectodomain and a much larger C-terminal endodomain that extends 6–8 nm into the viral particle [12]. The majority of M proteins do not contain a signal sequence despite being inserted in the ER membrane co-translationally. M proteins of coronavirus are highly diverse in amino acid contents; however, they maintain overall structural similarity within different genera. Altogether, the viral scaffold is maintained by M–M interaction. The study suggests that the M protein are dimeric in the virion and probably adopt two different conformations, allowing it to promote membrane curvature besides binding to the nucleocapsid [13].

4.3. Envelope (E) Protein

The E protein (~8–12 kDa) is present in small quantities within the virion. The coronavirus E proteins are highly divergent but have a common architecture. The E proteins membrane topology is not explored thoroughly; however,

studies suggest that it is a transmembrane protein. The E protein not only facilitates assembly and release of the virus but also has other functions. For example, the ion channel activity in SARS-CoV E protein is not required for viral replication but pathogenesis [14]. The coronavirus E protein is the most mystifying protein. It is the smallest of the major structural protein as well. It plays a multidimensional role in the pathogenesis, assembly and release of the virus [14, 15]. It is found to be a small integral membrane polypeptide, acting as a viroporin (ion channel). The absence or inactivation of the protein alters the pathogenicity of coronaviruses owing to conformational changes in morphology and tropism [16]. The E protein possesses an N-terminal ectodomain and a C-terminal endodomain besides having ion channel activity. In contrast to other structural proteins, recombinant viruses which lack E proteins are not always lethal, although this depends on virus type. The E protein comprises three domains: a short hydrophilic amino-terminal, a large hydrophobic transmembrane domain and an efficient C-terminal domain [15].

4.4. Nucleocapsid (N) Protein

The N protein is the only protein found in the nucleocapsid. It consists of two different domains – an N-terminal domain (NTD) and a C-terminal domain (CTD). Both of them are capable of binding RNA *in vitro*, but each domain employs different RNA binding mechanisms. Studies suggest that both domains contribute to optimal RNA binding [17]. The N protein is highly rich in ATP that is, heavily phosphorylated. This phosphorylation is considered to have triggered a structural change, leading to an increased affinity for viral and non-viral RNA. The protein binds the viral genome in a beads-on-a-string type conformation. Two specific RNA substrates the transcription-replication signals [18] and the genomic packaging signal [19] have been identified for N protein. Research indicates that the N protein also binds a key component of the replicase complex, nsp3 [17], and the M protein. The interactions among proteins, in turn, help to tether the viral genome to the replicase–transcriptase complex (RTC). Subsequently, they help in packaging the encapsidated genome into viral particles.

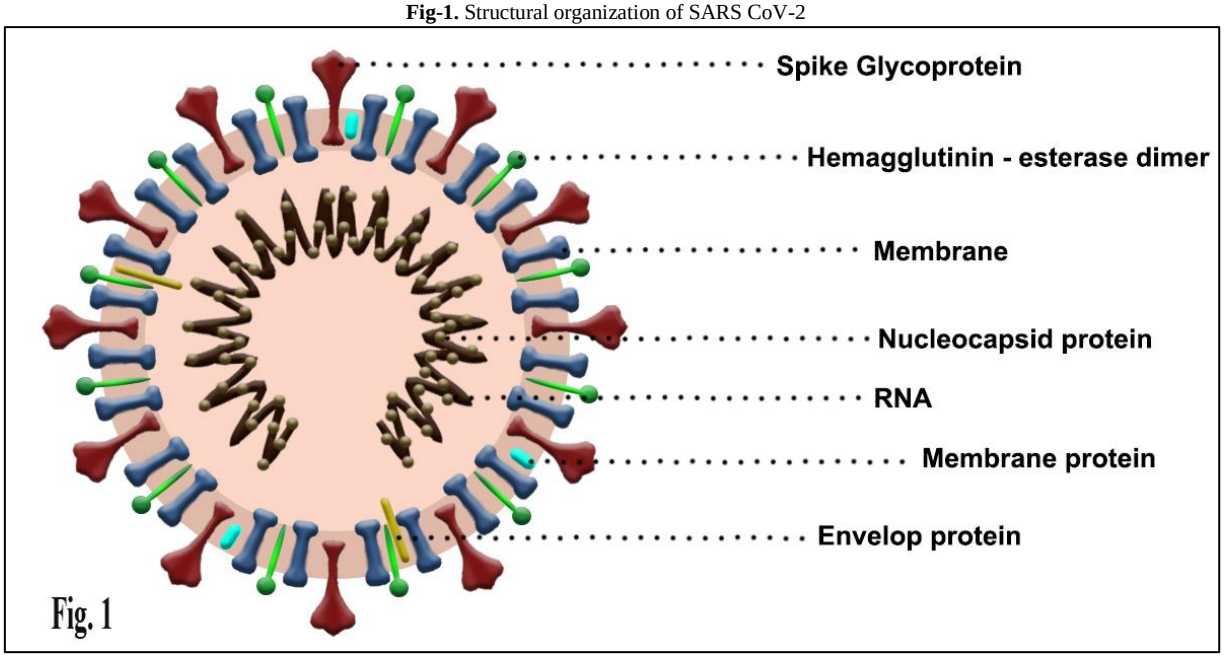
4.5. Accessory Proteins

The next protein in the line-up is the hemagglutinin-esterase (HE), which is present in a subset of β-coronaviruses. This protein acts as a hemagglutinin, binds sialic acids on the surface of glycoproteins and contains acetyl-esterase activity [20]. This act is considered to promote S protein-mediated cell entry and viral spread through the mucosa.

5. Non-Structural Proteins (nsps) and Accessory Proteins

In addition to the above-mentioned important structural proteins, the SARS-CoV-2 genome also contains fifteen nsps (nsp1–10 and nsp12–16) and eight accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b and ORF14) [21]. All these proteins play a major role in viral replication. Unlike the accessory proteins of SARS-CoV, SARS-CoV-2 does not contain 8a protein and has a longer 8b and shorter 3b protein [21]. Compared to the sequences of other coronaviruses, the nsp7, nsp13, envelope, matrix, and p6 and 8b accessory proteins do not have any amino acid substitutions.

Fig-1. Structural organization of SARS CoV-2



6. Virus-Host Cell Interaction

The comprehensive insight into the replication, growth phase of viruses during infection and the virus-host interaction is essential [22]. Viruses are obligatory parasites, depend on their host cells and exploit them by various mechanisms to accomplish their replicative phase of the life cycle which may damage the host cell or finally lead to

their death. To control any disease it is necessitated to understand the molecular events associated with viral replication.

7. Viral Replication and Infection through the Signalling Pathway

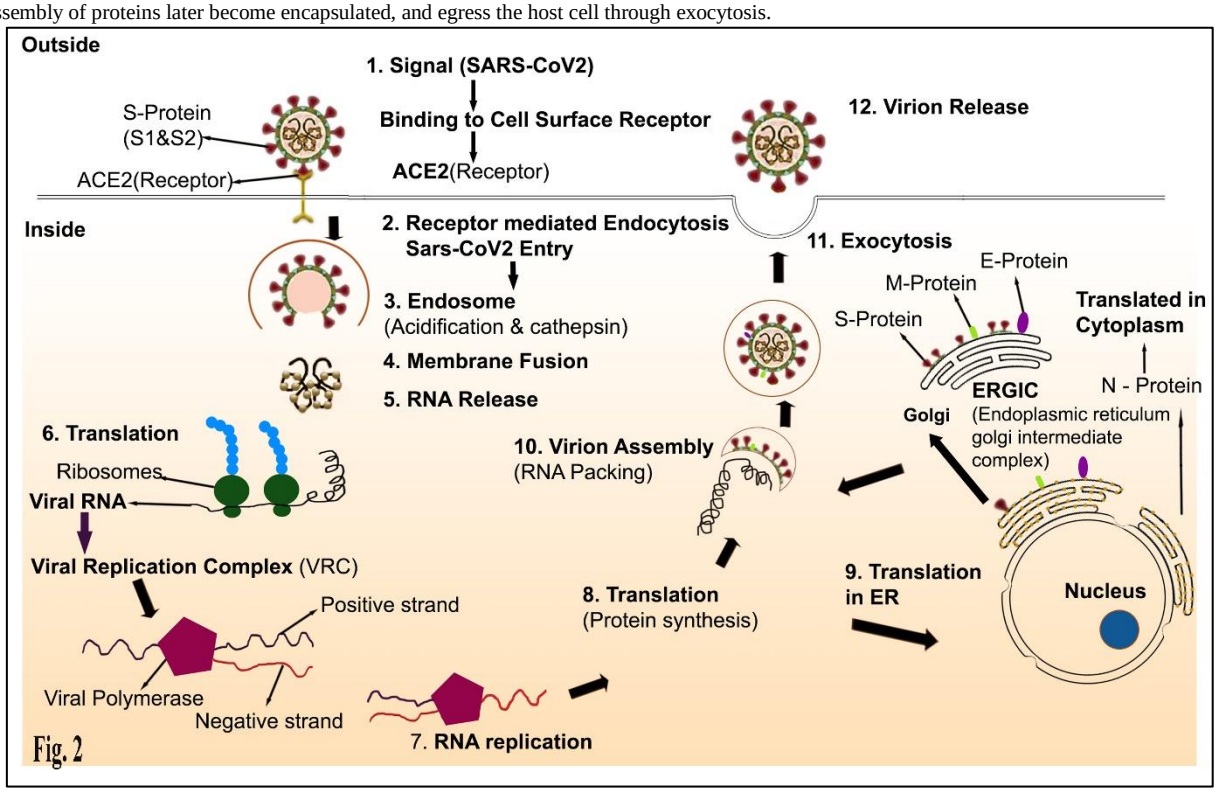
The coronavirus infection triggers many signalling pathways in the host cell (Figure 2). The signalling initiates the binding of S protein to the ACE2 receptor, internalised into the host cell later lead to apoptosis [23]. The recognition of host by the virus through one or more than one cellular receptor is the crucial step to synthesize viral mRNA [24]. The virus relies on host completely for the synthesis of proteins as they lack ribosomes. Further, the host cell glycosylates the viral proteins leading to the suppression of innate immune response. In specific to coronaviruses, the spikes bound to the membrane of coronaviruses attach to the host cell [25], enter the host cell through angiotensin-converting enzyme 2 (ACE2) receptors expressed on pulmonary alveolar epithelial cells [26]. The spike protein (subunit S1 & subunit S2) of coronavirus mediates into the host cell, initially binds to a receptor present on the cell surface and later fuse with viral and host membranes. The tropism of coronaviruses decided by the interaction of the cell surface receptor with the S1 subunit [27], subsequently leading to a change in conformation of S2 subunit, exposing the hidden peptide and insert into the cellular membrane. The spike protein subunit S1 is bound to ACE2, host cell serine proteases TMPRSS2 present on human cell's surface cleave the S1 protein at N-terminal receptor-binding domain and S2 at C-terminal allowing to fuse the viral and cellular membranes [28]. The virus entry into the host cell is released from endosomes by acidification or the action of an intracellular cysteine protease, cathepsin [29]. This is followed by the packing of the two heptad repeats in the three monomers into a six-helix bundle fusion core [30]. The close association of the viral and cellular membrane enables the fusion of the lipid bilayers, and the viral nucleocapsid finally delivered into the cytoplasm [31]. The expression of ACE_2 receptors is also found on the cell surface of heart, blood vessels and kidney. The key role of ACE_2 is to cleave the carboxy-terminal amino acid phenylalanine of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and hydrolyses into angiotensin (1-7) (H-Asp-Arg-Val-Tyr-Ile-His-Pro-OH) [32] thereby regulating mainly the blood pressure, wound-healing and inflammation. The gaseous exchange of oxygen and carbon dioxide occurs between lungs and blood vessels through the epithelial lining of the lung. When coronavirus attaches to the ACE_2 receptor, it blocks the ACE_2 action on regulating ANG II signalling, subsequently leading to the augmentation of ANG II to injured tissues such as lungs and heart in the subjects. The ACE_2 helps to modulate the many activities of a protein called angiotensin II (ANG II) which has a key role in acting as a vasoconstrictor that increases blood pressure and inflammation, increasing the damage of blood vessel linings leading to various types of tissue injury. ACE_2 converts ANG II to other molecules that neutralise the effects of ANG II. The COVID-19 positive people suffering from hypertension and other comorbidities evident of the mortality being observed due to cardiac arrest, kidney dysfunctioning and respiratory ailments [33]. The ACE2 receptors expression is a rate-limiting step for the virus to enter into the host cell [34]. The SARS CoV-2 pathogenicity affects the renin-angiotensin-aldosterone system (RAAS), the RAAS pathway utilises ACE1 which is a pulmonary capillary endothelial enzyme converts Angiotensin I to Angiotensin II, this predicts the COVID-19 positive subjects suffering from acute respiratory illness along with kidney dysfunctioning have inadequate ACE function lead to mortality [35]. Following the attachment of virion onto the host cell surface results in the delivery of viral genome across the membrane to the site of replication, cytoplasm. The replication/transcription complexes are attached to the membrane through transmembranes spanning proteins *viz.*, nsp3, nsp4 and nsp6 during the processes of replication and transcription of the coronavirus genome [36] lead to the formation of double-membrane vesicles [37]. The replication of coronavirus is initiated by the synthesis of genomic RNA which acts as a template, mRNA to translate replicase polyprotein following the uncoating of virion leads to the generation of multiple subgenomic mRNAs corresponding to both ends of genome, a process of discontinuous transcription [38]. The replicase comprises of two open reading frames (ORF1a and ORF1b), on translation produces the polyprotein processed to generate 16 non-structural proteins (nsp) comprises of polymerase and proteases [39]. The virion has four structural proteins *viz.*, spike (S), membrane (M), envelope (E) – these three proteins synthesized in ER and transported to the Endoplasmic reticulum-Golgi intermediate complex (ERGIC) for further processing while nucleocapsid (N) protein is translated in the cytoplasm and encapsulate the nascent genomic RNA to form the nucleocapsids, accessory proteins bind to structural proteins [40]. The assembly of virion occurs in ERGIC with the M-protein and aid of protein-protein interaction [31], later budded and exported in smooth-wall vesicles fuse with the plasma membrane and release the assembled virus [41]. Masters [31], indicated the S proteins which secrete to the plasma membrane escaping the virion assemble and fuses the infected cell with the uninfected cell thus forming multinucleated cell called syncytium facilitates the virus to infect other cells without being released into extracellular space.

8. Perturbation in Hosts' Endoplasmic Reticulum Mediated Through Unfolded Protein Response

The endoplasmic reticulum (ER) is the site of protein synthesis in eukaryotes. The infection of coronavirus in eukaryotes has a dire need to synthesize adequate amount of proteins in the host cell to accomplish its' life cycle. All proteins except N protein are synthesized in the ER. The protein folding exceeds the ER capacity. This results in the accumulation of unfolded proteins perturb the homeostasis of ER, thus paving the way to activate the signalling pathway of unfolded protein response (UPR) [42]. Pineau, *et al.* [43], indicated the ER stress can be marked by the excessive lipids or proinflammatory cytokines. There are three ER-transmembrane sensors linked to UPR *viz.*, the PKR-like ER protein kinase (PERK), the inositol-requiring protein1 (IRE1) and the activating transcriptional factor 6 (ATF6) signal ER through the negative feedback to decrease the protein synthesis and increase the protein folding

of unfolded proteins [44]. To compensate the ER stress, UPR accelerates the reversible translation attenuation, increases the protein folding capacity of ER, later activates the ER-associated degradation (ERAD), if ER stress prolonged, and UPR subsequently leads to apoptotic cell death [45]. The UPR in the host cell involves the signalling pathways namely, innate immunity, MAPK pathway, inflammatory response, autophagy, and apoptosis [46]. Finally, the mature virions are released through a process known as exocytosis by depleting the lipid content in the ER [47].

Fig-2. A schematic diagram showing the SARS CoV-2 replication with the following cascade of events. SARS CoV-2 with positive-sense RNA enters cells by binding its' spike protein to the host ACE-2 receptor following the fusion of host-viral membranes through endocytosis. The positive RNA strand is released into the cytosol undergoes translation by the host ribosomes. The viral replication complex protein recruits the positive RNA strand to subcellular membrane compartments, where functional viral replication complexes (VRCs) are assembled. The negative-sense stranded RNA is synthesized and serves as a template for the synthesis of many new positive-strand RNA. These nascent RNAs released from the VRC, while the negative-sense RNA is retained. The released RNA (+) enters a new cycle of translation in the endoplasmic reticulum, assembly of proteins later become encapsulated, and egress the host cell through exocytosis.



9. Immune Response Signals as a Cue of COVID-19

The immune system has a crucial role as a warrior in combating the pathogen entering the living system. As the virus enters the host epithelial cells, trespassing the physical barriers such as skin, mucosa in respiratory, gastrointestinal and urinary systems and chemical barriers (hydrochloric acid-stomach, lysozyme-sweat and tear glands and lactic acid-vagina), the innate immune response is initiated by the macrophages. This acquaints the virus with pathogen-associated molecular patterns (PAMP) and the viral RNA sensed by pattern recognition receptors (PRRs) on innate immune cells [Antigen-presenting cells (APCs) such as macrophages, dendritic and B-cells] later by a process known as phagocytosis, digesting the virus to harmless products. If the troupe of pathogens are too strong and innumerable, the macrophages signal the other immune cells by releasing cytokines, the signalling proteins leading to inflammation recruit the macrophages, monocytes, neutrophils. The inflammation involves vasodilation, increased vascular permeability, mast cell activation and degranulation, clotting system and kinin system. The inflammation stimulates macrophages and neutrophils to release cytokines in specific the interleukins, the IL-1 signals the brain to increase the temperature of the physiological system, IL-6 to the liver to produce acute-phase proteins (opsonins), IL 8 activates neutrophils and IL-2 and IL-12 activates natural killer cells [48]. Parameswaran and Patial [49], indicate the vital role of TNF-α which orchestrates the aforesaid roles and considered as "master regulator' in the inflammatory response and cytokine synthesis. The innate immune response to the infection deliberates the viral infections by the expression of the type I interferons (TI IFN), such as interferon (IFN)-α and IFN-β. The pattern recognition receptors (PRR) on endosomes include toll-like receptors (TLR-) 3, 7/8 and 9 also detect the SARS-CoV-2 [50]. The pathogen-associated molecular patterns (PAMPs) as viral RNA are sensed by pattern recognition receptors (PRRs) on innate immune cells including antigen-presenting cells (APCs), resulting in their activation. The dendritic cell has a key role in adaptive immune response, an antigen-presenting cell capture the antigen from the invading virus and directed to blood and lymphatic system to activate the T and B cells specifically [51]. The dendritic cell present antigen with MHC II to the CD4-T cells proliferate and become T-helper cells along with MHC I which present to CD8-T cells later become cytotoxic cells [51]. These T helper cells release cytokine to stimulate B cells to synthesize plasma cells and release antibodies. Further, the pathogen activates directly the complement system through lectin and alternate pathway [52]. The innate immunity interacts with the infectious microbe lead to the overproduction of tumour necrosis factor (TNF), interleukin-6 (IL-6) and IL-1β known as 'Cytokine storm' [53]. The high levels of cytokines attract the other immune cells to encounter the

infectious agent along with the activation and modified T cells direct the increased inflammatory response which causes the multi-organ failure.

10. Conclusion

Since six-months (2020) the research interests across different parts of the globe have increasingly striving hard to develop an efficient vaccine and target the SARS CoV-2. The prevailing health and economic crisis owing to SARS-CoV-2 pandemic have alarmed us as to how novel pathogens can rapidly propagate and spread through the human population causing the severe outbreak. To summarize, the inhibition of signalling pathways generate a targeted therapeutics in terms of suppressing the ACE2 expression, cytokine storm, unfolded protein response (UPR) to manage COVID-19.

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