

6-10-2020

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### Recommended Citation

Moenadjat, Yefta (2020) "Minireview: Covid-19: Criticism of The Pathogenesis Based on The Tropism," *The New Ropanasuri Journal of Surgery*. Vol. 5 : No. 1 , Article 12.

DOI: 10.7454/nrjs.v5i1.1076

Available at: <https://scholarhub.ui.ac.id/nrjs/vol5/iss1/12>

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## Minireview: Covid–19: Criticism of the pathogenesis based on the tropism

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Website: <https://scholarhub.ui.ac.id/nrjs/> DOI:10.7454/nrjs.v5i1.1076



### Introduction

Coronavirus disease 2019 or Covid–19 is the attribute given by The Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses,<sup>1</sup> despite the other attributes, i.e., the new–emerging SARS–CoV, novel coronavirus or SARS–CoV–2; whereas ‘new’ or ‘novel’ denoting the next generation of coronavirus with a distinct clinical entity to SARS–CoV (2002–2004) and middle east respiratory syndrome (MERS) outbreak (2012). Indeed, they comes from the same family, i.e. coronavirus.

Many reports showed that this coronavirus is leading to the pandemic higher than SARS–CoV 2002–2004 outbreak, it killed more people than SARS and MERS combined, despite lowercase fatality rate.<sup>2</sup> Following the first report in Wuhan, Hubei province of China in December 2019, the WHO warned coronavirus, dubbed Covid–19, was ‘public enemy no.1’ and potentially more powerful than terrorism;<sup>3</sup> as in February 2020 the disease spread out more than 66 countries globally. It believed it spread out through the droplet and aerosol, let the SARS–CoV–2 has a similar ability to spread as SARS–CoV, or even a higher. It remains unclear why the coronavirus of the same family becomes as that pathogenic and featuring the distinct nature of the disease that fatal. The learned lesson from SARS–CoV was the transmission predominantly through medical, i.e., the transmission through nebulizers, endotracheal suction and intubation, cardiopulmonary resuscitation, nasogastric feeding, and high flow rates of oxygen. Whereas from MERS was the transmission to the proper hand hygiene, sanitation, and physical barrier.<sup>4</sup> However, what precisely useful to this new–emerging SARS–CoV–2 is? To answer the question, introduction and a review of pathophysiology is the topic in this article.

### Coronaviruse

of common cold working group.<sup>5</sup> Tyrrell and Bynoe (1966) passaged a virus named B814 from endotracheal specimen patients with the common cold, highly similar to the previously described avian infectious bronchitis virus (IBV) and mouse hepatitis virus (MHV). In the same time, Hamre and Procknow found the virus named 229E from the kidney patient with the common cold,<sup>6</sup> and McIntosh succeeded in

growing the virus in the culture named the virus as OC.<sup>7</sup> The structures different from those of the myxoviruses. The viruses resembled the solar corona and giving rise to the name that ultimately assigned to the group.<sup>8</sup> At the time, Tyrell and colleagues classified the virus as coronaviridae.<sup>9</sup>

The Coronavirus family has a potential species specificity and interspecies transmission. The interspecies transmission of viruses from one host species to another is a significant factor responsible for the majority of emerging and re–emerging infections. The viruses are one of the most popular emerging viral families that threaten public health. An emerging infectious disease is defined as the incidence increased over the past 20 years and may increase in the future.<sup>10,11</sup> Currently, the viruses referred to a model in virology, as it infects more than 200 different hosts (mammals, birds, and human)<sup>11</sup> and has a global distribution.

In taxonomy perspective, five viruses associated with human disease, namely SARS–CoV which is the most categorically harmful, and four remaining human coronaviruses (HCoV), the alpha Coronaviruses HCoV–229E, HCoV–NL63, beta Coronaviruses HCoV–OC43 and HCoV–HKU1. Remarkably, HCoV–NL63 and HCoV–HKU1 were only discovered recently, despite the fact that each has a worldwide prevalence and has been in circulation for a long time (table 1). However, there were new variants discovered most recently (figure 1). Although generally associated with upper respiratory tract infections, the extant HCoV can also cause lower respiratory tract infections and have more serious consequences in the young, the elderly, and immunocompromised individual. In particular, HCoV–NL63 is strongly associated with childhood croup, and the most severe HCoV–HKU1, –OC43, and –229E infections are manifest in patients with comorbid.<sup>12</sup> However, after Wuhan 2019, more than 15 new variants of beta CoV discovered worldwide, including WSFMP\_Wuhan–Hu–1, 2019–nCoV/USA–CA1/2020, 2019–nCoV/USA–CA2/2020, 2019–nCoV/USA–IL1/2020, 2019–nCoV\_WHUO2, 2019–nCoV\_WHUO1, 2019–nCoV/USA–WA1/2020, 2019–nCoV\_HKU–SZ–005b–2020, 2019–nCoV\_HKU–SZ–002a–2020, 2019–nCoV/USA–AZ1/2020, WSFMP–WIV07, WSFMP–WIV02, WSFMP–WIV04, WSFMP–WIV05, WSFMP–WIV06, and SARS–like CoV\_WIV16.<sup>13</sup>

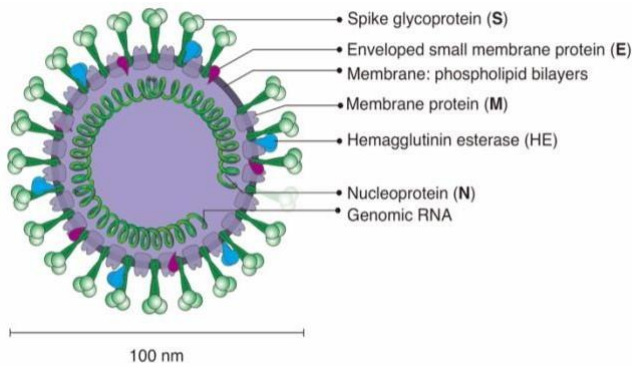


Figure 1. Schematic illustration of Coronavirus. Coronaviruses are spherical measuring 120–220 nm in diameter and presenting crown feature in the presence of pointed glycoproteins.

The genomic. Coronaviruses classified as RNA viruses measuring 120–220 nm (see figure 2). The genome structure is an unsegmented one measuring of 26–33 kb in length, generally of similar genomic and

structural and non-structural proteins. The structure is a single strand positive RNA (+ssRNA) start with 5' untranslated region (5' UTR) and 3' UTR poly-A tail. The RNA is used as the template to directly translate polyprotein (end open reading code, ORF) 1a/1b which encode the structural proteins (figure 3).<sup>12,13</sup> Then, structural protein containing 5'-end of ORF series encode non-structural proteins that are primarily involved in pathogenicity. (see table 2); and 3' tail. The number of ORF differs by species, and it has a significant portion of Coronaviridae genomes. More than two-thirds of the CoV genome is composed of an ORF coding for the replicase polyprotein 1a/1b. Followed by the coding regions of structural proteins and the remainder contains ORF encoding the structural proteins: spike, or spicule (S), envelope (E), membrane (M), nucleoprotein (N), and a variable collection of accessory proteins. The four proteins occur in the S–E–M–N order in all known CoV lineages (see figure 2). Among these SEMN genes, CoV encode species-specific accessory proteins, many of which appear to incorporate into virions at low levels, ranging from 1–16 genes.

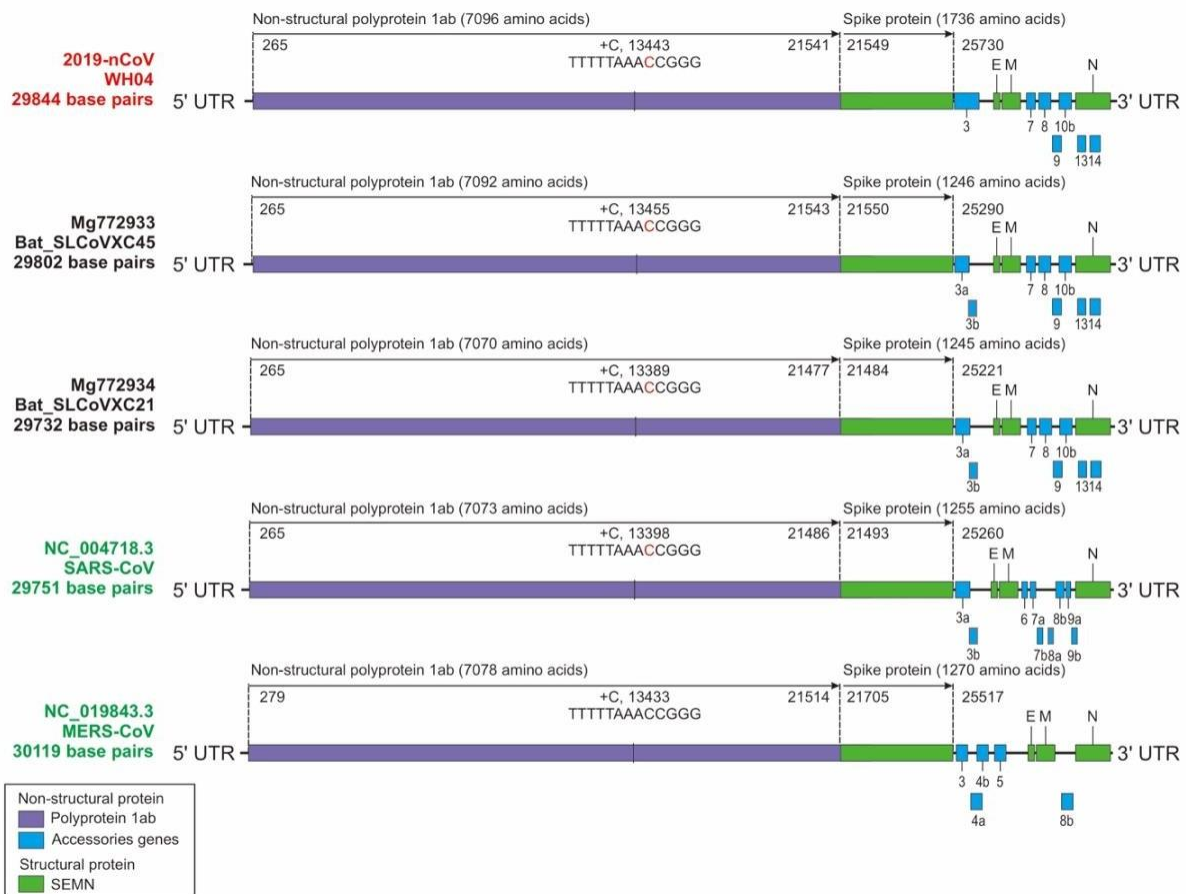


Figure 2. Construction of the protein Coronaviridae. There is a similarity between human and animal coronaviruses at the organization level of their genome. All coronaviruses encode non-structural protein (ORF replica 1a/1b, in purple) and structural proteins (in green) comprised of spike protein (S), envelope (E), membrane (M), and nucleoprotein (N). Each strain encodes several accessory proteins (in blue).

The spike glycoprotein (S) is an essential component in the species specificity, pathogenesis, and escape of immunity.<sup>14</sup> This protein referred to as a responsible gene that plays a critical role in virus pathogenicity.<sup>14</sup> The spike (S) glycoprotein is a class I viral fusion protein that binds to host cell receptors and mediates the earliest steps of infection.<sup>8,14</sup> Different characteristics and the virulence of human Coronaviruses to those in bat/rat are the S protein that was leading to the

speculation of engineered genes. The S monomer is a transmembrane protein of 128 to 160 kDa, composed of a large N-terminal ectodomain and a tiny C-terminal endodomain. A complete high-resolution structure has not yet been determined for any coronavirus S protein. However, a cryo-electron microscopic reconstruction of the SARS-CoV S protein confirms glycoprotein reveals a prerequisite conformational state for receptor binding.<sup>15,16</sup>

The most abundant structural protein in coronaviruses – the M protein – gives the virion envelope its shape. The M monomer, which ranges from 25 to 30 kDa, is a polytopic membrane protein that is embedded in the envelope by three transmembrane domains. At its amino terminus is a very small ectodomain; the C-terminal endodomain of M accounts for the significant part of the molecule and is situated in the interior of the virion or on the cytoplasmic face of intracellular membranes. Studies reveal that the large carboxy terminus of M extends some 6 to 8 nm into the viral particle and compressed into a globular domain, consistent with early work showing that the endodomain is very resistant to proteases. The E protein is a small polypeptide of 8 to 12 kDa found in a limited number in the virion envelope. The E protein is a small polypeptide of 8 to 12 kDa that found in limited amounts in the virion envelope. This protein has a hairpin conformation, placing both of its termini on the cytoplasmic face of membranes, or that E can have multiple membrane topologies. E protein correlated with transports calcium ion in ERGIC/Golgi membranes.<sup>17</sup> The N protein is the sole protein constituent of the helical nucleocapsid. Monomers of this 43–50-kDa protein bind along the RNA genome in beads-on-a-string configuration typical to other helical viral nucleocapsids. This phosphoprotein modified at a limited number of serine and threonine residues. Phosphorylation sites have been mapped for a representative Coronavirus from each genus, and targeted sites, collectively, fall in every domain and spacer region of the N molecule. The most conspicuous function of the N protein is to bind to viral RNA. Nucleocapsid formation must involve both sequences-specific and nonspecific modes of RNA binding. Specific RNA substrates that have been identified for N protein include the transcription-regulating sequence (TRS) and the genomic RNA packaging signal.<sup>8,14</sup>

The non-structural accessory proteins (nsp) are:<sup>13</sup> nsp1, known for cellular mRNA degradation, inhibiting IFN signaling; nsp2, unknown; nsp3, known as PLP for polypeptide cleaving, blocking host innate immune response, promoting cytokines promotion; nsp4, double membrane vesicle (DMV) formation; nsp5, 3CL<sup>Pro</sup> (chymotrypsin-like-protease), M<sup>Pro</sup> (main protease), which is polypeptide cleaving, inhibiting interferon (IFN) signaling; nsp6, function for restricting autophagosome expansion, DMV formation; nsp7, the cofactor with nsp8 and nsp12; nsp8, cofactor with nsp8 and nsp12, primase; nsp9, dimerization and RNA binding; nsp10, scaffold protein for nsp14 and nsp16; nsp11, remains unknown; nsp12, primer independent for RNA dependent RNA polymerase (RdRp); nsp13, RNA helicase, 5' triphosphate; nsp14, exoribonuclease, N7-MTase; nsp15, endoribonuclease, evasion of double stranded RNA (dsRNA) sensors; nsp16, or, 2'-O-MTase, avoiding MDA5 recognition, negatively regulating innate immunity.

## Pathogenesis

The virus binds to the glycocalyx on the epithelial surface for the initial attachment. The glycocalyx – a brush border composing glycoproteins, glycolipids, and proteoglycans – referred to the first barrier for the incoming viruses. The SARS-CoV are known to have specific receptors on the surface of the cell i.e., angiotensin converting enzyme (ACE2) as reported by Lu et al.<sup>18</sup> A study of Wrapp et al., recently released structure of the RBM ACE2 complex revealed that most S residues contacting

ACE2 are identical between SARS-CoV and 2019-nCoV. However, some are unique, including an important salt bridge that involves different amino acids in ACE2 to bind S of SARS-CoV and 2019-nCoV.<sup>16,19</sup>

SARS-CoV-2 binds to ACE2 by its spike and allows the virus to enter and infect cells. However, the spike protein has to be primed by an enzyme called a protease to complete the process. The used protease is similar to SARS-CoV, called transmembrane serine protease II or TMPRSS2.<sup>20</sup> This TMPRSS2 might promote viral spread and pathogenesis by diminishing viral recognition by neutralizing antibodies and by activating SARS S for cell-cell and virus-cell fusion.<sup>21</sup>

Previously there is a thought that a virus used either direct membrane fusion at the cell surface or endocytosis to enter cells. Later, studies showed that the endocytosis pathway involved in virus entry. Recently it is known that ACE2 receptor translocation and recycling, pH-dependent viral entry and early endosome location of the viral spike protein indicate that SARS-CoV pseudoviruses may enter cells via receptor-dependent, pH-sensitive endocytosis.<sup>22</sup>

The second barrier is the plasma membrane. Responsible for the cell's exchanges with the environment, it is the most complex and most dynamic of all cell membranes. Composition of plasma membrane property regulated by 1) the endocytic and secretory pathways and 2) continuous association and disassociation of proteins that interact with the cytosolic leaflet. The plasma membrane is a highly sensitive organ recognizing and responding to external stimuli. Viruses take advantage of this during entry. Here, the other important receptor is proteases, namely metalloproteinases. After clearing the plasma membrane through direct penetration or by exploiting endocytic pathways, viruses and viral capsids have to reach sites more in-depth in the cytoplasm. The cortical actin network underneath the plasma membrane and extreme crowding constitute major barriers to movement within the cytoplasm. Finally, because the viruses replicate in the nucleus, the genome and accessory proteins must travel to the nucleus and cross the nuclear envelope. This requires cooperation between the incoming virus and the nuclear import machinery.<sup>23</sup> The process of replication is unique in the cells and presented in figure 3.

## Tropism.

Viral particles have a single mission, i.e., to transport the viral genome from an infected host cell to a noninfected host cell, and to deliver it into the cytoplasm or the nucleus in a replication-competent form. The target can be a neighboring cell, a cell elsewhere in the host organism, or a cell in another organism. The process starts in an infected cell with the packaging of the viral genome and accessory proteins into a new virus particle, which is released into the extracellular space. When the virus contacts the surface of a new host cell, a complex series of events ensues tightly coordinated in time and space.<sup>23</sup>

ACE distribution is a crucial matter. ACE2 gene expressed in a great vary in humans, between organs, and between population.<sup>24,25</sup> Data showed that the most expressed tissue is the duodenum, gall bladder epithelium, testicular Sertoli cells and Leydig cells, glandular cells of seminal vesicle and cardiomyocytes., renal proximal tubules, colon liver, fat and brain.<sup>21,26</sup>

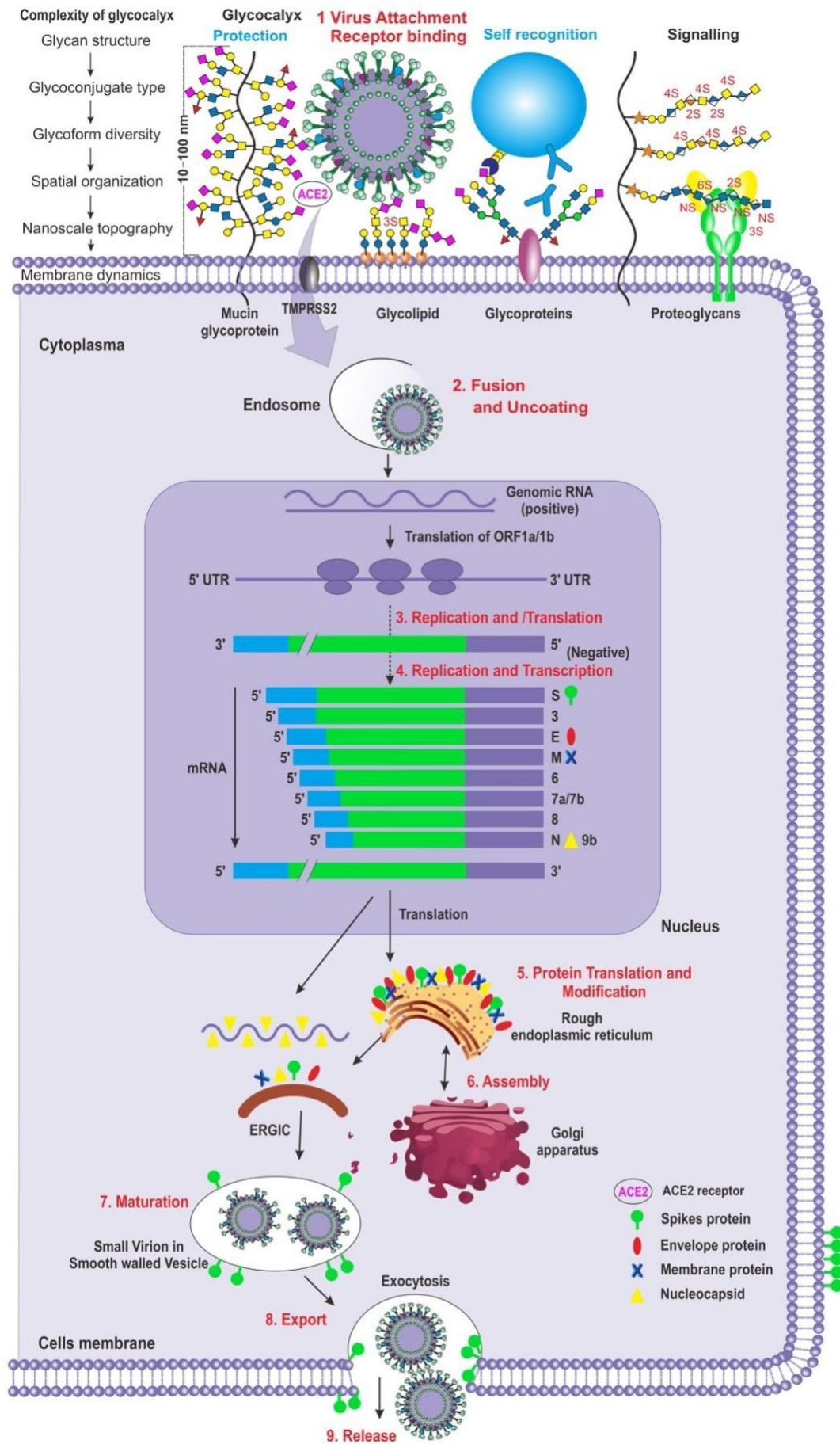


Figure 3. The Replication of Coronavirus infection following the attachment. The process including the fusion and uncoating, replication and translation, replication, and transcription, both in the nucleus. The protein translation and modification, the assembly proceeds in the rough endoplasmic reticulum, and maturation proceeds in the cytoplasm.

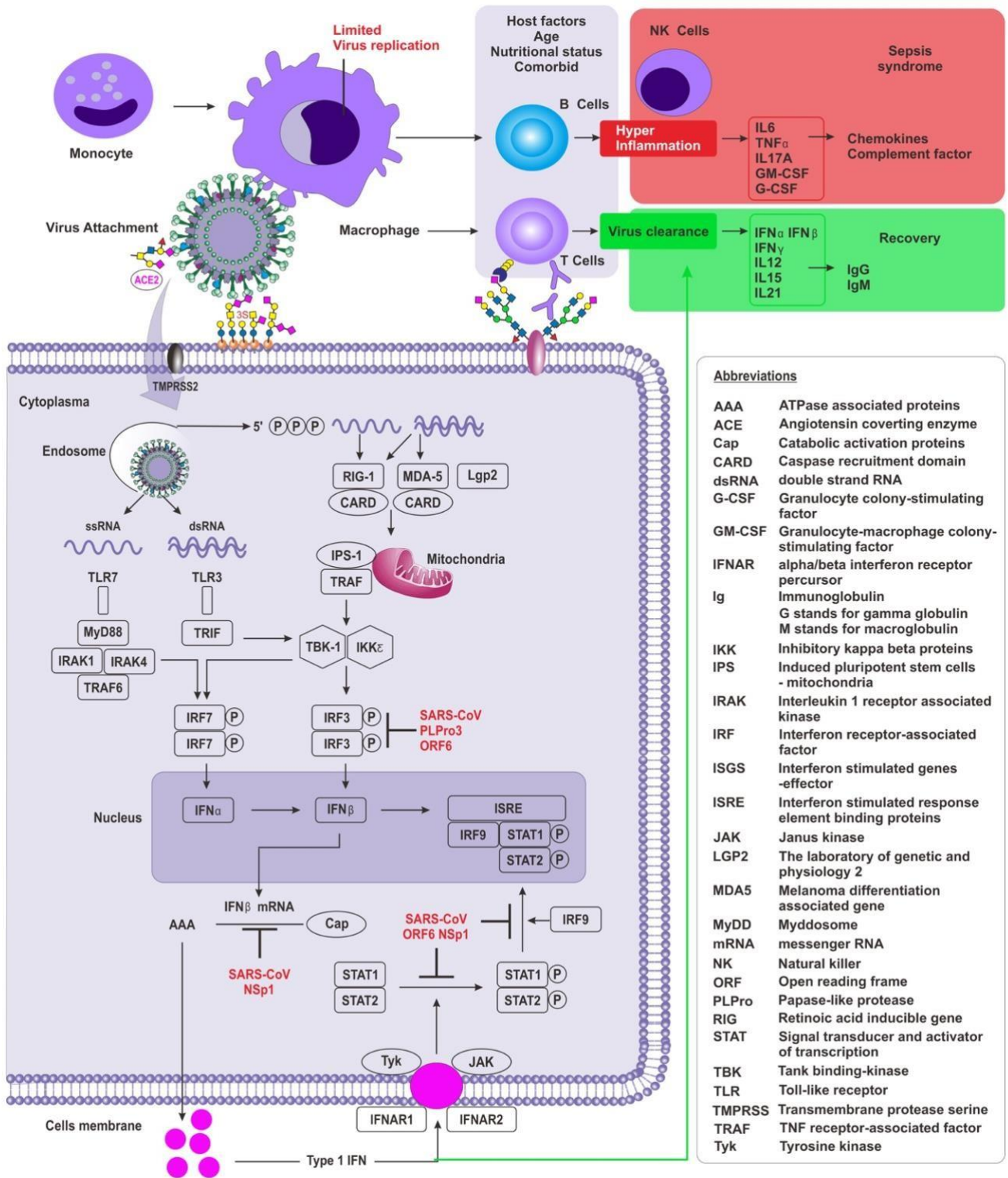


Figure 4. The molecular aspect of SARS-CoV infected cell. The viruses' characteristics included limited replication within the macrophage, blocking the IFN pathways within the cytoplasm that negatively modulated the immune system. Numerous molecular involved as the leading player in the orchestra referred to highly energy consuming.

This is very important because, to the knowledge, we do know that the Coronaviruses follow the general rules that can only infect cells to which they can bind, and whether the cells support the particular virus (namely tropism). If only we back to the basic knowledge that the ACE distributed in the glycocalyx – a brush border of epithelial cells – that predominant in endothelium in addition to intestinal mucosa, then the tropism should be focused on the endothelium – including blood brain barrier – as the starting point of an exaggerative systemic inflammatory response ever known.<sup>27-29</sup> In contrast, current knowledge told us the

pathophysiology the entrance is to inhaled infectious droplet, sneezing and aerosol that leading to the thinking that the site of infection starts in the respiratory mucosa and leading to severe respiratory distress (ARDS), but the study showed that the expression in reported locations, including alveolar type II (AT2) cells, could not be confirmed.<sup>30</sup>

Distribution of TMPRSS. In addition to ACE distribution, TMPRSS distribution is another subject of mater.<sup>31</sup> The TMPRSS2 node is a potentially interesting region of the interactome, as it has been

experimentally demonstrated that inhibition of TMPRSS2 with a protease inhibitor inhibits SARS-CoV-2 replication.<sup>20,32</sup> To the data, we know that TMPRSS2 highly expressed in prostate, duodenum and small intestine and lung<sup>33</sup> mostly serve to maintain basic homeostasis rather than re-establish homeostasis after external challenges, such as tissue injury and infection.<sup>34</sup>

### **The cytokine storm, hyperinflammation, the exaggerative inflammatory response, and immunosuppression**

The inflammatory response following viral infection is a natural physiologic process the way the body to eliminate the virus, and in repairing the damaged tissues – which is constructive. Naturally, the response paralleled the degree of severity of the infection, virulence, and, indeed, the host defense. As the virulence high, or more damage to the tissue caused as the impact of this viral infection, the response becomes exaggerated – excessive,<sup>35,36</sup> or even uncontrolled – leading to tissues and organ damage, the phenomenon formerly called systemic inflammatory response syndrome (SIRS) proposed by Mr. Bone in 1995.<sup>37</sup>

The inflammatory response following Coronaviruses resembling an orchestra triggered as the spikes attached to the receptors.<sup>18</sup> The receptor, ACE2, which is calcium dependent- activates TMPRSS, the toll-like receptors (TLR2), nod-like receptor family, the innate immune cells<sup>38–40</sup> in particular macrophage<sup>41,42</sup> and T cells, simultaneously.<sup>38</sup> Further, it is known that the viruses may replicates within the macrophage.<sup>43,44</sup> This phenomenon is one of the characteristics of the Coronaviruses, indeed. The inflammasome receptors are activated. The inflammasomes which are innate immune system receptors and sensors that regulate the activation of caspase-1, induce and regulates the secretion of proinflammatory cytokines interleukin 1 beta and interleukin 8.<sup>45</sup> Currently known, inflammasome of host proteins had implicated in host inflammatory disorders.<sup>46–48</sup>

NLRP3, the most investigated inflammasome in viral infection is activated by particular perturbations in the cell rather than directly binding to the ligand. Several cellular events upstream of NLRP3 activation have been described including ion flux (potassium efflux); mitochondrial dysfunction, such as production of mitochondrial reactive oxygen species (ROS) and release of mitochondrial DNA or cardiolipin into the cytosol; and lysosomal damage, resulting in release of lysosomal proteases, including cathepsins. NLRP3 activation is tightly regulated at both the transcriptional and posttranscriptional levels in two steps: an initial signal that activates NF- $\kappa$ B to induce pro-IL-1 $\beta$  and NLRP3 mRNA expression (priming), followed by an NLRP3 activator, which induces inflammasome formation.<sup>45,49,50</sup>

After contact to the viruses, the activation of NF- $\kappa$ B in cytoplasm proceeds and followed by the release of the early mediators (interleukin and surface antigens), that, in turn, activates the interferons (IFNs), which is the first-line defense against pathogen generally depends on the innate immune response. During the immune response process, the pattern recognition receptors (PRRs), that limited by the germline, used to identify the molecular organizations conserved among classes of pathogens, particularly viral double-stranded RNA. The IFN, which is the host-encoded secreted proteins comprised of three types (1, 2, and 3), plays a role in the interplays: induction and regulation of the innate

and adaptive response to viral infection. Following the viral infections, the expression of IFN type 1 (IFN- $\alpha$  and IFN- $\beta$ ) take place as a pivotal innate immune response. This IFN type 1 directly inhibits viral replication and mediate cellular immune systems - innate and adaptive immune system - providing resistance to the virus that maintains a long-term immunity. In addition to IFN type 1, IFN type 3, namely IFN- $\lambda$  or IFNL, also play essential roles in immune activities to the virus. IFN type 3 (IFN- $\lambda$ 1, 2, and 3) discovered as interleukin (IL)-29, 28a, and 28b showed the immune activities as IFN type 1. A later discovered IFN- $\lambda$ 4, which is expressed by the individual's gene symbol and directly mediated by PRRs found to be independent for both TLR and RIG-I pathways, namely IFN-independent manner. The information showed the early events of innate immune activities that much complex than previously known and may explain how the host-virus struggle (figure 4).<sup>49,51</sup>

A condition may provoke more IFN releases leading to a state of the complex interplay of an inflammatory orchestra. Viral virulence, including viral load and host defense that dependent on the innate immune system, a decay in the immune system as found in those with comorbid as well as in extreme age leading to a disharmony. A predominant cytokine release (SIRS dominant, 'first hit' theory of dr. Bone) followed by the anti-inflammatory cytokine release (compensated anti-inflammatory response syndrome/CARS, 'second hit' theory of dr. Bone) may lead to the cytokine flood. Cytokine storm syndrome (CSS) is a terminology proposed by dr. Behrens for the underlying hemophagocytic lymphohistiocytosis.<sup>52</sup> In contrast, SIRS belongs to Bone, which focused on the course of inflammatory response<sup>37</sup> and currently attributed to hyperinflammation. What a name the condition the same and results in a poor outcome, called immunosuppression.<sup>53–56</sup>

A condition, both of hyperinflammation in association of cytokine release syndrome comprised of both pro-inflammatory and anti-inflammatory mediators consumes a lot of energy. In accordance with the mechanism of acute physiologic derangement requiring energy from a non-adaptive manner of gluconeogenesis in metabolic perspective. Hence, the energy crisis resulting protein breakdown that in turn lead to exhausted metabolism. Then, energy crisis allowing the host defense decreased in non-sloping curve results in systemic failures.

A review elucidates an unknown mechanism, the pathophysiology, and the course of this Coronaviruses infection learned from SARS-CoV and MERS's lesson with its distinctive characteristics. There are out of the box thinking after a review. First, questioning the route of transmission. To the tropism, the virus may contact and replicate in epithelium with glycocalyx and ACE2 receptor, which is distributed not just in epithelial mucosa of the respiratory system, but intestinal, duodenum, gallbladder, and lymphatic vessels.<sup>57</sup> These cells may have a potential role in the transmission. In addition, knowing the Coronaviruses retains for 72 hours in surfaces, including the surgical and medical devices.<sup>58,59</sup> Hence, it is not just via aerosol.<sup>60,61</sup> In particular, endothelium as the port de entrée, and or the character of transmission, and the development of SIRS. In the perspective of the hyperinflammatory response, endothelial dysfunction plays a critical role in the inflammatory reaction that fatal due to viral infection such as Dengue hemorrhagic fever,<sup>62</sup> and Ebola,<sup>63</sup> etc., of which the same order to Coronaviridae. Third, criticism of the description regarding the course of the disease. The mucosa of the

respiratory tract may (or may not) be the port de entrée. Still, the acute respiratory distress syndrome referred to the impact of the excessive inflammatory response that manifests in 7<sup>th</sup> to 8<sup>th</sup> after infection, rather than the direct impact. This respiratory distress is a severe manifestation of multiple organ failure found in sepsis syndrome. Any etiology even caused by musculoskeletal trauma of non-respiratory systems. Criticism should be of one consideration that does not harm anybody, including him-/herself, to this pandemic virus, but do not go along with those unclear.

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