REVIEW

CYTOKINE STORMS MAY UNDERLAY THE MOLECULAR BASIS OF COVID-19 SEVERITY IN INFECTED PATIENTS

*Aja P.M.[1, 3, 4], Awoke J.N.[1], Ani O.G.[1], Ekpono E.U.[1], Ale B.A.2, Igwenyi I.O.[1] Agu P.C.[1], Ogwoni H.A.[1], Aja L.[6], Alum E.U.[1], Awuchi C.G.[5] and Obeten K. E.[7,8]

[1]Department of Biochemistry, Faculty of Science, Ebonyi State University Nigeria.
[2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria Nsukka.
[3]Department of Biochemistry, Faculty of Biomedicals, Kampala International University, Uganda
[4]Department of Biochemistry, Faculty of Medicine, Mbarara University of Science and Technology, Uganda
[5]School of Natural and Applied Sciences, Kampala International University, Kampala, Uganda
[6]Department of Science Education, Ebonyi State University, Abakaliki, Nigeria.
[7]Department of Anatomy, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Uganda.
[8]Department of Anatomy and Forensic Science, Cross River State University of Technology, Okuku, Nigeria.

ABSTRACT

Background:

The world is currently battling with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that originated in Wuhan, China late December 2019 and has now become a global pandemic.

Objective:

From the available scientific evidence, attempt was made to elucidate the possible molecular basis that underlays the severity of this disease with the aim of unveiling a novel therapeutic target for the development of effective therapy against this menace.

Methods:

Systematic MEDLINE (Ovid) and other databases such as EMBASE were searched for published articles up to June 2020. The subject heading/keywords – "novel coronavirus", "2019 novel coronavirus", "2019-nCoV", "COVID-19", "SARS-CoV-2 and Cytokines" were used.

Results:

The investigation revealed that SARS-CoV-2 having distinct tropism of nasal mucosa, negotiates its way into the host's cells prominently through type II pneumocytes of the lungs using its SPIKE (S protein) recognition binding domain (RBD) on the host's angiotensin-converting enzyme 2 (ACE2) receptors. Inside the cells, they rapidly replicate, avoiding the type I interferons (IFN-1) signaling crucial to the initial viral control. The delayed IFN-1 marshaling results to the encroachment of the neutrophils, mononuclear macrophages as well as cytokines/chemokines to the site of infections well above the threshold resulting in conditions known as hyper-cytokineamia (cytokine storm). This so-called "cytokine storm" orchestrates and exacerbates systemic hyper-inflammation, which induces lung injury and subsequently acute respiratory distress syndrome (ARDS), multiple system organ failure (MSOF) and eventual death of the patients.

Conclusions:

Modulating the expression and exacerbation of cytokines such as IL-6 and IL-1β may be a novel therapeutic approach against late stage COVID-19.

Keywords: Cytokine storm, COVID-19, molecular basis, SARS-CoV-2

*Corresponding Author
Patrick M. Aja, Department of Biochemistry, Kampala International University, Uganda, aja.patrick@kiu.ac.ug

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INTRODUCTION

Humans are among the many species of animals that can be infected by coronaviruses (CoVs) causing acute and chronic respiratory, central nervous system, enteric, and hepatic diseases [1, 2]. CoVs are the largest known single-stranded RNA viruses [3]. The positive-sense RNA enclosed in a viral envelope encodes the requisite information required for the virus functionality [4, 5]. According to the International Committee on Taxonomy of Viruses (ICTV), CoVs belong to the family, subfamily, and order of Coronaviridae, Coronavirinae, and Nidovirales respectively [6]. Further phylogenetic and genomic considerations divided the subfamily into four genera – Alpha (α)-coronavirus, Beta (β)-coronavirus, Gamma (γ)-coronavirus and Delta (δ)-coronavirus [2, 3, 7].

Since the dawn of the 21st century, there has been a series of reported clusters of pneumonia and diarrhoea disease cases that have been linked to β-coronaviruses. There was first reported case of deadly pneumonia outbreak caused by β-coronavirus - severe acute respiratory syndrome coronavirus (SARS-CoV) in Guangdong Province of China, in late 2002 [8] which spread to about 30 countries in 5 different continents and led to approximately 800 deaths in total [9]. A decade later, another deadly β-coronavirus – Middle East respiratory syndrome coronavirus (MERS-CoV) surfaced in the Middle Eastern countries [9, 10], which reportedly has a fatality rate of about 35% and has been implicated in recurrent pneumonia outbreaks in humans [5].

Most recently, in late December 2019, healthcare facilities in Wuhan, Hubei province, China, witnessed abnormally clusters of pneumonia cases of which the cause was enigmatic, thus attracting enormous attention within China and across the globe [11 – 14]. The timely molecular analysis by Chinese scientists led to isolation and characterization of a novel coronavirus (CoV) by Jan 7, 2020, which fingered the virus as the causative agent in the Wuhan patients [13 – 15]. The Chinese scientists named this newly discovered virus Wuhan coronavirus or 2019 novel coronavirus (2019-nCov) [16]. However, on 11 February 2020, World Health Organization (WHO) officially named the disease as coronavirus disease 2019 (COVID-19) and Coronavirus Study Group (CSG) of the ICTV proposed the name - severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) for the new coronavirus. However, this name is yet to be officially approved [17, 18]. According to WHO COVID-19 dashboard, as of 11:51am CEST, 21 April 2021, there have been 142,238,073 confirmed cases and 3,032,124 deaths globally due to COVID-19 [19]. This explosive tendency observed prompted the WHO to declare COVID-19 a Public Health Emergency of International Concern (PHEIC), on the 31st of January 2020 [12, 19].

In addition to four previously known CoVs that have been implicated in mild respiratory symptoms - HCoV-229E and HCoV-NL63 (α-CoVs), and HCoV-HKU1 and HCoV-OC43 (β-CoVs) there are now seven CoVs

known to infect humans [17]. There are growing scientific evidence that COVID-19 is phylogenetically and genomically similar to SARS-CoV and MERS-CoV but more closely related to Bat SAR-like coronavirus hinting at its zoonotic origin – perhaps its transmission from bat to humans [3, 20].

2. The Virion Morphology and Genome

2.1 Morphology

CoVs derived their name from the corona- or crown-like appearance of the particle [21]. Generally, CoVs are enclosed in a viral envelop and are pleomorphic with a diameter varying between 80–125 nm evident in most recent cryo-electron microscopy and cryo-electron tomography [7 – 23]. The helical nucleocapsid observed inside the viral membrane is a complex of the genome RNA with the phosphorylated nucleocapsid (N) protein [1]. The viral lipid-bilayer membrane envelope, however, is composed of three crucial structural proteins - Spike (S), a glycoprotein that forms the club-shaped surface projections or peplomers on the virion surface, giving the particles their characteristic fringed appearance [24]. Also the transmembrane glycoprotein (M) spans the membrane three times and binds to the nucleocapsid thereby promoting membrane curvature [6] and small transmembrane protein (E), a highly hydrophobic protein which facilitates the assembly and release of the virus, and thus crucial for its pathogenesis [6, 25]

2.2 Genome

CoVs are composed of non-segmented, positive-sense single-stranded RNA (+ssRNA) of ~30kb, making it the largest known family of virus [6, 7]. The genome is capped at the 5′-end and polyadenylated at the 3′-tail [24]. The 5′ end of the genome consists of leader RNA, which is an untranslated region (UTR) of 65 to 98 nucleotides. These are also present at the 3′-end with 200 to 500 nucleotides that are required for RNA replication and transcription [24].

Ultimately, the CoV genome is organized thus - 5′-leader-UTR- replicase-S (Spike)-E (Envelope)-M (Membrane)-N (Nucleocapsid)-3′ UTR-poly (A) tail with accessory genes interspersed within the structural genes at the 3′ end of the genome [22, 24].

Genomes and sub genomes of CoVs contain at least 6 open reading frames (ORFs). The first ORF which is two overlapping ORFs (1a and 1b) collectively function as the RNA Dependent RNA Polymerase (RDRP) [24, 26]. They constitute two-third of the viral RNA and serves as the template for translating two polypeptide – pp1a and pp1ab [17, 23]. These polypeptides are cleaved and processed into mature 16 non-structural proteins (NSPs) by virally encoded chymotrypsin-like protease 3CLpro (3C-like proteases) and PLpro (papain-like protease). The NSPs are crucial in the viral transcription and replication during infection for the synthesis of new viral genetic materials [27, 28]. The other one-third of the viral RNA

which constitute the remaining part of the ORFs encodes the four structural protein – S, E, N, and M, together with various accessory proteins that are not implicated in the viral replication that do interfer with host's first line of defence [6, 17, 29].

3. The Virion Entry and Replication in the Host

3.1 Entry

CoVs can be spread through airborne droplets. Humans can be infected with the SARS-CoV-2 via close contact with the person exhibiting symptoms of infection with the virus and possibly via faecal shedding [30 – 32]. Though the initial site of infection of SARS-CoV-2 is still unknown, inside the human system, the virus has a great propensity for tissues that express surface receptor which has precise specificity for receptor binding domain (RDB) on the viral membrane [5]. Several studies have extensively shown that SARS-CoV 2 acting in the same mechanism as SARS-COV, recognizes a cellular protein, human angiotensin-converting enzyme 2 (hACE2) receptors on the cell membrane through which it invades the cells of the host organisms [33 – 36]. ACE2 is mostly expressed on the surface of epithelial cells of the lungs (the alveolar epithelial type II or the type 2 pneumocyte), small intestine (luminal surface of intestinal epithelial cells where it functions as a co-receptor for nutrient uptake) [32], kidney, heart [37] and the brain [38]. The RDB of the SARS-CoV-2 like other CoVs resides in the S protein, hence, the viral attachment, fusion, and entry are mediated by the S protein [39], which also serves as the main target of antibody [33, 36]. The S protein working as a fusogenic substance after binding to ACE2 undergoes a conformational change [7] and facilitates the fusion of the viral and cell membrane utilizing the endosomal pathway with phosphoinositides, pore channel and cathepsin L playing a critical role [5, 16, 33, 40]. The spike fusion peptide attached to the cell membrane is cleaved by the host proteases [36] , leading to the release of the viral nucleocapsid into the cell cytoplasm and its subsequent uncoating [26] .

3.2 Replication

In the cytosol of the host cell, the replicase-transcriptase proteins encoded in ORF1a and ORF1b of the viral genomic RNA (gRNA) are translated initially as two large polyproteins, pp1a and pp1ab and are processed into NSPs, using the Grna as the template [6]. NSPs then provokes the rearrangement of the cellular membrane to form double-membrane vesicles (DMVs), where RNA replication is believed to occur [7, 41, 42]. The replicase-transcriptase proteins, together with other viral proteins and,

possibly, cellular proteins, undergo co-translational proteolytic processing to form replication-transcription complexes (RTC) anchored to the DMV [7, 43]. The RTC then makes an intermediate negative-sense single-stranded antigenomic RNA from the full-length gRNA. This serves as a template which RTC uses to synthesize a set of 3′ co-terminal nested genomic mRNAs (sgRNAs) by discontinuous transcription and finally translated into relevant viral structural and accessory proteins [6, 16, 44]. The viral proteins so synthesized are assembled with the gRNA into virions in the Endoplasmic reticulum-Golgi intermediate complex (ERGIC). Following assemblage and maturation, the virion in the vesicles are released via exocytosis through the smooth-walled vesicles [7, 16, 29]. The viral S protein that escaped assemblage migrates to the surface of the cell membrane facilitating fusion of adjacent cells (infected and uninfected) leading to syncytia (mass, multinucleated cells) thereby propagating the virus spread in a host manoeuvring detection or neutralization by host's immune responses [22].

4. The Concept of the Cytokine Storm and Immunopathology of the SARS-CoV-2.

The study of Huang et al. [11] highlighted the severity of the COVID-19 establishing fever, cough, and myalgia or fatigue as the most common symptoms of the disease while sputum production, headache, haemoptysis, and diarrhoea as the less common symptoms. These conditions are so because the assembled and mature viruses released as the virus gains entrance into the cells, infect various host cells where they engage the innate immune system first. The innate immune system serves as the first line defense system against the invading infectious agents in humans, in this case SARS-CoV-2. Additionally, the SARS-CoV-2 possesses specific molecular pattern called Pathogen Associated Molecular Patterns (PAMP) that recognizes and binds to the Pattern Recognition Receptors (PRRs) like Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) on the cells of the innate immune system thereby occasioning immune responses [7, 45, 46]. This essentially induces the activation of at least one of the following pathways: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), interferon regulatory factor (IRF)-3 and 7, activator protein 1 (AP-1) and activating transcription factor (ATF)-2/jun, leading to induction of proinflammatory cytokines [7, 45, 47]. In effect, the transcription of IFN-stimulated genes (ISGs) in the nuclei is initiated provoking the production of type I interferons (IFN-1), IFN-α, and IFN-γ [45]. Ideally, the successful upregulation of this IFN-1 inhibits virus replication and its spread at the

initial stage [48].

Conversely, CoVs – SARS-CoV and MERS-CoV that are genetically similar to SARS-Cov-2 have been reported to suppress IFN-1 intervention by acquiring the requisite machinery that manoeuvres the IFN-1 signalling [48, 49] . Perhaps, this delayed IFN-1 signalling further compromises the immediate viral control and orchestrates the influx of hyper-inflammatory neutrophils and monocytes-macrophages resulting in elevated lung cytokine/chemokine levels. This is further followed by vascular leakage, and impaired virus-specific CD4+ and CD8+T cell responses and ultimately leading to fatal pneumonia or acute respiratory distress syndrome (ARDS) and eventually death of the infected patient [47, 48, 50, 51] . It is believed that the accumulated mononuclear macrophages are signalled via the IFN-α/βreceptors to produce more monocyte chemo-attractants (such as CCL2,CCL7, and CCL12), which recruit more mono-nuclear macrophages to the site of infection leading to exuberant pro-inflammatory cytokines (TNF, IL-6, IL1-β, and inducible nitric oxide synthase) hence, inflammation ensues leading to the severity of COVID-19 [23] . We propose that the accumulated effects of these cytokines now working in the negative leads to exacerbation and severity of COVID 19 especially at the late stage of infection.

Furthermore, Huang et al. [11], highlighted the hallmark of cytokine storm (hypercytokinemia) in COVID-19 severity as elevated levels of IL1B, IFNγ, IFN-γ-induced protein 10 (IP-10; CXCL10), and monocyte chemo-attractant protein 1 (MCP1) were observed in infected patients. In addition, they noted that granulocyte colony-stimulating factor (GCSF), IP-10, MCP-1 (CCL2), macrophage inflammatory protein 1A (MIP1A) - (CCL3), and TNFα levels in patients in an intensive care unit (ICU) were markedly elevated when compared to non-ICU patients. Interestingly, Liu et al. [52] recently reported hypercytokinemia in COVID-19 patients and implicated cytokines such as, IFN-α2,IFN-γ, IL1ra, IL2, 4, 7, 10, 12 and 17, chemokine IP-10, G-CSF, and M-CSF. More so, pro-inflammatory cytokines and some helper T cells (Th1, Th2, and Th17) were all found to be linearly associated with viral load and lung injury hence underlying the disease's severity in the patients especially at the late stage of the infection.

Therefore, it is evident from the above studies that SARS-CoV-2 likely employs a similar mechanism as observed in SARS-CoV and MERS-CoV. Perhaps, SARS-CoV-2 having suppressed the initial clearance effect of the innate immune system replicates rapidly and

are engulfed by the antigen-presenting cells (APC) mainly major histocompatibility complex-I (MHC-I). The particles are subsequently presented to the virus-specific CD4+T cells activating them to proliferate and differentiate into T-helper (Th1) cells leading to secretions of IL-6, interferon-gamma and granulocyte-macrophage colony-stimulating factor (GM-CSF) and other pro-inflammatory cytokines [53]. The GM-CSF and IL-6 are both implicated in COVID-19 severity due to cytokine storm as the GM-CSF activates monocytes releasing further IL-6 and other factors leading to ARDS, multiple system organ failure (MSOF) and eventual death in patients infected [53]. On the other hand, the viruses are presented to the virus-specific CD8+T cells by MHC-1 leading to their proliferation and differentiation and as such exerting its cellular cytotoxicity [53]. Moreover, the results of Xu et al. [54] revealed the over activation of T-lymphocytes partly accounting for the severe immune injury observed in COVID-19 patients and their eventual apoptosis leading to the collapse of the entire immune system and invasion of other pathogens.

The explicit and rapid viral replication in addition to cytokine storm further elicits the induction of apoptosis in lung epithelial and endothelial cells. Apoptosis of endothelial cells and epithelial cells damages the pulmonary micro vascular and alveolar epithelial cell barriers and causes vascular leakage and alveolar oedema, eventually leading to hypoxemia which are hallmarks of ARDS [23]. The hypoxemia that ensues leads to an array of systemic dysfunction evident in COVID-19 patients. It has been observed that even in patients who recovers from the pneumonia of COVID-19 that there is onset or the progression of cognitive deficits associated with behavioural abnormalities [38] . Steardo et al. [38] hypothesized that these observations resulted due to the assault and subsequent breakage of the blood-brain barrier (BBB) by the exacerbated cytokine storm, which further instigates uncontrolled and persistent neuro-inflammation resulting in damage to the hippocampus and cortical areas responsible for cognitive functions and behavioural attributes in such recovered patients. Studies have recommended the identification and treatment of hyper-inflammation (cytokine storm), perhaps using existing, and approved therapies, which have shown proven safety profiles to enhance the reduction of the current rising global mortality cases of COVID-19 [55].

a.Emerging Cytokine Storm-Based Therapeutic Approaches against COVID-19

Due to the rapid increase in research and advancement of knowledge in general understanding of COVID-19 pathology,

several therapeutic approaches are emerging to be deployed in tackling the menace [56, 57, 58, 59]. Few of these therapeutic approaches are geared towards addressing the pathology of cytokine storm in COVID19 whereas others target other aspects of the infection. One of such approach that targets cytokine storm is the use of immunotherapy. Polyclonal antibody by plasma therapy, polypeptide hormone for the maturation of T cells, immunoglobulins, ACE2 immunoadhesin, and a monoclonal antibody against the interleukin-6 are currently undergoing different stages of development for the therapeutic management of COVID-19 [15, 35, 56]. Although, these therapeutics are mostly in the experimental stages of drug development, however due to their success in treatment of SARS-CoV and MERS-CoV, there are hopes that they may work effectively for 2019-nCoV [32].

b. Immunotherapy

With the emerging understanding that, hyper-inflammation may be responsible for the severity of end-stage COVID19, it is imperative that immunosuppression be considered as therapeutic option to modulate this derangement [60, 61, 62, 63]. However, as obtained with rheumatoid arthritis, patients diagnosed with end-stage severe COVID-19 should firstly be screened for the occurrence of cytokine storm with the aid of various biomarkers of hyper-inflammation such as increasing ferritin, decreasing platelet counts, or erythrocyte sedimentation rate in addition to the Hidradenitis Suppurativa (HS) core of the patients [55]. Various immunotherapy approaches such as rapamycin, monoclonal antibodies working through various mechanisms of action have been proposed for the treatment of cytokine storm envisaged in end-stage COVID-19 [64, 65]. However, monoclonal antibodies are preferred due to their specificity, purity, low risk of blood-borne pathogen contamination, and safety compared to serum therapy and intravenous immunoglobulins preparations [65, 66].

Albeit, clinical investigations into the use of monoclonal antibodies for COVID-19 are limited and therefore requires caution in its usage on patients [67]. Moreover, a monoclonal antibody cocktail or the combination of different monoclonal antibodies that recognize different epitopes on the viral surface may increase the effectiveness of virus-neutralization [15, 68, 69]. Some monoclonal antibodies have been repurposed to target and mitigate specific cytokines such as IL-6, which contribute in the abnormal elevation of cytokines (cytokine storm) which is a major pathology of late stage COVID-19 [70, 71, 72]. Some of these include Siltuximab,

ruxolitinib and tocilizumab monoclonal antibodies (mAb), which target IL-6 and its receptor (IL6R) [73, 74, 75, 76]. Tocilizumab has received several investigations in the recent times and is currently on top of the list of these mAb that have been proposed for the treatment of COVID-19 [77].

Despite major progress in the development of monoclonal antibody-based passive immunotherapy for coronavirus infection, there is currently no marketed monoclonal antibody due to the laborious, expensive and time-consuming demand in the large-scale production of monoclonal antibodies for clinical application. More so, caution would be required in administration of monoclonal antibody based therapy due to individual differences in response to such new therapeutics [78]. Moreover, designing and developing advanced protein production platforms and expression systems is urgently needed to provide efficient monoclonal antibodies at an affordable cost in a short time for the use of wider population of the world. Efforts towards this direction should be encouraged. Other therapeutic approaches that could target cytokine storm in COVID19 include corticosteroids [6, 79], antimalarial (Artemisia, chloroquine and hydroxychloroquine) [80, 81, 82, 83].

Another non-therapeutic option is nutritional intervention [84]. Generally, nutritional intervention has proven to help in enhancing the recovery and human defence against infectious diseases [85, 86, 87]. Nutritional interventions especially the use of vitamins such as vitamin C, E and D and mineral elements such as Zinc have been reported to enhance the positive outcome of patients with severe COVID19 [88, 89, 90, 91, 92]. Proper intake of good diets are therefore encouraged especially during pandemic to help boost the immune system [84, 93, 94]. Moreover, how nutritional intervention enhances the pathology of cytokine storm in COVID-19 patients are currently not clear and hence would require further investigative studies to enhance our understanding.

5. Conclusion and Perspective

Based on the available scientific evidence, we have posited that cytokine storm is a strong factor to be considered in the pathology and severity of late stage COVID-19. We hypothesize that treatment options/strategies targeting the overexpression of these cytokines may offer possible therapeutic remedies against late stage SARS-CoV-2. The high transmissibility and severity of SARS-CoV-2 makes it a big threat to humanity. As our knowledge of this disease continues to increase through further research, every therapeutic strategies must be deployed to ensure the world

is able to curtail this menace in the shortest possible time.

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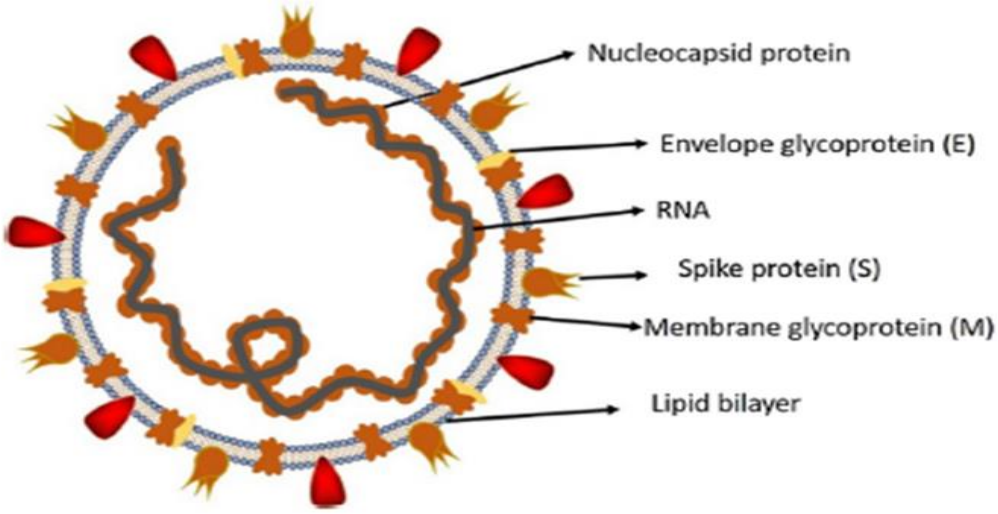

Figure 1 Structure of SARS-CoV-2 [16].

Figure 2 shows the Coronavirus Genome Organization [22].

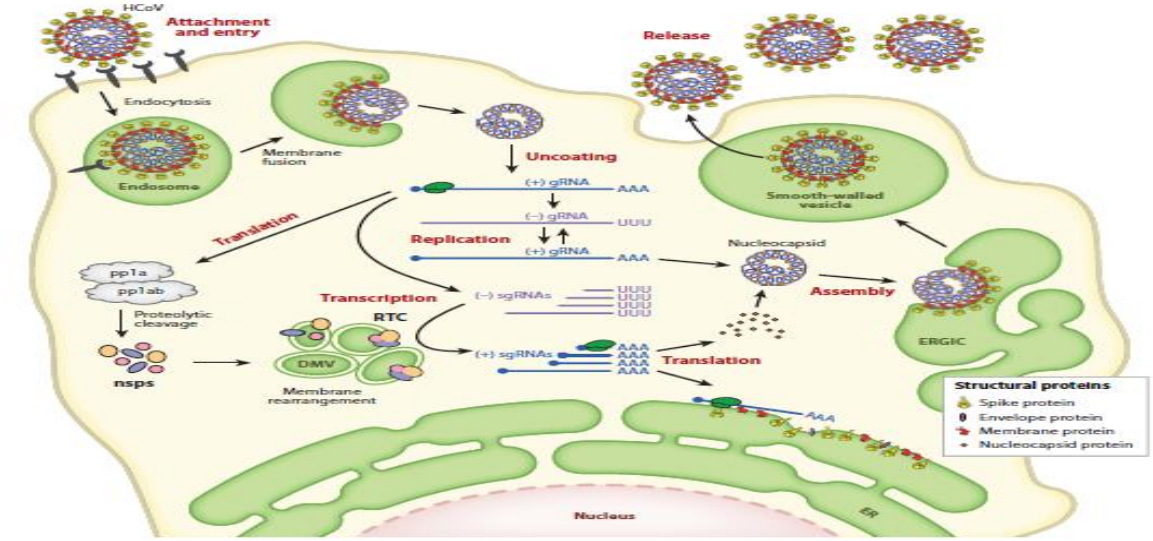

Figure 3 Replication cycle of SARS-CoV-2 [7]