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Review Article

Immune Responses During Human Coronavirus Infection: Suggestions for Future Studies

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Summary: Severe Acute Respiratory Human Coronavirus 2 (SARS-hCOV 2) infection which began in December 2019 has rapidly disseminated worldwide due to non-availability of anti-viral treatment or vaccine, no knowledge of virus-human interaction, lack of prognostic factors for stages of illness and ability of hCoV 2 to rapidly mutate and infect multiple cell types. Host inflammation and evasion of host immune responses by viruses are believed to play major roles in disease severity of human Corona viruses (hCoVs), thus uses of anti-inflammatory and immune-boosting agents apart from complete multi-disciplinary approach are suggested to combat the ranvaging SAR-hCOV 2 infection. This paper related the structural proteins and life cycle of CoV with host immune responses to CoV. This is to bring out gaps in knowledge for possible future researches.

Keywords*: Antibodies, Coronaviruses, Inflammation, Phagocytes, Vaccine.*

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INTRODUCTION

Coronaviruses are easily spreading, enveloped, nonsegmented, positive-sense single-stranded RNA virus with unelucidated mechanisms of pathogenesis and complex host immune response (Li *et al*., 2020). The causative agent of 2019–2020 ongoing coronavirus disease 2019 (COVID-19) was discovered in January 2020 to be a novel betacoronovirus of the same subgenus as Severe Acute Respiratory Syndrome Coronavirus 2 (WHO, 2020).

Since there are no antiviral treatments or vaccine available, efforts to prevent spread of hCoV includes confinement, screening, restricted body contact and use of nose/mouth mask (UNESCO 2020). Thus, researches on coronaviruses will continue to seek understanding of CoV-host interaction which will significantly improve ability to design vaccines and reduce disease burden. This paper highlights the role of immune responses during coronavirus infection.

Structural Proteins of hCoV

Coronaviruses are enveloped non-segmented, positive-sense and single stranded RNA viruses having four major structural proteins, as follows: Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) proteins. The S protein (approximately 150 kDa) makes up the distinctive spike structure on the surface of the virus and mediates attachment to the host receptor. In most, but not all, coronaviruses, S protein is cleaved by a host cell furin-like protease into S1 and S2 (Abraham *et al*., 1990). S1 makes up the large receptor-binding domain of the S protein while S2 forms the stalk of the spike molecule. The M protein is a small (approximately 25–30 kDa) most abundant structural protein in the virion. It has 3 transmembrane domains and is thought to give the virion its shape (Li *et al*., 2020). The E protein is small sized (approximately 8–12 kDa) highly divergent protein with ion channel activity required for pathogenesis. It facilitates assembly and release of the virus (Beniac *et al*., 2006). The N protein is present in the nucleocapsid capable of binding RNA, binds nsp3 a key component of the replicase complex, and the M protein (Chang *et al*., 2006).

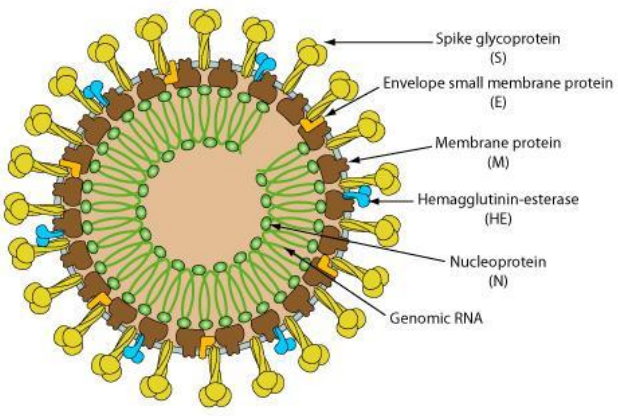

Figure 1:
Diagramatic Structure of a Corona Virus
(Source: Li et al., 2020)

A fifth structural protein, the hemagglutinin-esterase (HE), which binds sialic acids on surface glycoproteins contains acetyl-esterase activity is present in a subset of β-coronaviruses. The HE activities were proposed to enhance S protein-mediated cell entry and virus spread through the mucosa (Molenkamp and Spaan, 1997).

Life Cycle of Coronavirus

It takes 14 days from CoV infection to detection of simplest symptoms as a result of the following stages of CoV life cycle (attachment and entry into cells, replication and transcription of the sub-genomic RNAs, translation and assembly of the viral replicase complexes). Thus, blocking attachment or any of these stages of CoV cycle may be considered in treatment or preventive strategies of COVID-19. SARS-CoV is spread through infectious aerosols containing the virus by breathing, or when someone with the virus sneezes or coughs into their hands which contaminates objects other people touch or when un-infected person(s) breath in air close to an infected patient who sneezes or coughs. CoV from these sources attach to exposed surfaces especially epithelial lining of respiratory system. *A question is "why wont CoV penetrate through intact human skin, eye or mouth" The likely answer is the innate immune factors of these organs which require further investigations. However in the case of eyes, one may think of feeling the taste of an eye-drop in the mouth after dropped in the eye due the intimate link between the eye,nose and throat.*

The initial attachment of the virion to the host epithelial cell is initiated by interactions between the S protein and its receptor (Bosch *et al*., 2003). Many coronaviruses utilize peptidases for instance angiotensin-converting enzyme 2 (ACE2) as their cellular receptor which is commonly found on the surface of cells in the respiratory and digestive systems (Li *et al*., 2003). By binding ACE2, SARS-CoV leads to the downregulation of ACE2 expression (Hamming *et al*, 2004) and might therefore negate the protective effect of ACE2. Following receptor binding, the virus gain access to the host cell cytosol accomplished by acid-dependent proteolytic cleavage of S protein by a cathepsin or another protease. S protein cleavage occurs at two sites. The first cleavage separates the receptor binding domain (RBD) and fusion domains of the S protein (Belouzard *et al*., 2009) and the second cleavage exposes the fusion peptide that inserts into the membrane or within acidified endosomes. This allows for the mixing of viral and cellular membranes, resulting in fusion and ultimately release of the viral genome into the cytoplasm. The next step in the coronavirus lifecycle is the translation of replicase gene from the virion genomic RNA (Snijder *et al*., 2003; Baranov *et al*., 2005).

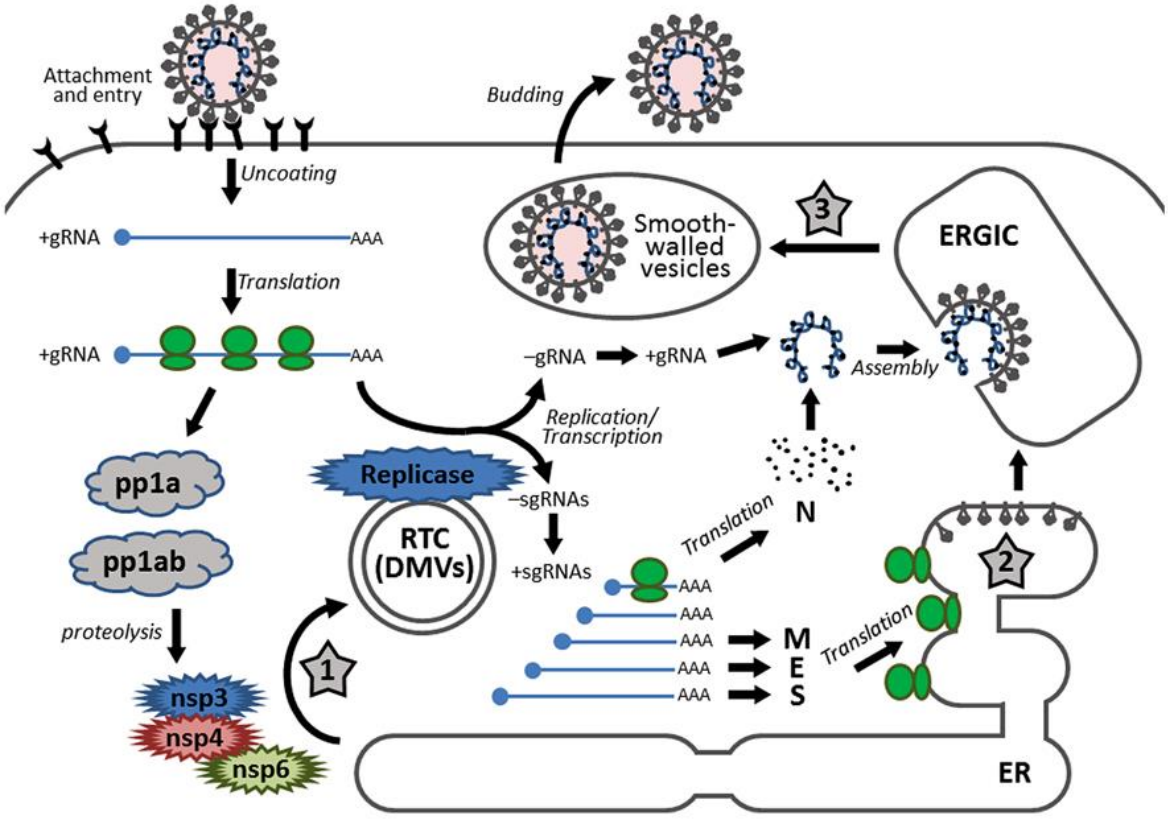

Figure 2:
Schematic Diagramatic of Life Cycle of Coronavirus.
(Source: Fung and Ding 2014).

Viral RNA synthesis produces both genomic and sub-genomic RNAs follow the translation and assembly of the viral replicase complexes.

After replication and subgenomic RNA synthesis, the viral structural proteins (S, E and M) are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) (Krijnse-Locker *et al*., 1994). There after, viral genomes encapsidated by N protein bud into membranes of the ERGIC containing viral structural proteins, forming mature virions (de Haan and Rottier 2005) After assembly, virions are transported to the cell surface in vesicles and released by exocytosis. *It is not known if the virions use the traditional pathway for transport of large cargo from the Golgi or if the virus has diverted a separate, unique pathway for its own exit.* In several coronaviruses, S protein that does not get assembled into virions transits to the cell surface where it mediates cell-cell fusion between infected cells and adjacent uninfected cells (Fehr and Perlman, 2015). This leads to the formation of giant multinucleated cells, which allows the virus to spread within an infected organism without being detected or neutralized by virus-specific antibodies.

Human Immune responses To CoV

Innate immune factors are chemical, cellular and mechanical/physical barriers that prevent entry and establishment of foreign materials (Edem and Arinola 2015). The nasal cilia filteration, mucus trapping action and muco-ciliary movements are assumed to be relevant during COVID-19, thus the advice to put on nose-mask (example of mechanical/physical barrier). It was also reported that CoV thrives on skin for few hours. *But the relevance of these innate factors requires further investigations.* Prevention of direct entry of CoV into alveoli of the lungs forms part of innate protection against entrance by CoV. However, the ability CoV to attach to the lung epithelial cell through S protein leads to formation of dsRNA of CoV during CoV replication in host cell cytoplasm (Bosch *et al*., 2003), thus S protein may form the basis for the development of a vaccine.

The host innate immune system detects dsRNA (a Pathogen-Associated Molecular Patterns, PAMPs) of CoV using Pattern Recognition Receptors (PRRs). This is followed by NF-kB activation which promotes the synthesis of type I IFNs and other proinflammatory cytokines (Schneider at al., 2014). The most studied members of the Type I family of interferons are the multiple IFNα isotypes and IFNβ. The mammalian types of IFN are designated IFN-α (alpha), IFN-β (beta), IFN-κ (kappa), IFN-δ (delta), IFN-ε (epsilon), IFN-τ (tau), IFN-ω (omega), and IFN-ζ (zeta, also known as limitin) (Cheung CY *et al*., 2005). Type I IFNs promote the release of antiviral proteins for the protection of uninfected cells, limit virus spread, and enhance macrophage phagocytosis, NKC-, T lymphocytes- and B lymphocytes- functions. Other pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF-β and MCP-1) produced by infected cells in response to CoV infection attract, retain and activate lymphocytes and leukocytes to the site of infection (Edem and Arinola 2015). *An evaluation of these factors during COVID-19 progression is desirable.*

Other PRRs involved in human immune response to CoV are Toll-like receptors TLR 2, TLR 4, mannose receptor, scavenger receptor; mannose-binding lectin and C-reactive protein (Li *et al*., 2020). This stage is associated with high fever, hypoxemia and progression to pneumonia-like symptoms despite progressive decline in virus titers (Peiris*et al*., 2003). However, hCoV evades host immune responses by reaching high titers very early after infection producing multiple proteins that inhibit IFN response and induction of T cells apoptosis (Channappanavar *et al*., 2016).

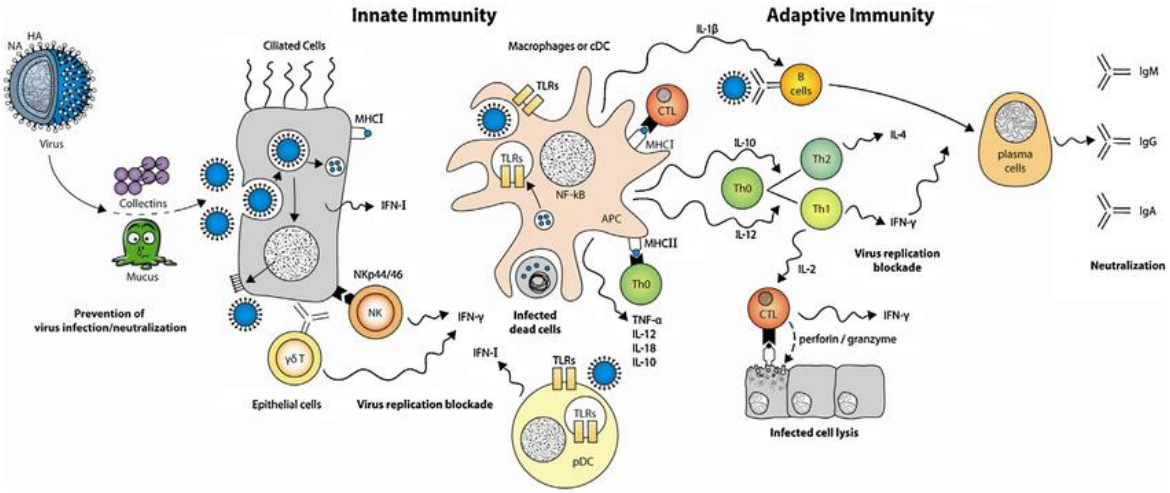

Figure 3:
Schematic Diagram of Human Immune Resposes to hCoV.
(Source: Li et al., 2020)

In the lungs, consequences of rapid virus replication and exuberant pro-inflammatory cytokine/chemokine responses are lung epithelial and endothelial cell apoptosis, compromised lung microvascular and alveolar epithelial cell barrier resulting in vascular leakage and alveolar edema resulting in hypoxia (Rodrigue-Gervais *et al*., 2014). *This may be one of the ways by which CoV leaked to blood circulation.* During this phase, there is progressive decline in virus titers and approximately 20% of patients progressed to ARDS which often resulted in death (van den Brand, 2014) proposed to have resulted from exuberant host production of inflammatory mediators including IL-6, IL-8, IL-1β, and GM-CSF, reactive oxygen species, and chemokines such as CCL2, CCL-5, IP-10, and CCL3 (Jiang Y *et al*., 2005; Zhao J *et al*., 2010; Drosten *et al*., 2013).

It is unknown whether or not people who have recovered, clear CoV infection and mounted strong immune response can get reinfected. However, two immune lung cells [alveolar macrophages (AM) and nerve/airway associated-macrophages (NAM)], four types of blood immune cells (antibody-secreting cells, follicular helper T cells, activated CD4+ T cells and CD8+ T cells) and immunoglobulin M and IgG antibodies that bound the CoV activate immunity to CoV. NAMs are distinct from other lung-resident macrophage subsets and highly express immunoregulatory functions under steady-state and inflammatory conditions (Ural *et al*., 2020). Apart from dissemination of CoV into circulation at the alveoli (Fehr and Perlman, 2015), macrophages present viral peptides to B- and T- lymphocytes leading to adaptive immunity.

A recent study reported increased antibody-secreting cells (ASCs), follicular helper T cells (T_{FH} cells), activated $CD4^+$ T cells and $CD8^+$ T cells and immunoglobulin M (IgM) and IgG antibodies that bound the COVID-19-causing coronavirus SARS-CoV-2 were detected in blood of COVID-19 patients before symptomatic recovery (Thevarajan *et al*., 2020). These immunological changes persisted for at least 7 days following full resolution of symptoms (Wu *et al*., 2007). T cells, CD4+ T cells, and CD8+ T cells particularly play a significant antiviral role by balancing the combat against pathogens (Arinola, 2003) and the risk of developing autoimmunity or overwhelming inflammation (Cecere *et al*., 2018). CD4+ T cells promote the production of virus-specific antibodies by activating T-dependent B cells. However, CD8+ T cells are cytotoxic and can kill viral infected cells. CD8+ T cells account for about 80% of total infiltrative inflammatory cells in the pulmonary interstitium in SARS-CoV-infected patients and play a vital role in clearing CoVs in infected cells and inducing immune injury (Maloir *et al*., 2018). The depletion of CD8+ T cells do not affect and delay viral replication at the time of infection with SARS-CoV (Channappanavar *et al*., 2014; Ng *et al*., 2016). Depletion of CD4+ T cells is associated with reduced pulmonary recruitment of lymphocytes and neutralizing antibody and cytokine production, resulting in a strong immune-mediated interstitial pneumonitis and delayed clearance of SARS-CoV from lungs (Chen *et al*., 2010). Additionally, T helper cells produce proinflammatory cytokines (IL-1, IL-6, IL-8, IL-21, TNF-β, and MCP-1) which recruit monocytes and neutrophils to the site of infection (Arinola *et al*., 2014). SARS-CoV-specific T cells have been screened in SARS convalescent patients. It was found that all detected memory T cell responses were directed at SARS-CoV structural proteins. Further, these reactions are found to last up to 11 years after infection (Li *et al*., 2020). Results of the research showed that the T cell response to S protein and other structural proteins (including the M and N proteins) is long-lasting and persistent. *This provides evidence for the design of the COVID-19 vaccine composed of viral structural proteins with long-term memory cell responses.*

Humoral immunity which is B-lymphocyte mediated controls the spread of infection (Olaniyi and Arinola, 2011). Reports show that humoral immunity is essential to control the persistent phase of CoV infection because antibodies isolated from patients who have survived Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection have been described (Niu *et al*, 2018a and b). In the peak period of viremia, 75% of the blood samples of patients diagnosed as SARS in the first 1 to 2 weeks before symptoms have virus RNA and circulating IgG. IgG production persists from acute SARS-CoV infection and during recovery (Wu *et al*., 2007). *This is an important subject that needs further study.*

Virus encoded proteins help them evade the detection of the Complement System, suggesting that complements are vital to the antiCoV response. C3a and C5a have potent proinflammatory properties and can recruit or activate neutrophils. Anti-C5a antibody shows protection against SARS-CoV infection activates Complement pathway (Gralinski *et al*., 2018). *These are pointers to the need to assess the levels of these humoral factors in CoV patients. Possiblity of developing monoclocal antibodies against structural or attachment strategy of CoV is worthy since* CoV uses its spike proteins as an adhesion factor to facilitate host entry through a special receptor. Human monoclonal antibody (m336) to receptor-binding region of MES coronavirus spike protein showed high neutralization activity to MES-CoV in vitro (Ying *et al*., 2014) and reduced the MES-CoV RNA titer of lung by 40 000 to 90 000 folds (Houser *et al*., 2016).

Dendritic cells (DCs) play a key role in innate immune and adaptive immune responses by effectively stimulating activation of T-lymphocytes

and B-lymphocytes (Arinola, 2003). DC precursor cells differentiate into DCs in the presence of GM-CSF, IL-4 and TNF-α (Guo *et al*., 2012). In addition, HIV-1 attenuates the major histocompatibility antigen I (MHC I) on the surface of DCs, thereby reducing the ability of DCs to present the viral antigens. HIV-1 infection enhances the expression of DC-specific intercellular adhesion molecule-3-grabbing nonintegrity (DC-SIGN), thus inhibiting CC chemokine receptor 7 (CCR7) and MHC-II, which are important receptors of DC homing (Fairman *et al*., 2012; Cardone *et al*., 2015). *These previous results indicate that CoV infection may interfere with the differentiation and function of DCs*.

Conclusion and suggestions for Further studies

CoVs infection is a periodical unpredictably threat to human life which spread rapidly. Of more concern is lack of approved vaccines or drugs for the treatment of CoV infections. To fulfill the pressing need of effective therapeutic measures, targeted immunotherapy and vaccine development, there is need for further elucidation of host immune responses during hCoV infection. This paper vindicates that inflammation is heightened during hCoV infection, therefore therapeutic interventions to control the inflammatory processes may be useful in the management of hCoV infection. More importantly, startegies directed at reducing the viral load at early stage of hCoV infection is strongly advocated.

Future studies should develop strategies that (a). Develops monoclonal antibodies against structural and non-structural proteins or blocks receptor sites. (b). Improves the understanding of pathways and mediators of inflammation during hCoV-infection in relation to the timing of therapeutic interventions. (c). Determines the roles of antioxidants and inflammatory agents in amelioration of symptoms during hCoV infection. (d). Determines host genetic factors which have been directly implicated in hCoV infection e.g characterise the polymorphisms of host Cathepsin, C3a and C5a or MHC Class 1 gene products (e). Creates forum for interdisciplinary collaborative researches between all professionals. (f). Usefulness or otherwise of surfactants and defensin, salivary and skin proteins during CoV infections.

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