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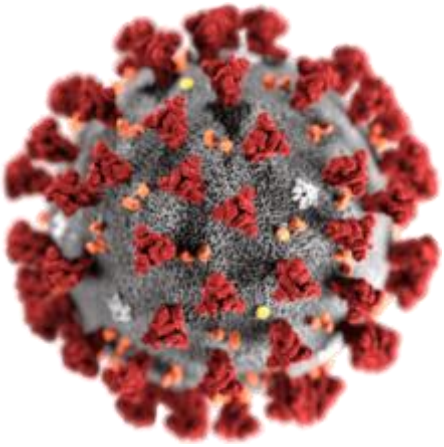

COVID-19 Science Report:

Pathogenesis and Host Immune Response to SARS-CoV-2

Jointly Developed by:

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Contents

Executive Summary

SARS-CoV-2 pathogenesis is triggered by viral infection, and amplified by dysfunctional immune responses. Severe disease takes two major forms: acute respiratory distress syndrome (ARDS) where lung damage occurs to an extent where the patient is unable to get sufficient oxygenation, and sepsis where uncontrolled inflammation leads to septic shock and systemic dysfunction of multiple organs. Thus, therapeutics targeting either viral infection or the ensuing dysfunctional immune responses are likely to be useful in combating COVID-19. In addition, adaptive T cell (cellular) and B cell (antibody) immune responses also play important roles in resolving disease, and observations of the mechanics of these adaptive immune responses from infected and convalescent patients provide clues as to how effective vaccines and monoclonal antibody therapeutics can be developed against COVID-19.

Objective of Report

This report aims to provide public health officials, MOH and policy makers with updates and insights on the pathogenesis, host-immune responses to the infection of COVID-19 with a comparison with previous coronavirus outbreaks (SARS, MERS) and how this translated or would translate to the different clinical presentation and management of the infection. Sources include publications, reports, opinions of overseas experts familiar with Singapore, and local research.

Pathophysiology of SARS-CoV-2

There are seven coronaviruses known to cause disease in humans. Four of them (229E, NL63, OC43, and HKU1) only infect the upper respiratory tract, and therefore typically cause minor colds. The other three (SARS-CoV, MERS-CoV, SARS-CoV-2) can replicate in the lower respiratory tract, and can thus cause pneumonia with associated higher disease and fatality rates. Infection with SARS-CoV-2 has been shown to reach a peak viral load 5-6 days after symptom onset, earlier than SARS-CoV which peaks at about 10 days after symptom onset [1-4]. Notably, SARS-CoV-2 in upper respiratory tract secretions has been found in asymptomatic individuals [1, 5] – this supports the notion that even asymptomatic or minimally symptomatic patients can potentially transmit COVID-19.

SARS-CoV-2 causes acute respiratory distress syndrome (ARDS). Based on preliminary studies of Chinese patients infected with SARS-CoV-2, the pathophysiology of this disease closely resembles that of SARS-CoV [6-8]. The pattern of increasing severity with age is broadly consistent with the epidemiology of SARS-CoV and MERS-CoV, though these underlying mechanisms remain unclear. Studies conducted during the last SARS-CoV outbreak indicated that lung injury found in patients with ARD resulted from aggressive inflammatory responses that were initiated by virus replication in the airways [9]. In other words, it is the host response to the virus (rather than the virus itself) that is the likely critical determinant that defines the degree of morbidity and pathology found in the patient. “The virus matters, but the host response

matters at least as much, and probably more," says Professor Stanley Perlman, a virologist and paediatric infectious disease specialist at the University of Iowa.

ARDS is characterised by symptoms of dyspnoea and hypoxaemia. Patients are also susceptible to secondary bacterial and fungal infection [6]. ARDS can lead directly to respiratory failure, which is the major cause of death (70% of death cases) [10]. Cardiac arrest can also occur (15% of death cases), potentially triggered by hypoxaemia. In some cases, the large amount of cytokines released by the immune system in response to the viral infection and/or secondary infections result in a severe immune overreaction – a cytokine storm, leading to symptoms of sepsis (28% of death cases). In some cases, the cytokine storm can cause multi-organ failure, including renal failure (4% of death cases). In SARS-CoV infection, most patients who progressed to renal failure eventually died [11].

Target Cell Infection and potential Interventions

The first step in a viral infection is the virus binding to a host cell through a target receptor on the host cell. Previous studies on SARS-CoV indicated that the virus predominantly infects airway epithelial cells, alveolar epithelial cells, vascular endothelial cells, type II pneumocytes, and macrophages in the lung, through the angiotensin convertase enzyme 2 (ACE2) host target receptor [12-14]. Given that SARS-CoV-2 uses the same entry receptor, it is likely that these cell subsets are also implicated in the current outbreak [15, 16]. SARS-CoV infection induces the down-regulation of ACE2 in the lung cells. This may be important for disease progression because the loss of pulmonary ACE2 function is associated with acute lung injury [17-20]; the reduction in ACE2 function can cause dysfunction of the renin-angiotensin system (RAS) which is a hormone system that regulates blood pressure and causes inflammation and vascular permeability in the airways.

Of interest is the fact that the ACE2 gene is located on the X-chromosome. Given the difference in the fatality rate between males (2.8%) and females (1.7%)[21], it is possible that specific gene variants of the receptor confer greater susceptibility. However, this requires further research for confirmation as differential immune promoting versus immune suppressing activities have also been linked to oestrogen versus testosterone. Another interesting interaction is found with nicotine – nicotine exposure regulates that RAS system that influences the expression of ACE2. This may go some way towards explaining the relative low prevalence of smokers in disease cohorts [22].

Both SARS-CoV and SARS-CoV-2 share a high degree of similarity in their genetic makeup [16]. In both viruses, the Spike (S) protein is expressed on the surface of the virus particles which gives them the appearance of crowns. This protein consists of two main subunits, the S1 and S2. The S1 subunit consists of an N-terminal domain and the receptor binding domain (RBD), of which the latter spans from amino acid sequence 270-510 of the SARS-CoV [23]. This RBD recognises and binds to angiotensin converting enzyme 2 (ACE2) as its host target receptor, which represents the critical starting point of the infection process [16]. The S2 subunit consists of a fusion peptide (FP) region and two heptad repeat regions HR1 and HR2 [24, 25]. After

binding to the receptor, the S1 subunit is cleaved away from the spike protein, exposing the fusion peptide that inserts into the host membrane. The S2 region then folds upon itself to bring HR1 and HR2 regions together. This folding pulls the host and virus membranes together and leads to fusion, which releases the viral package into the host cytoplasm.

There are several factors that may account for the increased infectivity of SARS-CoV-2 relative to SARS-CoV. The ACE2 receptor binding domains (RBDs) of SARS-CoV and SARS-CoV-2 share 72% identity in amino acid sequence, with highly similar ternary overall structures, but molecular modelling suggests that SARS-CoV2 RBD has a stronger interaction with ACE2 [26]. In addition, there is an extra genetic insertion unique to SAR-CoV-2 that translates to a furin-like cleavage site in the S protein [27]. This may in part account for the increased infectivity of SARS-CoV-2 relative to SARS-CoV. In addition to furin pre-cleavage, the serine protease 2 TMPRSS2 is also required to properly process the SARS-CoV-2 spike protein and facilitate host cell entry [28].

One avenue for therapeutics against SARS-CoV-2 is to block the host target ACE2 receptor or TMPRSS2 protease. There are several candidates which target them that are already approved for use in humans. Against ACE2, machine learning algorithms found that baricitinib, a drug approved for rheumatoid arthritis, could inhibit ACE2-mediated endocytosis [29]. Against TMPRSS2, nafamostat mesylate [30, 31] and camostat mesylate [28] are clinically proven inhibitors, and they are currently approved in several countries for other conditions. However, there are no clinical trials specifically testing these drugs against COVID-19 as of the time of writing. If this is validated, the rapid re-purposing of these drugs can provide a timely and effective response in the fight against Covid-19.

Inflammation and Dysfunctional Immune-Mediated Damage

While virus infection is the trigger for pathogenesis, a dysfunctional immune response is also a major player in the development of severe disease. SARS-CoV2 is a *cytopathic* virus [32] – it induces the death and injury of cells and tissues it infects as part of its natural replicative cycle. Thus, infection of epithelial cells in the airways and subsequent replication of the virus in these tissues likely causes high levels of virus-linked apoptosis/pyroptosis with associated vascular leakage (like SARS-CoV [33]). Pyroptosis is a pro-inflammatory form of programmed cell death that is strongly associated with other cytopathic viruses [34]. This is the most obvious trigger for the inflammatory response that follows initial infection [35]. In most people, it should induce a wave of local inflammation, recruiting immune cells from the blood into the infected site to eradicate the pathogen. These deal with the infection at the local site (in the lung), the immune response then recedes, and patients recover. However, in some people, a dysfunctional immune response occurs. The prolonged bioavailability of viral antigens combined with the continued release of pro-inflammatory factors from infected cells causes an uncontrolled immune response that triggers the massive proliferation of immune cells and the overproduction of cytokines. Thus, local inflammation can turn into a cytokine storm that mediates widespread inflammation of the lungs, which then has ripple effects across all organs of the body and potentially results in the myocardial damage and circulatory failure observed in some patients [36]. For reasons that are

poorly defined, the elderly are more likely to have a dysfunctional immune system that fails to successfully eradicate the pathogen.

It remains unclear whether ongoing virus infection itself is necessary to drive the ongoing damage. For both SARS-CoV and SARS-CoV-2, viral titres in respiratory tract samples could be on the decline even before the onset of symptoms of pneumonia [2, 4]. Nevertheless, a large retrospective cohort study showed that viral RNA was detectable in non-survivors up till the point of death, showing at least that failure of the immune response to clear the virus is a hallmark of poor outcome [37]. In addition, some studies on SARS-CoV have found that the virus may infect targets apart from lung cells, notably T lymphocytes [38], macrophages [39-41], and monocyte-derived dendritic cells [42]. This could contribute to the observed lymphopenia in patients [38]. Such infection might also further drive aberrant cytokine production, regardless of whether the virus is able to achieve productive infection [39-42]. The degree to which SARS-CoV-2 also targets these cells is currently unknown. Studying the precise drivers of immune dysfunction is necessary to inform the application of appropriate immunomodulatory treatments.

Researchers have discovered the identities of several cytokines involved in the destructive aberrant cytokine storm. Patients infected with SARS-CoV-2 have It has been observed that SARS-CoV-2 infection causes increased secretion of pro-inflammatory cytokines and chemokines IL-6, IL-1β, IFN-γ, MCP-1, IP-10, IL-4, and IL-10 in the blood of afflicted patients [8, 10] and this parallels observations made for SARS-CoV and MERS-CoV [43]. Moreover, there is an observed correlation between patients with severe disease who require hospitalisation in Intensive Care Units (ICUs) with higher blood plasma levels of IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1A, and TNF-α. One source of these cytokines could be macrophages: there exists a highly inflammatory monocyte-derived $FCN1^{+}$ macrophage population in the bronchoalveolar lavage fluid of patients with severe COVID-19 disease but not patients with mild COVID-19 disease [44]. These macrophages secrete inflammatory cytokines, including MCP-1, IP-10, and MIP-1A from the above-listed cytokines. While these studies represent important inroads, a full picture of the critical host immune factors that underlie the development of more severe inflammatory responses in some patients (versus others) remain poorly defined.

Several immunosuppressive therapies aimed at limiting immune-mediated damage in COVID-19 are at various phases of development. While corticosteroids were not recommended for SARS-CoV infection, trials are underway to determine if they are beneficial for COVID-19 [45]. IL-6 antagonists are also being tested – a clinical trial (188 persons) is underway to test the efficacy of Actemra (Tocilizumab) [46], and Kevzara (Sarilumab) being explored as well [47]. One novel therapy is Cytosorb, which acts to absorb a broad spectrum of cytokines, damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) to reduce their circulating levels [48]. An off-label use of an agent with immunomodulatory properties, thalidomide, has also been reported [49].

T Cell Responses Against SARS-CoV-2

Around one week after onset of symptoms, adaptive immune responses (both T and B cells) against SARS-CoV-2 become detectable. These adaptive immune responses are specific for the virus and/or virus-infected cells, and are highly potent. T cells play two major roles: CD8+ T cells directly attack and kill virus-infected cells, while CD4+ T cells are responsible for priming both B cells and CD8+ T cells as well as cytokine production for immune cell recruitment. The first autopsy that was reported on a patient who succumbed to COVID-19 infection revealed that accumulation of lymphocytes (likely T cells) was observed in the lungs, and the low levels of T cells that are found in the peripheral blood are hyperactive [50]. Together with reports of lymphopenia in patients [7], these suggest that T cells are attracted to the infected site to attempt to control the infection. Other than this study, there is a paucity of data in T cell responses in COVID-19 infection. Thus, data on the T cell response during coronavirus infection are mostly inferred from SARS-CoV and MERS-CoV.

T cell responses are severely impaired in patients during the acute phase of SARS-CoV infection [51-53]. Weakened T cell activation in these patients leads to increased duration of viral load in these patients [54]. One reason for this weakened T cell response is the altered dendritic cell (DC) maturation and migration to the lymphoid organs [55], since DCs are important for T cell activation. Infection with MERS-CoV resulted in slight increase of CD8, but not CD4+ T cells in patients, but their activation profiles remain dysfunctional [56]. Despite the impaired response, convalescent patients develop coronavirus-specific memory T cells, which can be found in patients after recovery for up to two years [57, 58]. This pro-inflammatory profile may be an aggravating factor during infection. However, CD4+ T cells have been hypothesised to prevent SARS-CoV disease occurrence, as depletion of these cells in mouse studies demonstrated slower virus clearance from the host and more severe lung inflammation [59]. Using a mouse-adapted strain of SARS-CoV, reactivation of the DCs resulted in higher numbers of virus-specific CD4+ and CD8+ T cells that accumulated in the lungs and improved survival [60, 61]. Also, transfer of SARS-CoV-specific CD4+ and CD8+ T cells into immunodeficient mice resulted in better protection when challenged with the mouse-adapted strain of SARS-CoV [61].

Coronavirus-specific T cells are clearly important in eliminating the virus and controlling disease development, and are important for vaccine strategies. However, whether T cell responses alone are capable of preventing infection in human settings remains to be investigated.

B Cell Responses and Antibodies Against SARS-CoV-2.

The principal protective role of B cells in immune responses to SARS-CoV and MERS-CoV is in the secretion of antibodies that neutralise the viruses. These antibodies bind specifically to receptors displayed on the surface of the virus and block its access to target receptors on host cells. The key determinants for neutralising antibodies have been defined. These are found on the viral Spike (S) glycoprotein [62]. There is evidence of an overlap in the antibody repertoire that targets SARS-CoV and SARS-CoV-2. Some human and rodent antibodies raised against antigens derived from SARS-CoV do cross-react with SARS-CoV-2 and these have been

proposed as potential therapeutic or prophylactic modalities that can be deployed to fight this disease [63]. Antibodies represent powerful candidate therapeutic agents that can bind to the virus, block its infectivity and effect its clearance from the circulation of infected patients.

However, the selection of therapeutic antibody candidates should include a consideration of potential unwanted side-effects. Previous studies in animal models have shown that in SARS-CoV infection, anti-S protein-neutralising antibodies (anti-S-IgG) can also cause severe lung injury by altering inflammatory responses [64]. Results from these animal studies also appear to mirror some of the clinical observations in SARS-CoV infected patients: the development of acute respiratory disease coincides with antiviral IgG seroconversion in 80% of patients [4]. In addition, it was found that patients who developed the anti-S-neutralising antibody faster had a higher chance of dying from the disease; it took an average of only 14.7 days for the deceased patients to reach their peak levels of neutralising antibody activities, as opposed to 20 days for the recovered patients [65]. It has been proposed that anti-S antibodies in patients can potentially augment the pro-inflammatory activity of Fc receptor expressing cells in the lung (such as alveolar macrophages). The binding of neutralising antibody-virus immune complexes (Virus-Nab complexes) to activatory FcRs on lung macrophages induces the expression of pro-inflammatory factors such as IL-8 and MCP-1 that add to the immune stimulatory milieu [66]. Such complexes may also activate the complement system leading to further unwanted inflammation [64]. Thus steps should be taken to engineer therapeutic antibodies into formulations that have little or no pro-inflammatory activity but retain their virus-binding/neutralising activity. There are well defined protein engineering approaches that can be exploited to achieve this aim [67].

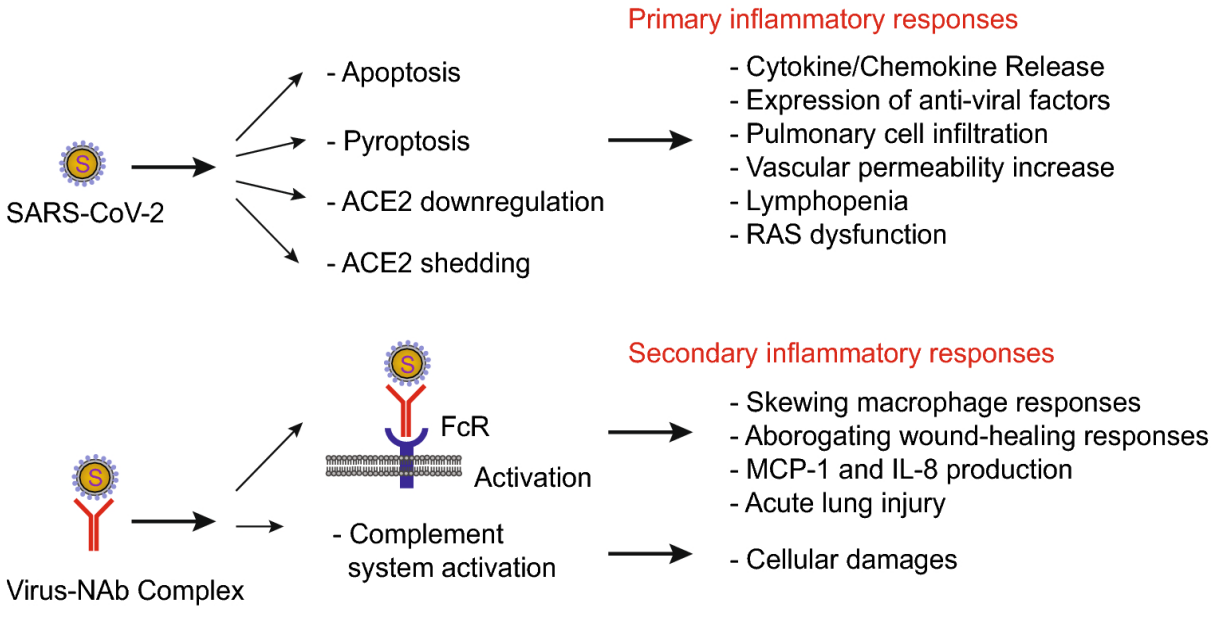

Figure 1. Possible mechanisms of SARS-CoV-2-mediated inflammatory responses. Based on previous studies of SARS-CoV, we separate the inflammatory responses in SARS-CoV-2 infection into primary and secondary responses. Primary inflammatory responses occur early after viral infection, prior to the appearance of neutralising antibodies (NAb). These responses

are mainly driven by active viral replication, viral-mediated ACE2 downregulation and shedding, and host anti-viral responses. Secondary inflammatory responses begin with the generation of adaptive immunity and NAb. The virus-NAb complex can also trigger FcR-mediated inflammatory responses and acute lung injury. Adapted from [68].

Conclusion

In conclusion, we present the various mechanisms that potentially underlie SARS-CoV-2 induced inflammation and pathology (Figure 1). Anti-viral therapies aimed exclusively at blocking or removing the virus must also factor in the virus-induced inflammatory processes that underlie morbidity and pathology. The association with immune dysfunction and disease presentation should also serve as a note of caution in vaccine development and evaluation. Vaccines that induce the wrong type of immune response have the potential to augment disease activity (rather than reduce it). Thus due consideration must be given to controlling or modulating the inflammatory response in treated patients alongside targeting the virus.

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