Primer on the Pathogenesis of Severe COVID-19: Part Two

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Abstract

In the following continuation article, the author will expand on how the mechanisms discussed in Part One capitalise on host characteristics to produce the organ specific damage seen in severe coronavirus disease (COVID-19), with specific reference to pulmonary and cardiac manifestations. Pneumonia is the primary manifestation of COVID-19; presentation varies from a mild, self-limiting pneumonitis to a fulminant and progressive respiratory failure. Features of disease severity tend to directly correlate with patient age, with elderly populations faring poorest. Advancing age parallels an increasingly pro-oxidative pulmonary milieu, a consequence of increasing host expression of phospholipase A2 Group IID. Virally induced expression of NADPH oxidase intensifies this pro-oxidant environment. The virus avails of the host response by exploiting caveolin-1 to assist in disabling host defenses and adopting a glycolytic metabolic pathway to self-replicate.

Although not a cardiotropic virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can induce arrhythmias, a myocarditis-like syndrome, and myocardial infarction. Monocyte activation as a consequence of a surge of cytokine expression is the driver of these processes. Induced expression of cluster of differentiation 147 (CD147) and TNF-α may also have a role. SARS-CoV-2 fluently harnesses the immune mechanisms of the host to its advantage, rendering it a formidable systemic pathogen. Future effective treatments are contingent upon improved aetiological understanding.

INTRODUCTION

In Part One of this narrative review examining the pathogenesis of severe coronavirus disease (COVID-19), the author addressed the mechanism by which the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus subverts the innate immune response while remaining largely invulnerable to its effector functions. Critical SARS/SARS-CoV-2 infection is notable for an apparent biphasic (dysregulated) immune response, initially characterised by muted interferon-ß (IFNß) production which becomes robust and persistent (mostly derived from plasmacytoid dendritic cells) with the advent of clinical features. This response is associated with

impaired T-cell and antibody responses.[1] The virus itself is ostensibly invulnerable to cellular antiviral mechanisms, impeding all of them with the notable exception of the protein kinase R (PKR) pathway, which is activated in response to the intercellular presence of replicating double stranded (ds)RNA.[2-4] It is this PKR activation which amplifies IFNß expression and also causes copious overexpression of IL-6. This overexpression of IL-6 in a T-cell depleted milieu results in the characteristic cytokine storm (T-cell response would normally keep such cytokine storm in check).[5,6] The NLRP3 inflammasome pathway, as opposed to the mutually exclusive PKR, is activated in paediatric patients and leads to consequent milder manifestations.

The author also discussed the overproduction of cluster of differentiation 147 (CD147), also known as extracellular matrix metalloproteinase inducer or basigin, and how this dovetails with viral entry into host cells. The viral spike protein binds to the angiotensin-converting enzyme 2 (ACE2), precipitating the overproduction of NADPH oxidase as a downstream consequence. In Part Two, the focus is shifted to the systemic mechanisms of host-viral interaction.

THE GENESIS OF PULMONARY MANIFESTATIONS OF COVID-19

Much speculation regarding the noncardiogenic pulmonary oedema seen in COVID-19 centred on its physiological similarity to high altitude pulmonary oedema and its unconventional acute respiratory distress syndrome characteristics. This arose from a loose thread of comparison, prefaced on the presence of hypoxaemia that was out of proportion to the reported dyspnoea, the extent of the radiographic opacities, and a higher than typical respiratory system compliance on a ventilator (with reduced work of breathing). High altitude pulmonary oedema is characterised by exaggerated hypoxic pulmonary vasoconstriction and elevated pulmonary arterial pressures (45–65 mmHg). The latter is substantially at odds with the COVID-19 pneumonia phenotype, and these early speculations have been the subject of firm rebuke.[7-8]

The spectrum of COVID-19 pneumonia spans two phases, referred to as types L and H. The L-type perhaps best characterises the earlier stages of the infection when there is a loss of hypoxic pulmonary vasoconstriction while pulmonary arterial pressures remain near normal. The lungs remain very compliant in spite of worsening hypoxia, characterising the 'happy hypoxic' phenotype. During this phase, radiologically apparent subpleural and parafissural ground-glass opacification depict the limited extent of early oedema. However, it is the loss of hypoxic pulmonary vasoconstriction which accentuates the apparent ventilation/perfusion mismatch (vascular perfusion of the nonaerated lung). This eventuates in the H-type pneumonia, which is highly oedematous, has high elastance (low compliance), and a high right-to-left shunt phenotype.[9]

The Pro-Oxidant Pulmonary Milieu

The key feature that underpins the pathogenesis of lung disease in SARS/COVID-19 is a rather hostile pro-oxidant pulmonary micro-environment. The Perlman group[6] identified secreted phospholipase A2 Group IID (PLA2G2D) as a phospholipase that is preferentially and abundantly expressed in dendritic cells and lymphoid organs. This expression was enhanced in the lungs of aged animals. The source of this increase appeared to be largely CD11+ cells (i.e., respiratory dendritic cells, monocytes, and neutrophils). PLA2G2D is a 'resolving serum PLA2' that ameliorates dendritic cell-committed innate and adaptive immune responses by mobilising anti-inflammatory lipid mediators. In cases of SARS infection, when oxidative stress is enhanced, PLA2G2D is responsible for the pulmonary mobilisation of prostaglandin D2, which, by acting on its anti-inflammatory receptor D-type prostanoid receptor 1, dampens dendritic cell migration and thereby T-cell-driven antivirus response. In elderly populations wherein PLA2G2D levels are already high, viral clearance is impaired. Highlighting that SARS incurred this effect through oxidative stress, this group were able to demonstrate substantially ameliorated survival rates in aged (Bagg Albino [BALB]/c) mice exposed to the virus who were treated with the antioxidant N-acetylcysteine.[10] Mitochondrial reactive oxygen species, elaborated by host-viral metabolism and host antiviral response, are a known principal cause of hypoxic pulmonary vasoconstriction.[11] However, this enhanced hypoxic pulmonary vasoconstriction is contrary

to what is observed in COVID-19 pneumonia. Hence, a pro-oxidant milieu *per se* is insufficient to explain the phenotype.

The Role of IFN1 and Protein Kinase R

As established in Part One of this review, a late surge of IFN1 after peak viraemia provokes an escalation in monocyte-macrophage activation with concomitant curtailment of T-cell activation and antibody responses. The clinical consequence of this is worsened alveolar oedema and a failure to clear the virus efficiently.[12,13] It is also the case that papain-like proteases, along with other viral and induced host mechanisms, preclude production of IFN1.[2,14] This is certainly advantageous to the virus early in the course of infection when exposure to IFN1 might otherwise prevent viral replication. IFN production has been highlighted as biphasic. Critically, the IFN peak trails, rather than matches, peak viraemia.[12] The most likely explanation for this switch to elevated IFN1 expression is the emergence of PKR because of the presence of replicating dsRNA. Indeed, PKR is regulatory and may be required for IFN mRNA integrity.[15]

PKR promotes inducible nitric oxide synthase (iNOS) production via interferon regulatory factor-1 and NF-κB. iNOS reduces the hypoxic pulmonary vasoconstriction by relaxing pulmonary vascular smooth muscle.[16] Furthermore, the nucleocapsid protein of SARS-CoV activates the expression of cyclooxygenase-2 (COX-2).[17] iNOS specifically binds to COX-2 and S-nitrosylates it, enhancing COX-2 catalytic activity and thereby accentuating the inflammatory cascade.[18] This contributes to hypoxic pulmonary vasodilatation and the intense inflammatory process observed in COVID-19 pneumonia.

The Role of Caveolin-1

Caveolae are plasma membrane invaginations, which form in the Type 1 squamous alveolar cells lining the lungs and play a role in mechanoprotection. Caveolin-1 (Cav-1) is a scaffolding protein and a major component of caveolae. Molecular modelling and simulation of SARS-CoV has confirmed eight caveolin-binding sites.[19] Cav-1 has been touted, in at least one confirmatory study, as having the ability to induce protein-mediating viral endocytosis.[20] To date, no studies have been performed to evaluate the specific immune-pathogenic role of Cav-1 in SARS/SARS-CoV-2 infections. As such, it may be informative to extrapolate some data from the influenza A virus. The M2 matrix protein of human influenza A was shown to interact with Cav-1, facilitating Cav-1 influence on viral replication. Indeed, dominant-negative Cav-1 mutants resulted in a decrease in virus titre in infected cells.[21]

Enhanced Cav-1 expression may constitute a normal, adaptive response in host pulmonary epithelium. Cav-1 is a negative regulator of NADPH oxidase-derived reactive oxygen species.[22] As described in Part One of this review, angiotensin II binding to its Type 1 receptor, as a consequence of the viral spike protein binding to the ACE2 receptor, mediated enhanced signalling through various subtypes of NADPH oxidase to produce reactive oxygen species.[23,24] Also, Cav-1 was shown to suppress COX2 expression.[25]

Caveolin-1 and viral manipulation of host metabolism

Dominant-negative Cav-1-mutant mice have been shown to exhibit increased mitochondrial reactive oxygen species. However, 2-deoxy-D-glucose attenuated this increase, implicating that Cav-1 is in control of glycolytic pathways. Metabolomic analyses revealed that Cav-1 knockdown led to a decrease in glycolytic intermediates, accompanied by an increase in fatty acids, suggesting a metabolic switch.[26] Notably, a recent proteomic analysis of SARS-CoV-2-infected cells revealed host pathway changes such that a glycolytic profile was adopted. Glycolysis was necessary for viral replication, in that blocking glycolysis with nontoxic concentrations of 2-deoxy-D-glucose prevented SARS-CoV–2 replication in Caco–2 cells (a cancer cell line devoid of Cav-1).[27]

Caveolin-1 and mechanotransduction in the pulmonary epithelium

Cav-1 is a key regulator of pulmonary endothelial barrier function and is required for mechanical stretch-induced lung inflammation and endothelial hyperpermeability, both *in vitro* and *in vivo*. As such, Cav-1 has been shown to be central to the pathogenesis of pulmonary oedema in ventilator-induced lung injury.[28] Cav-1 is a major contributor to pulmonary

compliance.[29] The presence of hypoxia causes reduced Cav-1 expression as a routine adaptation of lung epithelial cells, leading to disassembly of cholesterol domains/caveolae.[30] The paradoxical overabundance of Cav-1 in the hypoxic pulmonary microenvironment of COVID-19 pneumonia may account for the clinical observation of high lung compliance in severely hypoxic patients, the so-called 'happy-hypoxics'.

Lung mechanical stretch employs a series of adaptive cellular elements. Components of the Hippo pathway, the transcription factors Yes-associated protein/Tafazzin (YAP/TAZ), were previously identified as key downstream elements and mediators of mechanical cues.[31] When a cell is subjected to mechanical stretch, large tumour suppressor kinase 1/2 (LATS1/2), which binds YAP/TAZ in the cytoplasm, prevents YAP/TAZ translocation to the nucleus where the transcription factor can positively influence cell division and other processes such as the induction of Cav-1 transcription.[32] In the setting of mechanical stretch, the co-chaperone protein BCL2-associated athanogene 3 (BAG3), facilitates the autophagic degradation of mechanically damaged cytoskeleton components. BAG3 utilises its WW domain to bind the YAP/TAZ inhibitors LATS1/2 or AmotL1/2 and thereby promotes nuclear translocation of YAP/TAZ, as well as concomitant transcriptional activation of proteins involved in cell adhesion and extracellular matrix remodelling, including Cav-1.[31,33] Cav-1, in turn, positively regulates YAP transcription.[34] Notably, YAP negatively regulates IFNβ expression and antagonises innate antiviral immunity.[35] BAG3 is a stress-inducible host protein that is specifically required for efficient replication of SARS-CoV.[36] The method through which BAG3 accomplishes this is unknown but may be similar to some herpes viruses. Varicella-zoster virus redistributes BAG3 and its co-chaperones Hsp70 and Hsp90 into nuclear replication/transcription foci in infected cells to efficiently complete its replicative cycle.[37]

Caveolin-1 and pulmonary hypertension

It should be noted that Cav-1 also functions as a negative regulator of pulmonary hypertension by inhibiting endothelial NOS (eNOS) uncoupling.[38] This may explain the near normal pulmonary arterial pressures seen in the context of apparently severe COVID-19 pneumonia. Murata et al.[39] reported that chronic hypoxia (10% oxygen levels for 1 week) induced the atrophy of endothelial cells, impaired calcium ion increase, and led to tight coupling between eNOS and Cav-1. This, in turn, blocked several eNOS-activation processes in the rat pulmonary arterial endothelium.[39] Furthermore, similar changes, such as atrophy of endothelial cells and condensation of eNOS into caveolae, were observed in hypoxic organ-cultured pulmonary endothelium.[40] This group demonstrated that dexamethasone could block this hypoxia-induced endothelial dysfunction in organ-cultured pulmonary arteries.[41] Dexamethasone disrupts glycolysis, likely through promotion of phosphofructokinase-1, which is likely to impede the host metabolism necessary for viral proliferation.[42] Dexamethasone may also exert possible beneficial effects through induction of claudin-4, which is protective of the alveolar epithelial barrier;[43] it may also suppress the virally-induced expression of NADPH-oxidase.[44] However, dexamethasone appears to promote the induction of Cav-1 in pulmonary epithelial cells, thereby exposing a limitation of its utility in COVID-19 pneumonia.[45]

As alluded to in Part One of this review, the production of haem oxygenase-1 also protects against oxidative lung injury. However, this protection is thwarted by Cav-1 expression through competitive inhibition.[46-48]

Although direct evidence for the role of Cav-1 has not been empirically demonstrated, there is a high likelihood that it plays a critical role in SARS-CoV-2 immunopathogenesis.

THE GENESIS OF CARDIAC MANIFESTATIONS OF COVID-19

For most patients, COVID-19 pneumonia constitutes the earliest and most virulent clinical manifestation of the condition. However, early in the course of the pandemic, it became apparent that cardio-specific manifestations such as myocarditis and arrhythmia constituted a major source of morbidity and mortality.[49,50] Although cardiovascular complications such as hypotension and tachycardia were common in patients with SARS, they were usually self-limiting. Bradycardia and cardiomegaly were less common, while cardiac arrhythmia was rare.[51,52]

During the Toronto, Canada, SARS outbreak in 2003, however, SARS-CoV viral RNA was detected in 35% of autopsied hearts.[53]

A clear departure from SARS infection has been witnessed with the high morbidity of cardiac manifestations of COVID-19. COVID-19, as a thromboinflammatory condition, may induce myocardial infarction.[54] Also, given the virulence of the pulmonary/systemic features of the condition, it is possible that those with an underlying cardiac condition might be induced to transition into cardiac failure.[55] These are rational assumptions; however, typically the cardiac manifestations of COVID-19 appear to trail behind the peak of the inflammatory processes when viral titres are in decline. This discussion will focus on the myocarditis-like syndrome because acute viral myocarditis can be fulminant and may sometimes mimic acute myocardial infarction and cardiac failure, as well as cause arrhythmias.[56]

Pathology of the COVID-19 Myocarditis-Like Syndrome

Myocarditis is an inflammatory disease of the myocardium and is diagnosed by established histological, immunological, and immunohistochemical criteria.[57,58] Histopathological reporting of endomyocardial biopsy and cardiac autopsy findings has been sparse and somewhat inconsistent during the pandemic so far.[59-61] The diagnosis has instead been prefaced on surrogate markers such as a raised troponin, electrocardiogram, and transthoracic echocardiogram changes.[62,63] However, no significant brisk lymphocytic inflammatory infiltrate, consistent with the typical pattern of viral myocarditis, has been apparent in any specimens examined so far.

Histopathological analysis of an endomyocardial biopsy specimen from a patient with COVID-19 myocarditis revealed sparse monocytic inflammatory infiltrates with significant interstitial oedema and limited focal necrosis.[60] One study even found endothelial cell infection in several organs, including the heart vessels, with no sign of lymphocytic myocarditis.[61]

Investigations have found that cardiac myocytes show nonspecific features consisting of focal myofibrillar lysis and lipid droplets. Viral particles in myocytes and endothelia were not observed, and small intramural vessels were free from vasculitis and thrombosis. Endomyocardial biopsies did not show significant myocyte hypertrophy or nuclear changes; interstitial fibrosis was minimal, focal, and mainly perivascular.[59] It should be noted that the sensitivity of endomyocardial biopsy for lymphocytic myocarditis is variable and depends on the duration of illness. In subjects with symptom duration of <4 weeks, up to 89% may have lymphocytic myocarditis,[64] but generally this is lower, between 10% and 35%, depending on the 'gold standard' used.[65-67]

There has been speculation that ACE2 expression is a likely reason for myocardial involvement in COVID-19.[68-70] This is questionable, given that no SARS-CoV-2 genome has been detected within myocardial cells, at least so far. Also, although the myocardium does express ACE2, its expression of TMPRSS2, which is necessary for viral entry, is negligible.[71-73] Further speculation around cardiac pericyte involvement inducing focal myocyte necrosis may be flawed, given that pericyte expression of TMPRSS2 is also modest. There is very limited evidence to suggest that SARS-CoV-2 is a cardiotropic virus.[68,74]

The Contribution of Monocytes

The specific role of monocytes in myocardial inflammation in COVID-19 infection may be caused by viral spike protein glycans binding to host monocyte lectins.[75,76] Alternatively, the macrophages seen in biopsy and autopsy specimens may be an enhanced population of cardiac resident macrophages. The development of advanced gene fate-mapping techniques has shown that, in the steady-state, two resident cardiac macrophage subsets are present: MHC-II^{low} CCR2- and MHC-II^{high} CCR2- cells. Under inflammatory conditions, a third macrophage subtype can be found in the heart and is classified as MHC-II^{high} CCR2+ cells. Originating completely from bone marrow-derived monocytes, the population of this macrophage subtype is recruited during inflammation and ultimately replaces embryo-derived cardiac macrophages because their proliferative properties diminish with age. Circulating CCR2+ monocytes interact with the CCR2 ligand, monocyte chemoattractant protein-1 (MCP-1/CCL2), which is a chemotactic cytokine that potentiates macrophage recruitment and invasion.[77,78] It should be noted that in addition to inflammatory (and reparative) processes, macrophages are key mediators of

electrical conduction in the heart and as such, their derangement by an inflammatory process may trigger arrhythmias.[79] MCP-1 is produced in excess as a consequence of the SARS-CoV-2-induced overproduction of PKR, both directly and through IL-6 overproduction, and by PKR-endoplasmic reticulum kinase which can promote MCP-1 production through activating transcription factor-4.[80-83] Bindarit is a safe inhibitor of MCP-1 and may be a useful to attenuate macrophage inflammatory activity in the context of cardiac disease seen in COVID-19.[84,85]

There is only sparse evidence for a substantive contribution to myocardial inflammation by infected T cells. It has been demonstrated that T cells may become infected by SARS-CoV-2, but virions are unable to propagate within T cells. The virus was demonstrated to enter T cells via the CD147 integral membrane receptor.[86]

The Contribution of CD147

In Part One of this review, the topic of IL-6 overproduction was addressed. One downstream consequence of this was the resultant induction of CD147.[87,88] CD147 has been shown to function as a signalling receptor for extracellular cyclophilins A and B and to mediate chemotactic activity of cyclophilins towards a variety of immune cells.[89] In this capacity, it has been demonstrated that in coxsackievirus B3 myocarditis, cyclophilin A/CD147 induces chemotaxis of T cells and monocytes/macrophages through matrix-metalloprotein-9 (MMP-9) induction. MMP-9 is required for adequate lymphocyte migration under inflammatory conditions and is thought to directly 'remodel' myocardium. When cyclophilin A is deleted, or in the presence of an antibody directed against CD147, there is reduced lymphocyte infiltration and myocardial infarct size, as well as preserved left ventricular function, in mice upon ischaemia and reperfusion injury.[90,91] Similar results have been extrapolated to humans with congestive heart failure, wherein remodelling secondary to MMP-9 plays a major role.[92] Thus, while it is not a specific viral effect, the induction of CD147 may be critical to the clinical manifestation of myocarditis.

Intriguingly, Cav-1 has been shown to negatively regulate CD147 clustering; however, this effect was most apparent at 4 °C and absent at body temperature.[93] It should be noted that azithromycin may be able to disrupt CD147 ligand interactions, establishing some of its basis in the treatment of malaria. However, given the inherent risks of QT prolongation in the context of an already diseased heart, caution would be advised with azithromycin in COVID-19. A preferable alternative might be meplazumab, an anti-CD147 humanised antibody, currently on orphan drug designation and approval by the U.S. Food and Drug Administration (FDA) for the treatment of malaria.[94]

The Putative Role of TNF-α

TNF-α, which may be overexpressed as a consequence of COVID-19-induced cytokine storm, aggravates myocarditis, and the neutralisation of TNF-α by antibodies or soluble receptors attenuates viral myocarditis.[95-98] Although the exact mechanism through which TNF-α contributes to decreased contractile performance in myocarditis is not well known, a number of studies have emphasised the negative impact mediated by NO.[99-101] It appears that IL-1α may co-operate with TNF-α to potentiate its effects in viral myocarditis.[97] In prolonged COVID-19 illness, the quantity of replicating virus starts to diminish and the relative burden of PKR expression is reduced. The protracted inflammatory process leads to some release of neutrophil serine proteases, such as neutrophil elastase, which processes pro-IL-1α to IL-1α independently of caspase (i.e., independently of inflammasome activation).[102-104] Furthermore, the diminished PKR signalling begins to surrender its inhibition of the NLRP3 pathway, which is triggered by the SARS viroporins ORF3a and ORF8b through cell membrane permeabilisation.[105,106]

The Role of the Renin-Angiotens in System

Because of viral spike proteins binding to ACE2 receptors, much has been made of the resultant effects on the renin-angiotensin system. There is a sustained boost in renin and angiotensin II expression in COVID-19.[107,108] These contribute to an endocrine backdrop that is supportive of the maintenance of the myocarditis-like condition. In this regard, angiotensin II receptor antagonists have been demonstrated to reduce myocardial damage in animal models of myocarditis.[109,110]

CANDIDATE PHARMACOTHERAPY

In establishing a platform for future treatments, real consideration needs to be given to antioxidation as a means of ameliorating the pro-oxidant milieu of the lungs in COVID-19 pneumonia. Lead candidates here would include N-acetylcysteine and the flavonoid quercetin. Quercetin has many desirable properties, including its lipid solubility in the surfactant rich environment of the lung, and it may have some efficacy in specifically blocking viral entry into cells and blocking airway epithelial cell chemokine expression, including MCP-1.[111-114] It may not be desirable to pharmacologically reduce Cav-1 expression because of the possible cardiac side effects.[115] The RECOVERY Trial, based in Oxford University, Oxford, UK, has found specific utility for dexamethasone as a significant treatment for severe COVID-19 pneumonia.[116] As discussed in Part One, elevated PKR was suggested to be amenable to remediation.[117] As relatively novel agents, bindarit and/or meplazumab may have a role in preventing/treating the myocardial injury seen in COVID-19.

CONCLUSION

In conclusion, the preceding narrative review has offered an overview of the pathogenesis of severe COVID-19 infection, as borne out through pulmonary and cardiac effects. The author acknowledges that all of the information synthesised in this review does need to be subjected to rigorous evaluation and investigation.

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