Understanding COVID-19 from innate immune perspective and develop possible therapeutic interventions.

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Summary:

The present hypothesis is envisioned in trying to develop therapeutic intervention measures against SARS-CoV2, causative agent for COVID-19. Studying the innate immune response to SARS-CoV2 gives us a proper understanding of the pathogenesis phenomena by the virus. Possibly, SARS-CoV2 is suppressing the host interferon system and over stimulating the pro-inflammatory cytokines, leading to cytokine storms culminating in tissue damages and other ensuing pathophysiology. Hence, combinatorial approaches using repurposed anti-inflammatory and antiviral drugs has been thought of as good intervening measures against COVID-19. Anti-inflammatory for blocking the IL1 β production and antivirals (IFNs or viral polymerase inhibition) for curbing viral load would be perhaps a good combination.

Introduction:

On Dec 2019, a new infectious respiratory disease (COVID 19) emerged in Wuhan province, China. The etiological agent was identified to be a novel coronavirus, SARS-CoV2, which is closely related to previously known SARS-CoV. Despite drastic containment measures the virus wreaked havoc causing a pandemic, infecting about 27,00,000 people around the world with more than 190,000 fatalities. Coronaviruses (CoV) are positive single stranded enveloped RNA viruses. As the virus is single stranded RNA virus, its mutation frequency is expected to be much higher than the DNA viruses. SARS-CoV2 are known to bind to ACE2 receptors of target epithelial cells of lung, intestine, blood vessels and initiate infection¹. As of now there are no specific drug known to effectively manage the virus, hence there is an unmet need on pressing exigency.

Innate immune response to SARS-CoV2:

The fact that a fraction of the population infected with SARS-CoV2 are asymptomatic, with mild symptoms, and others severely symptomatic raised the question of involvement of innate immune system in understanding the pathogenesis phenomena properly. More so, a proper dissection in this direction would perhaps lead to design strategies for possible intervention against COVID-19.

Following pathogen (virus/bacteria/fungi) sensing by the host pattern-recognition receptors (PRRs), the host induces the production of molecules limiting the growth of pathogens and pro-inflammatory cytokines. The PRRs include Toll like receptors, RIG-I like receptors and

NOD like receptors. The pro-inflammatory cytokines, when produced in controlled fashion are also capable of protective action against pathogens by limiting their growth. However, the deregulated production of the pro-inflammatory cytokines contributes to inflammation, tissue damages and further ensuing pathophysiology². It's the fine tuning between the molecules restricting the growth of pathogens and pro-inflammatory molecules which maintains the balance and helps in running the host system effectively.

The interferons (IFNs) provides the first line of innate defence against viral infection in vertebrates. Upon viral infection, host PRRs sense viral components (RNA) and initiate a signaling cascade resulting in the secretion of IFNs. These IFNs further leads to the transcriptional induction of many IFN-stimulated genes (ISGs), out of which many have antiviral activity specifically against RNA viruses (OAS1, MX1, ISG1, etc). Type 1 IFNs (IFN- α , IFN- β , IFN- ω , IFN- ϵ , IFN- ζ , IFN- κ , IFN- τ , and IFN- δ) are the most recognised viral inducible cytokine having antiviral properties³. Coronaviruses are known to inhibit host Type 1 IFN production, which may account for viral pathogenesis⁴. The other RNA viruses- Influenza virus⁵ Ebola virus⁶ Dengue virus⁷ are also known for suppressing host IFN production for self propagation. Bats are known to constitutively express high amount of IFN- α which may explain the antiviral state behind the ability of bats to host many of these pathogenic viruses asymptotically⁸. Of the PRRs, TLR7 (endosomes) and RIG-I (cytosolic) like receptor are recognised to be single stranded RNA sensor for IFN production⁹.

Amongst the pro-inflammatory cytokines, (IL1 β) is one of the most known to be induced by RNA viruses thru the involvement of NLRP3 inflammasomes¹⁰. SARS Coronavirus is also known to activate NLRP3 inflammsomes¹¹. The transcriptional priming of NLRP3 transcript was also found to be impaired in bats leading to a defective (IL1 β) production¹², which might attribute to reason for harbouring these viruses asymptotically. Clinically, ACE2 (+) lung cells from patients infected with the virus have also shown high expression of pro-inflammatory cytokines (IL1 β , IL6, TNF α)¹³. Interestingly, the patients who got infected in China, Italy and Spain had reported comorbidities with diabetes, heart diseases and hypertension. Notably, these pathological conditions are attributed to hyper activated IL1 β production^{14,15,16}

Possible intervention measures taming the hyper inflammatory cytokine storm:

Acute COVID-19 patients have shown deposition of fibrin and platelet microthrombi in the lung vasculature, which contribute to progressive respiratory dysfunction and eventual deaths. Abnormal bleedings were also observed in Catheter and IV channels, with increased ferritin level and CRP in blood. These were perhaps due to activation of the coagulation cascades (similar to sepsis), the aftermath of cytokine storm produced by the deregulated immune system. Anti-clotting Tissue Plasminogen activator (tPA) has been shown to very effective in this aspect¹⁷. Clinical trials to be started soon on this. Coincidentally, the sepsis drug (Xigris) was also an anti-coagulant.

Chloroquine/Azithromycin combination has seen some mixed efficacy data against SARS-CoV2. Azithromycin, a macrolide antibiotic is much known to be a bacterial translational inhibitor, specifically for gram negatives. Azithromycin is also known to exert antiviral activity in cystic fibrosis patients by the induction of IFNs and ISGs¹⁸. Perhaps in COVID-19 patients too, the antibiotic takes care of secondary bacterial infections which develop with the host system becoming compromised following the cytokine storm.

IL6 Receptor blocker Tocilizumab is also in contention as intervention measure against SARS-CoV2. Interestingly, IL6 KO mice showed increased mortality with Influenza virus and HSV-1 virus challenge, conferring a protective action of IL6^{19,20}. Bacterial endotoxin induced ARDS also showed reduced pulmonary inflammation with recombinant IL6, reconfirming the protective action of the cytokine²¹. However, perhaps it's the timing and the amount of induction of the cytokine which decides whether it is going to be protective or detrimental to the system. It's really tempting to speculate how IL6 blocker turns up as intervention measure in clinical trials.

TLR7 is a single stranded RNA sensor located in the endosomes of lung epithelial cells and airway smooth muscles. TLR7 has been of importance in asthma, the disorder of lungs characterized by hyperactivated Th2 adaptive response (excessive broncho constriction and inflammation). The TLR7 agonist Imiquimod (R837) has found therapeutic application in attenuating airway inflammation by reducing the Th2 response (IL4, IL5, IL13) and shifting the equilibrium towards activating Th1 response (IFN γ , ISGs)²². Imiquimod might be helpful in reversing the airway hyper responsiveness of COVID-19 patients too.

IL1 β , a pro-inflammatory cytokine is known to contribute towards inflammation (sterile and pathogen mediated). Hyper stimulation of the cytokine leads to adverse pyroptosis and cell death. Sterile inflammation leads to different pathological disorders like Rheumatoid arthritis, Gout, Diabetes, Alzheimers, Atherosclerosis, etc. Blocking IL1 β production by IL1R antagonist Anakinra have shown promising results in these conditions^{23,24}. In COVID-19 patients too, the IL1 β production is robust leading to cytokine storm, which attribute to hyper inflamed state and pathophysiology. Hence, IL1 β inhibitor might be helpful in the management of COVID-19. Combinatorial approaches using anti-inflammatories and anti-viral repurposed drug (anti-inflammatories for dampening the IL1 β production and antivirals for curbing the viral load with IFNs or viral polymerase inhibition) might be good intervening measures.

Type 1 IFN has been shown to play protective role in SARS-CoV2 pathogenesis in human bronchial epithelial cells and A549 expressing ACE2. COVID-19 patients were seen to express high levels of C-X-C Chemokines (CXCL2, CXCL8, CXCL9, CXCL16) and Interleukins (IL1 β and IL6)²⁵. The Chemokines are also being known to be positively regulated by IL1 β , along with neutrophil infiltration in lungs during inflammations²⁶. Autopsies of COVID-19 lungs have shown to be filled with jelly like material causing severe respiratory distress probably by the accumulation of hyaluronic acid. And, the pro-inflammatory cytokines IL1 β and TNF α are known to be inducers for hyaluronic acid²⁷.

Summing up together the following combinatorial approaches may be tried empirically :

- 1) Recombinant Type I interferon as antiviral, in combination with a IL1 β inhibitor as anti-inflammatory (Anakinra)
- 2) TLR7 agonist Imiquimod as antiviral, in combination with a IL1 β inhibitor as anti-inflammatory (Anakinra).
- 3) IL1 β inhibitor (Anakinra) as anti-inflammatory, in combination with CoV RNA Polymerase inhibitor (Remdesivir/ Favipiravir)

The patients who are mildly asymptomatic, in whom the the cytokine storm hasn't started or not caused enough damage to be managed perhaps would be the best respondent of these interventions. While, asymptomatic patients should only need antivirals.

Conclusion:

The present hypothesis is postulated on the fact that a fraction of the population which get infected by SARS-CoV2 remain asymptomatic or mildly infected, while another fraction being severely symptomatic. In the asymptomatic or mildly infected population, the pro-inflammatory cytokines perhaps play a protective role. On the contrary, in the severely infected population, the pro-inflammatory cytokines are detrimental, causing the cytokine storm with increasing viral load, tissue damages and pathophysiologies. Coincidentally, the patients in Europe who fell sick have reported comorbidities (diabetes, heart disease, hypertension) with high IL1 β production. The intervention measures which have been hypothesised are combinatorial approaches by blocking the hyper activated IL1 β production along with stimulation of the type 1 IFNs or viral polymerase inhibitor. These interventions can be perhaps tried for other deadly zoonotic RNA viruses, which evade the host immune surveillance for self propagation. However, further basic science research is required to explore these intervention measures and large scale clinical trials to validate it along.

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Contributions:

RG did the literature survey, hypothesised the concept and wrote the manuscript.

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Declaration of Interest:

I don't have any competing interest.