REVIEW ARTICLE

Defeating the COVID-19 Pandemic by Targeting the Critical Interface between SARS-CoV-2 Virus Infection and Its Destructive Immune System Effects

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Abstract

Having recently made the evolutionary transition from bats to humans, the novel Coronavirus SARS-Cov-2 has single-handedly created a defining moment in human history as the world reluctantly embraces a new paradigm in which the devastating effects of rapidly emerging diseases underscore the fragility of human life. The purpose of this review is to take a broad-spectrum view of the challenges that lie ahead in defeating this ongoing pandemic. In the absence of a complete understanding of the SARS-CoV-2 virus and its pathogenic potential, the accomplishments of modern medicine in the molecular age, nevertheless, allow unprecedented insight into fine-tuned molecular mechanisms of infection and our increasing ability to monitor and assess this disease and its global consequences. This review attempts to define the virulence mechanisms and pathophysiological consequences of the SARS-Cov-2 virus that, based on our current understanding, will most likely respond to preventive and therapeutic approaches.

Introduction

Around the world, major health care and research institutes are searching for protective vaccines and therapeutic candidates for the treatment of COVID-19, including the National Institutes of Health (NIH) and The European Medicines Agency (EMA). In addition, the World Health Organization (WHO) is currently conducting clinical trials under the name of Solidarity-2 to evaluate up-and-coming vaccines and therapeutic drug candidates to treat this disease. This effort involves more than 70 countries to date. Despite the enormity of the task, it is possible to condense the therapeutic approaches to specific clinical modalities based on our understanding of the unique properties of SARS-CoV-2 infection and the molecular biology tools that have facilitated a comprehensive understanding of the structure, function and pathogenicity of this virus. In addition, new rapid-paced approaches have streamlined the many complexities of vaccine development by using viral RNA, DNA and protein subunit vaccine models to accelerate the path from bench to bedside. Rather than presenting a moment-by-moment summary of the clinical data, which inevitably will change rapidly even as this paper goes to print, the focus here is on the rationale for preventative and therapeutic approaches, as well as the overall results obtained thus far, if available, to make calculated predictions as to their potential efficacy.

Key Elements Driving Vaccine Design and Therapeutic Targets

Targeting the SARS-2 Coronavirus genes responsible for Infection

One of the most important determinants of human infection by SARS-CoV-2 virus is the structural spike "S" protein, that has recently evolved the capacity to bind very effectively the human angiotensin converting enzyme 2 (ACE-2) receptor found on the surface of lung alveoli, endothelial tissue and several other organ systems[1]. The importance of the "S" protein as an anti-viral target is underscored by the identification of the D614G missense amino acid mutation in SARS-CoV-2 virus strains prevalent in the USA and Europe, which increases the formation of functional spike proteins on the virus surface and is associated with significantly enhanced infectivity[2]. Virus uptake by membrane fusion in cells of the upper respiratory tract initially involves a host type 2 trans membrane serine protease called TMPRS S2 which cleaves the spike protein, facilitating the fusion of the virus to the host cell membrane in order to deposit the viral contents and begin the cycle of infection. Once the virus is inside the cell, the viral gene encoding the replicase enzyme complex is translated on host ribosomes, facilitating the synthesis of many copies of the viral genome. Later steps involve the translation of viral genes to form the structural proteins of the virus capsid and envelope. The viral proteins required for each of these stages of infection represent a suitable potential target for antiviral drugs and vaccines; however, the viral spike protein is a preferred target since it is essential to the earliest stages of infection; blocking this entry step is a logical preventive/therapeutic target that is also a major focus in vaccine development.

Genomic sequencing of SARS-CoV-2 early on, when the virus was first identified in China, showed that this unique RNA sequence represents a novel Coronavirus[3]. The DNA sequence is approximately 88% identical to two bat-derived SARS Coronaviruses; its genome displays 50% sequence identity to MERS Coronavirus and 79% identity to the original SARS Coronavirus. This novel virus was termed SARS-CoV-2 by the International Virus Classification Commission. The

genomic sequence information facilitated a rapid assessment of the structure and function of the virus and its mechanisms of pathogenicity in humans. SARS-CoV-2 contains genes encoding polyproteins with viral replication functions, including the RNA polymerase. Additional Open Reading Frames (ORFs) code for structural proteins, including spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins[4] (see Figure 1).

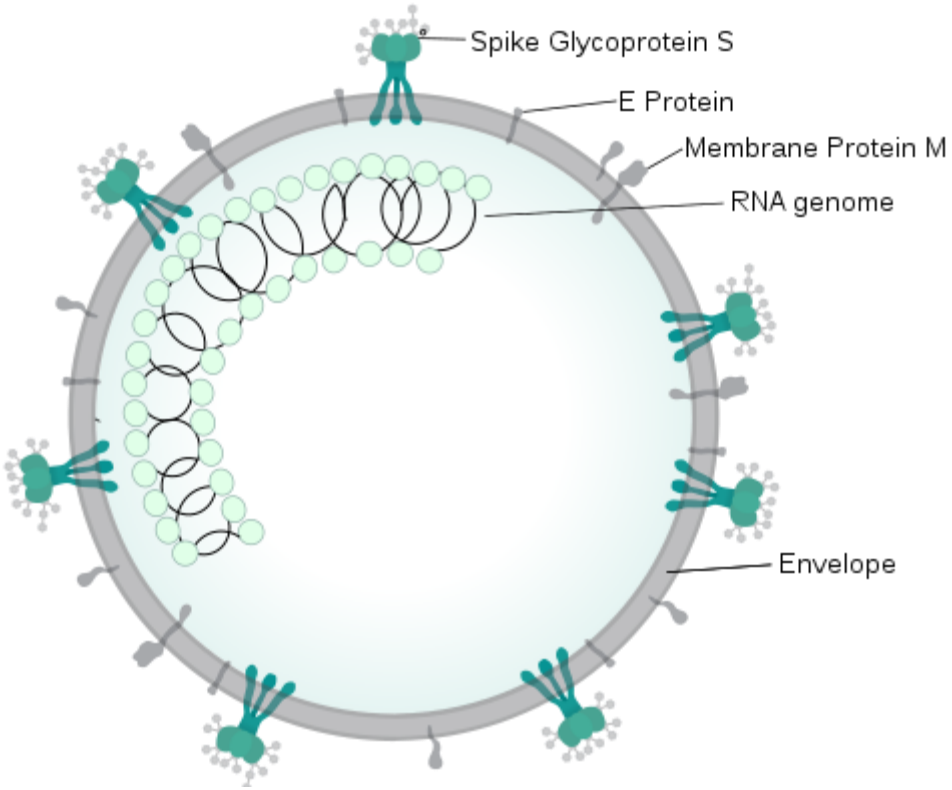

Figure 1. Coronavirus virus structural proteins, including nucleocapsid (N), membrane (M), envelope (E), and spike (S) critical to infection and immune system responses. (Courtesy Centers for Disease Control, USA)

The therapeutic strategies most commonly employed target key events required for virus reproduction and spread, including receptor binding to host cells by viral spike attachment protein, replication of its RNA genome, and/or assembly of the virus particles in the infected cells. Since there are key differences between these virally directed processes and human metabolism, this approach often yields therapeutic modalities with minimal side effects on the body. A key model system illustrating this principle is Human Immunodeficiency Virus (HIV) in which the unique properties of the reverse transcriptase, protease and attachment proteins have spawned a plethora of multi-drug "cocktails" that have transformed a once-fatal disease into one that is clinically manageable and life-sustaining. Many of these HIV-targeted drugs were developed in the context of viral genome analysis in conjunction with experimental assessment of the precise role of individual viral genes in infection. Currently, a similar approach is in play to develop SARS-CoV-2

targeted therapeutics, using the genomic data that have been generated subsequent to the identification of this novel infectious disease agent in early 2020.

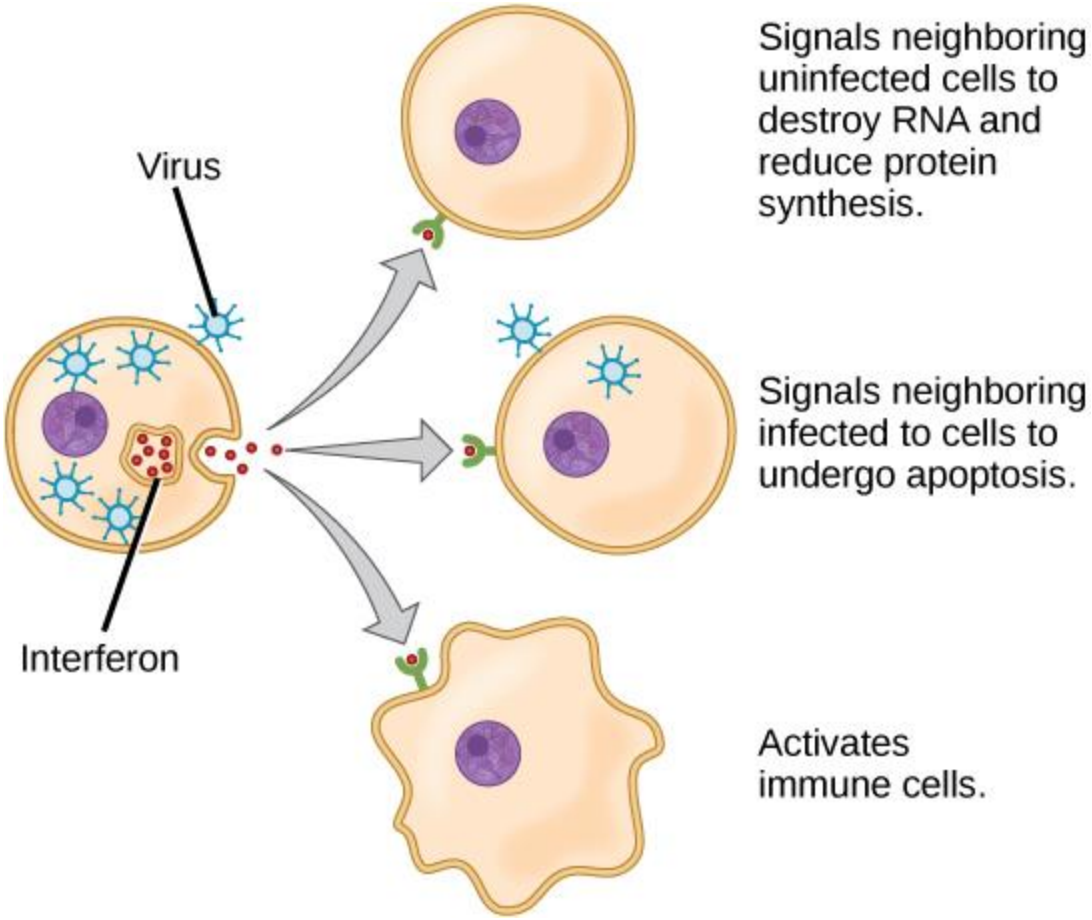

Figure 2. Interferon: general pathways in immune system activation. (Courtesy of Concepts of Biology - 1st Canadian Edition by Charles Molnar and Jane Gair is licensed under a Creative Commons Attribution 4.0 International License).

Targeting the critical interface between SARS-CoV-2 and dysregulated immune system responses

The use of IFN-1 in patients with SARS-CoV-2 may be especially important as virus infection appears to block host cell IFN-1 production at early stages of infection[5] (see Figure 2). Due to the very recent genesis of SARS-CoV-2, it is important to assess very carefully the transmissibility and pathogenicity of previously identified members of this virus Family responsible for disease outbreaks. To this end, the closely related SARS-CoV (79% genomic identity) and MERS-CoV (50% genomic identity) viruses may be instructive. Early indications are that SARS-CoV-2, like SARS-CoV and MERS, blocks Interferon Type 1 (IFN-1) early in infection, which may

be correlated with the severity of infection, as is the case with its viral relatives[5, 6]. The structural proteins "M" and "N" as well as non-structural ORFs are involved in this interferon blockade. Research suggests that Type 1 IFN (IFN-αβ) is the most important IFN responder to early stage infection by SARS-CoV-2[7]. The presence of viral RNA in the infected cell activates RIG-1 (retinoic acid-inducible gene 1) and MDA5 (melanoma differentiation-associated protein 5) that stimulate IFN Type 1 synthesis via IRF3[8]. Studies of the protective effect of IFN on Coronavirus infections in the 1980s showed that intranasal recombinant IFN-α decreased viral load and duration of illness in approximately 80 healthy volunteers enrolled in the clinical study[9]. SARS-CoV-2 shares many of the same proteins used by other Coronaviruses to block IFN Type I, including non-structural proteins nsp1, nsp3, nsp16, ORF3b, ORF6 as well as the M and N gene products. Significantly, increased virulence among Coronavirus members may be linked to enhanced IFN Type 1 blocking activity. This has been shown in both MERS and SARS-CoV[10]. In contrast, Coronaviruses with lower virulence, such as HKU1, have a more limited ability to block IFN-Type 1 activation[11]. These data implicate IFN Type 1 targeting in early stage Coronavirus infection as a critical determinant of virulence. Pharmacologic targeting of viral RNA and critical gene products involved in IFN blockade is, therefore, a logical approach to mitigating early stage SARS-CoV-2 infection. Due to the broad-spectrum effects of interferons, however, this approach must be carefully evaluated with respect to the timing and dosing regimens as their effects on the immune system can be difficult to regulate.

Patient clinical studies aimed at monitoring the course of infection at the virus:host interface play an extremely important role in defining both the temporal disease course and the key cause and effect interactions that can inform therapeutic approaches to disease mitigation. Defeating COVID-19 requires a design to interfere with the devastating consequences of advanced infection that involve systemic immune system hyperstimulation. There are, of course, two players in this treacherous infectious cycle between the virus and the host. Scientists agree that some of the more serious and sometimes fatal consequences of infection involve hyper-sensitization of the immune system, associated with what has been termed the "cytokine storm". Therefore, the immune system itself can be seen as a potential target for therapeutic approaches designed to mitigate the unchecked immune system responses that can result in death. It is also critical to distinguish among the multifaceted stages of infection and diverse clinical pathologies in order to develop an effective therapeutic paradigm for this complex virus. Clearly, a one-size-fits-all approach will not work on all COVID-19 patients. Much still needs to be learned about this important dynamic but, at present, there is some understanding of the ways in which the immune system responds to the virus and the ways in which that process may become derailed, resulting in collateral damage to the body. The goal of this research approach is to mitigate the untoward responses of the immune system while, at the same time, enhancing the ability of the immune system to defeat the virus.

Previous research on SARS-CoV and MERS-CoV have provided us with some background towards understanding the complex interactions between these human Coronaviruses and the host immune system as they relate to the potential to develop a successful vaccine[12]. Importantly, the presentation of viral antigens depends on Major Histocompatibility Complex (MHC) Class I molecules mostly, although MHC Class II molecules also play a role in virus recognition by the immune system. Research on these related Coronaviruses suggests that

IgG antibody is primarily responsible for long-term protection against reinfection and that these antibodies are primarily directed against the "S" spike antigens of the virus[13]. With respect to the molecular pathology involved, researchers have determined that neither SARS-CoV-2 nor MERS-CoV displays pathogen associated molecular patterns (PAMPS) that usually trigger recognition by host pattern recognition receptors (PRRs) to initiate immune responses to viral infection[14]. The mechanism by which this resistance occurs is via the production of double membrane vesicles that lack PRR display. The viruses replicate in these vesicles, thereby shielding them from detection from the host innate immune system which ordinarily would represent a first line defense against infectious disease. This allows the virus to gain a foothold within the respiratory tract and the lungs prior to eliciting a significant immune response. It is probable that this decoy mechanism also facilitates the systemic consequences of SARS-CoV-2 infection[14]. This sophisticated immune evasion strategy of the virus may complicate efforts to develop an immune based approach to prevent or mitigate infection.

Virus-associated PAMPs are known to activate Toll-Like Transmembrane Receptors (TLRs); studies of patients with SARS-CoV and MERS-CoV showed that TLR3, in particular, was involved in immune system activation[15]. TLR3 induction is associated with downstream IFN- α and IFN-β expression, as well as TNF, IFN-gamma, IL-6, IRF-3 interferon regulatory factor-3 and NFkB[16, 17]. Animal studies of SARS and H1N1 flu virus suggest that lung infection is associated with the production of oxidized lipids that, in turn, activate TLR4, MyD88 and TRIF to induce inflammatory cytokine release[18]. In particular, IL-6 is associated with pulmonary damage [19]. To this end, research reported from China suggested that increased circulating levels of Interleukin-6 (IL-6) and fibrinogen correlate with poor prognosis in patients with COVID-19[20]. In contrast, CD4+ and CD8+ T cell levels are generally depressed in acute stage COVID-19 infection[21].

One of the most important physiological consequences of advanced COVID-19 infection is acute respiratory distress syndrome (ARDS). This is a systemic inflammatory response to viral infection initiated by the secretion of proinflammatory cytokines and chemokines by immune effector cells in response to the infection. In addition to acute respiratory distress, COVID-19 can also cause multiple organ system failure and death. ACE-2 receptors on endothelial cells appear to mediate vascular inflammation, blot clots and systemic hypoxia[22]. Genetic studies of the related SARS-CoV virus showed that Open Reading Frame 8 (ORF-8) activates intracellular stress pathways, damage to lysosomes and autophagy[23]. Additional research has shown that ORF-8 activates intracellular inflammasomes, involved in innate immune responses, via NLRP3 (Cryopyrin)[24]. This process results in the production of inflammatory cytokines that stimulate pyroptosis, a specialized type of programmed cell death that occurs in response to intracellular pathogens. Pyroptosis induces further cytokine release, triggering tissue infiltration of inflammatory cells. Importantly, IL18 production, in response to pyroptosis, activates IFN-ϒ and its adaptive immune system targets[25, 26]. Similar mechanisms may occur also in SARS-CoV-2 pathogenicity, as its ORF8 protein is closely related to that of SARS-CoV. This inflammasome mediated pathway may be associated with more severe stages of COVID-19[27, 28]; moreover, later stage macrophage associated IL-1 production appears to play an important role in the "cytokine storm" linked to advanced disease[29]. COVID-19 patient autopsies have revealed that diseased tissues contained high levels of monocytes, macrophages and CD4+ T cells[30].

Thus, a critical issue in play is the multifaceted role of individual immune system components that respond to early versus later, more severe, stages of disease. The "ideal" IS response to viral infection is a rapid recruitment of innate system components that trigger the adaptive immune system to destroy virally infected cells and create a permanent antigenic memory that blocks re-infection by the same agent. Instead, SARS-CoV-2 employs strategies to minimize initial innate responses to infection, and, later, to trigger dysregulated immune system hyperstimulation associated with virally-induced tissue damage that characterizes advanced stages of infection in approximately 20% of infected individuals[31]. Therapeutic approaches designed to reprogram IS responses must address these early versus late infection profiles differently, lest these approaches exacerbate the dysfunctional IS pathology induced by SARS-CoV-2.

Key questions for follow-up studies related to SARS-CoV-2 prevention and treatment

1. **Age differential: why are younger individuals statistically at lower risk for severe COVID-19?**

Approximately 80% deaths from COVID-19 occur in patients age 65+, even though infection by SARS-CoV-2 has been documented in all age groups[32]. Recently published data from the Centers for Disease Control (CDC) showed that 70% of individuals in the U.S. testing positive for SARS-CoV-2 are younger than age 60[33]. Clearly, there are many important reasons for this demographic difference; however, there are certain implications that may relate to the mechanisms by which the IS responds to SARS-CoV-2 infection that may have important preventive and therapeutic applications. Research on SARS-CoV-2 pathogenesis at early stages of infection has shown that viral gene products specifically target IFN 1 and that, as a consequence, innate IS responses designed to prevent disease progression are blocked[34]. Research on the effects of ageing on the IS *in vitro* have shown that mononuclear cells from individuals 50+ years display reduced levels of IFN-α, β in response to virus infections, in general, as compared to younger individuals[35]. That SARS-CoV-2 immunosuppression involves early stage targeting of IFN-1 suggests a physiologically relevant explanation for the increased risk for advanced disease in older patients.

Another striking observation is that children under 19 years are dramatically less susceptible to SARS-CoV-2 infection symptomatology than the rest of the population in the U.S[36]. Moreover, this trend was observed also during the SARS-CoV and MERS outbreaks[37]. At this juncture, one can only speculate on the reasons for this surprising differential. Part of the explanation may involve innate IS functions that are intrinsically more responsive to infection early in life. In addition, the extent to which routine infant and childhood vaccinations may contribute to broad-spectrum enhancement of IS activity in response to unrelated viral infections has come under scrutiny as a potential contributory explanation[38]. Previous research suggests that early childhood vaccines, such as live, attenuated Measles, Mumps, Rubella (MMR) and Bacille Guerin Calmette (BCG) , a live attenuated tuberculosis vaccine, may provide so-called "nonspecific effects" (NSE) that reduce the risk of some of the more serious pathological consequences of infection by unrelated viruses[39, 40]. This possibility has received increased scrutiny in the wake of the COVID-19 pandemic. The protective effect of BCG vaccine against infant mortality from all causes is most likely the result of enhanced innate IS activity; moreover, evidence suggests that geographical

regions where BCG vaccination is routine may display lower mortality rates from COVID-19[41]. An elegant epidemiological study recently published by Escobar *et al.* [42] provides convincing evidence of a relationship between COVID-19 mortality rates and long-term/continuous BCG vaccination national policies in a broad-based assessment of approximately 150 countries. Their study also showed a quantitative relationship between less developed nations and lower mortality rates, which may be at least, in part, linked to their BCG infant vaccine programs (see Figure 3). These observations have led to the suggestion that high level trained innate IS responses early in SARS-CoV-2 infection may be a primary determinant of disease outcome. There is preclinical evidence to suggest that this bystander effect is due to generalized effects on bone marrow leukocytes that enable these immune system components to react more vigorously to viral antigens[43]. Additional work has shown that vaccine-induced "trained" immunological responses affecting Myeloid Derived Stem Cells (MDSCs) may reduce severe lung inflammation, highly relevant to infection by SARS-CoV-2[44].

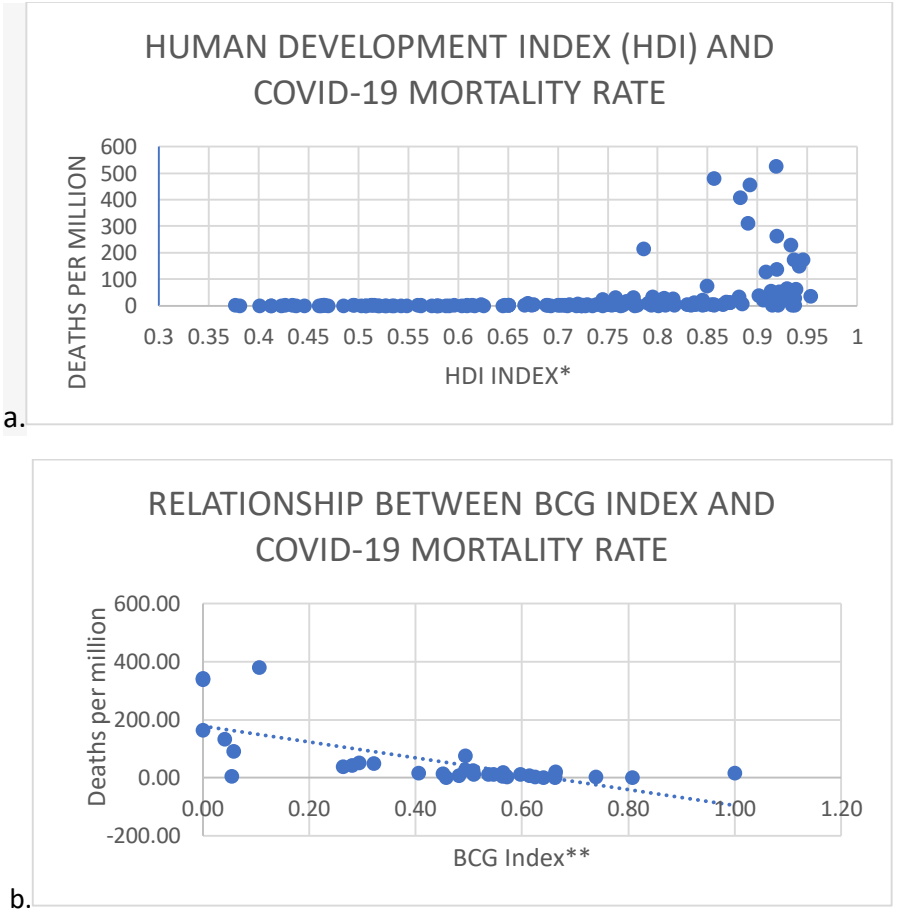

Figure 3. COVID-19 mortality rate data compiled by Escobar et al.42 as a function of: a. Human Development Index (HDI)*: the higher the standard of development, the higher the ranking up to 1.0; and b. BCG Index** calculated as = (age of oldest vaccinated cohort × total number of years of vaccination campaign)/standardization parameter (5,625) × Mean BCG vaccination coverage. All data shown were obtained from the COVID-19, BCG and related data accrued by the study authors.

2. Is it possible to block the "cytokine storm" in infected patients responsible for advanced COVID-19?

An overriding principle in the treatment of COVID-19 is the importance of blocking SARS-Cov-2 infection before immune system hypersensitization begins the relentless destruction of the body's organ systems. Therapeutics currently approved for use in specific countries include dexamethasone, Remdesivir and Favipiravir (Avigan). Recent data from the RECOVERY Trial suggest that the administration of glucocorticoids such as dexamethasone to patients with advanced COVID-19 may improve patient recovery statistics [45]. Approximately 2,000 patients were enrolled in the randomized trial; a 35% decrease in mortality in patients with advanced disease requiring ventilators, and a 20% reduction in mortality among patients on oxygen supplementation, were observed. The well-known physiological effects of glucocorticoids, such as dexamethasone, involve suppression of inflammation by NF-KB and AP-1 [46]. Other immunosuppressive effects relevant to COVID-19 include inhibition of vascular permeability and decreased leukocyte involvement in areas of inflammation, as well as transcriptional repression of mediators of inflammation, including cytokines implicated in the "cytokine storm" linked to advanced stages of COVID-19 [47]. Additional research studies have shown that the anti-inflammatory effects of glucocorticoids also involve the transcriptional activation of immunosuppressive genes, including IKB, DUSP, and IL-110 [48].

Remdesivir (Gilead Sciences) was originally developed as a treatment for Ebola and Marburg viruses, with limited success[49]. This anti-viral medication specifically targets RNA replication. Clinical data obtained thus far has shown that the drug may decrease the duration of illness in patients with COVID-19, without having a significant effect on overall mortality[49].

Favipiravir (Avigan) was developed in Japan as a treatment for influenza and much later used in patients with Ebola virus infections[50]. Like Remdesivir, the drug targets RNA virus replication. Recent studies in patients with COVID-19 showed an average reduction in recovery time from 11 days to 5 days[50].

Hydroxychloroquine (HCQ), a repurposed anti-malarial drug that has been shown to block SARS-CoV-2 virus uptake into human cells *in vitro*[51], has had a controversial introduction as a potential treatment for COVID-19. Conflicting data have created significant confusion about the potential effectiveness of this drug; however, a clinical study from the Henry Ford Hospital in USA, a retrospective analysis of a large cohort of over 2,500 patients who were treated with hydroxychloroquine, showed a significant reduction in mortality rates in patients who received it as a single agent (13.5%) or in combination with the antibiotic azithromycin (20.1%) as compared to a 26.4% mortality rate for patients who received neither drug[52]. These findings directly contradicted a RECOVERY trial conducted by researchers at Oxford University that showed no significant beneficial effect of hydroxychloroquine administered to a cohort of over 1,500 patients[53]. A possible explanation for this discrepancy is that the Michigan study assessed patients who had been treated at earlier stages of infection than the RECOVERY patient cohort. It appears that Remdesivir and Favipirivar, that target viral replication, may also show an increased benefit when administered at earlier stages of disease. SARS-CoV and MERS-CoV immunosuppression, occurring in advanced disease, is associated with extensive neutrophil and monocyte-macrophage infiltration of

infected sites linked to Acute Respiratory Distress Syndrome (ARDS), and other inflammatory complications that can be fatal. These later stage systemic effects follow from suppression of early innate IFN-associated immune responses by these human Coronaviruses to establish infection. Later, advanced stages of disease appear to be mediated at least in part by a subsequent hyperstimulation of IFN which results in dysregulated immune responses associated with the "cytokine storm" and multi-system organ damage[54]. The timing of therapeutic interventions designed to potentiate effective IS responses to SARS-Cov-2 infection may, therefore, be of the utmost importance in achieving successful therapeutic responses.

3. What are the potential implications of IS responses in patients with COVID-19 on the development of effective vaccines?

General categories of SARS-CoV-2 vaccine design include the conventional approaches using inactivated whole virus, spike "S" protein vaccines, virus-like particles (VLPs), vectors containing inserted viral genes, DNA and RNA viral sequences[55] (see Figure 4). Earlier vaccines prepared against SARS-CoV, included whole virus, a recombinant spike protein vaccine, Virus Like Particles (VLPs) containing the S, N, E and M proteins from mouse hepatitis Coronavirus. Preclinical testing of these earlier vaccine formulations showed that vaccinated mice post-challenge developed immunopathologic reactions in lung tissues associated with Th2-type eosinophil hypersensitivity, also seen in some children who developed Respiratory Syncytial Virus (RSV) infections subsequent to receiving a vaccine against RSV, leading to the alarming conclusion that vaccination exacerbated the effects of natural infection in these children, resulting in severe lung inflammation and death in some cases[56]. Follow-up research in mice showed that the RSV vaccine induced high levels of CD4+ T cells, primarily Th2, that caused massive lung infiltration by Type 2 cytokines and eosinophils[57].

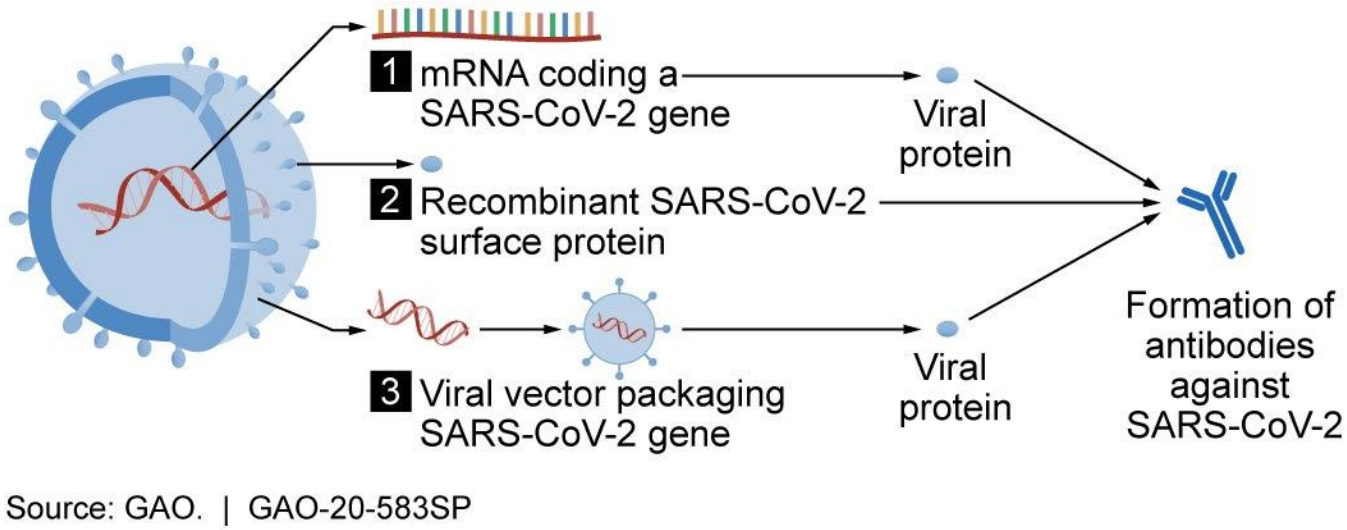

Figure 4. Prototype vaccines against COVID-19 (Courtesy U.S. Government Accountability Office from Washington, DC, United States)

Additional animal studies on the effects of these early Coronavirus vaccines were also instructive. Mice inoculated with several different SARS-CoV vaccine formulations showed a similar pattern of IS hyperstimulation and serious disease following challenge; however, the response to an "S" protein vaccine was not as severe as with inactivated whole virus preparations[58]. In the vaccinated mice, lung pathology, resulting from vaccine-associated IS hyperstimulation, was observed even in the absence of detectable virus post challenge. Since challenge with inactivated virus did not produce this response, it was concluded that early viral replication may be required to induce this pathological response. It appeared that, although vaccination halted the spread of the virus, it caused the immunopathology characteristic of advanced disease. Similar effects were observed in mice vaccinated with a Venezuelan equine encephalitis virus containing the SARS-CoV "N" gene[59]. In contrast, the inclusion of the "S" gene in this recombinant vaccine did not produce this pathological response, leading to the suspicion that the "N" gene product may be responsible for this effect. Notably, vaccine formulations containing the "S" protein, but NOT the "N" gene product, provided anti-virus protection in the animal studies.

Human clinical trials of SARS-CoV experimental vaccines showed induction of a protective antibody response and no reports of pathological effects, though the human (versus mouse) patients were not challenged with live virus[60]. Thus, the potential of SARS-CoV vaccines to induce this Th2-associated lung pathology is currently unknown. The conflicting data on the capacity for the "S" gene product to induce hypersensitivity may mean that the configuration of the protein in different vaccine formulations may affect IS responses[61]. Additional research suggests that IgG antibodies directed against the SARS-CoV "S" protein (anti-S-IgG) may contribute to lung pathology by stimulating the release of cytokine IL-8, MCP1 and inflammatory macrophages, as well as negatively affecting the levels of TGF-β and wound-healing macrophages[62].

Previous studies on SARS-CoV showed that IS responses to infection include short-lived IgG production generated against the "S" and "N" proteins, and a long-lasting T cell response[63], suggesting that Coronavirus vaccines might induce long term protective effects if they evoke a significant T cell response; moreover, T cell responses to the viral structural proteins (especially "S" and "N") were found to be most pronounced in post-infection patient mononuclear cells . In recovering patients with SARS-CoV, CD8+ T cell levels were significantly higher than CD4+ levels[64]. IS responses were found to target epitopes of the "S", "M" and "N" viral antigens most commonly.

Preliminary data on SARS-CoV-2/COVID-19 suggest that poor patient outcome is associated with low anti-viral antibody levels, also the case with SARS-CoV and MERS[65.] Recently, significant CD8+ T cells have been documented in 70, and CD4+ T cells in 100%, of patients recovering from COVID-19[66]. CD4+ responses were primarily targeted to the structural proteins "S", "N" and "M", whereas a lesser response was detectable against the nonstructural proteins (including nsp3, nsp4, ORF3a and ORF8). CD8+ responses against "S", "M" and numerous nsps were detected. Interestingly, CD4+ T cells directed against SARS-CoV-2 were also identified in approximately 50% of healthy individuals never exposed to this new virus, suggesting that exposure to other human Coronaviruses may produce long-lasting cross-reactive IS responses. Nevertheless, the IS protective profile for SARS-CoV-2 is incomplete, and these clinical data suggest that some patients do not display significant protective levels of immunity post infection.

How these disparities might affect the scope and duration of protective immunity following SARS-CoV-2 vaccination is currently unknown.

Due to the extreme urgency presented by the COVID-19 global pandemic, accelerated efforts to develop a vaccine depend on functional validation of viral antigens/epitopes most likely to elicit a protective immune response against subsequent infection. Much of the data has been obtained from analyzing the IS profiles of post-infection patients in comparison to unexposed healthy controls. Preliminary results have shown that the "S", "M" and "N" structural proteins comprise the major equivalent immune targets of CD4+ T cells, identified in virtually all recovered COVID-19 patients[67]. In addition, nsp3, nsp4, nsp12, ORF3s, ORF7a, and ORF8 were also identified as CD4+ targets. Significant differences were observed in CD8+ targets elicited by SARS-CoV-2; namely, "S" and "M" were the single most important targets, but 50% of CD8+ responses were detected against nsp6, ORF3a and "N" gene products. Importantly, recovering patients displayed a predominantly Th1 cytokine profile, absent the Th2 component linked to advanced and fatal disease.

Conclusion

The purpose of this paper was to define the virulence mechanisms and pathophysiological consequences of the SARS-CoV-2 virus that, based on our current understanding, will most likely respond to preventive vaccine and therapeutic approaches. The focus on therapeutic rationale and overall results obtained thus far in the study of SARS-CoV-2 and the related Coronaviruses, SARS-CoV and MERS-CoV, was designed to make calculated predictions as to their potential efficacy. This inquiry has led to the following conclusions:

1. The therapeutic strategies most likely to mitigate infection target key events required for virus reproduction and spread, including receptor binding to host cells by viral spike "S" attachment protein, replication of the RNA genome, and/or assembly of the virus particles in the infected cells. Since there are key differences between these virally directed processes and human metabolism, this approach should yield therapeutic modalities with minimal side effects on the body.

2. Preliminary data implicate IFN-Type 1 targeting in early stage Coronavirus infection as a critical determinant of early stage virulence. To this end, studies of the protective effect of IFN on Coronavirus infections in the 1980s showed that intranasal recombinant IFN-α decreased viral load and duration of illness in approximately 80 healthy volunteers enrolled in the clinical study. Due to the broad-spectrum effects of interferons, however, this approach must be carefully evaluated with respect to the timing and dosing regimens as their effects on the immune system can be difficult to regulate.

3. Defeating COVID-19 requires a design to interfere with the devastating consequences of advanced infection that involve systemic immune system hyperstimulation. It is critical to distinguish among the multifaceted stages of infection and diverse clinical pathologies in order to develop an effective therapeutic paradigm for this complex virus. Therapeutic approaches designed to reprogram IS responses must address these early versus late infection profiles differently lest these approaches exacerbate the dysfunctional IS pathology induced by SARS-CoV-2.

4. Childhood resistance to COVID-19 may involve innate IS functions that are intrinsically more responsive to infection early in life. In addition, the extent to which routine infant and childhood vaccinations may contribute to broad-spectrum enhancement of IS activity in response to unrelated viral infections has come under scrutiny as a potential contributory explanation. There is preclinical evidence to suggest this bystander effect is due to generalized effects on bone marrow leukocytes that enable these immune system components to react more vigorously to viral antigens.

5. An overriding principle in the treatment of COVID-19 is the importance of blocking SARS-CoV-2 infection before immune system hypersensitization begins the relentless destruction of the body's organ systems. It appears that Remdesivir, hydroxychloroquine and Favipirivar, that target early stages of infection, may show an increased benefit when administered at earlier stages of disease. The timing of therapeutic interventions designed to potentiate effective IS responses to SARS-CoV-2 infection may, therefore, be of the utmost importance in achieving successful therapeutic responses.

6. Preclinical testing earlier SARS-CoV vaccines showed that vaccinated mice developed immunopathologic reactions in lung tissues associated with Th2-type eosinophil hypersensitivity, also seen in some children who developed Respiratory Syncytial Virus (RSV) infections subsequent to receiving a vaccine against RSV. The potential of SARS-CoV-2 vaccines to induce this Th2-associated lung pathology is currently unknown. The conflicting data on the capacity for the "S" gene product to induce hypersensitivity may mean that the configuration of the protein in different vaccine formulations may affect IS responses.

7. Previous studies on SARS-CoV showed that IS responses to infection are associated with a long-lasting T cell response, suggesting that Coronavirus vaccines might induce long term protective effects if they evoke a significant T cell response; moreover, T cell responses to the viral structural proteins (especially "S" and "N") were found to be most pronounced.

8. CD4+ T cells directed against SARS-CoV-2 were also identified in approximately 50% of healthy individuals never exposed to this new virus, suggesting that exposure to other human Coronaviruses may produce long-lasting cross-reactive IS responses.

9. The IS profile for SARS-CoV-2 is incomplete, and clinical data suggest that some patients do not display significant protective levels of immunity post infection. How these disparities might affect the scope and duration of protective immunity following SARS-CoV-2 vaccination is currently unknown.

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