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Innate immunity in COVID-19 patients mediated by NKG2A receptors, and potential treatment using Monalizumab, Choloroquine, and antiviral agents

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\textbf{ABSTRACT}

Following the outbreak of a novel coronavirus (SARS-CoV-2), studies suggest that the resultant disease (COVID-19) is more severe in individuals with a weakened immune system. Cytotoxic T-cells (CTLs) and Natural Killer (NK) cells are required to generate an effective immune response against viruses, functional exhaustion of which enables disease progression. Patients with severe COVID-19 present significantly lower lymphocyte, and higher neutrophil, counts in blood. Specifically, CD8\textsuperscript{+} lymphocytes and NK cells were significantly reduced in cases of severe infection compared to patients with mild infection and healthy individuals. The NK group 2 member A (NKG2A) receptor transduces inhibitory signalling, suppressing NK cytokine secretion and cytotoxicity. Overexpression of NKG2A has been observed on CD8\textsuperscript{+} and NK cells of COVID-19 infected patients compared to healthy controls, while NKG2A overexpression also functionally exhausts CD8\textsuperscript{+} cells and NK cells, resulting in a severely compromised innate immune response. Blocking NKG2A on CD8\textsuperscript{+} cells and NK cells in cancers modulated tumor growth, restoring CD8\textsuperscript{+} T and NK cell function. A recently proposed mechanism via which SARS-CoV-2 overrides innate immune response of the host is by over-expressing NKG2A on CD8\textsuperscript{+} T and NK cells, culminating in functional exhaustion of the immune response against the viral pathogen. Monalizumab is an inhibiting antibody against NKG2A which can restore the function of CD8\textsuperscript{+} T and NK cells in cancers, successfully ceasing tumor progression with no significant side effects in Phase 2 clinical trials. We hypothesize that patients with severe COVID-19 have a severely compromised innate immune response and could be treated via the use of Monalizumab, interferon \(\alpha\), chloroquine, and other antiviral agents.

\textbf{Introduction}

Following the outbreak of a novel coronavirus (SARS-CoV-2), COVID-19 has rapidly spread throughout the entire globe and has severely affected the capacity of the global public health community [1]. COVID-19 has been reported to cause more severe disease in older men and individuals with comorbidities [2], potentially indicating a weakened immune system in individuals presenting with increased severity of disease.

Cytotoxic T-cells (CTLs) and Natural Killer (NK) cells are required to generate an effective immune response against viruses [3], functional exhaustion of which results in disease progression [4]. Indeed, patients with COVID-19 presented with significantly lower lymphocyte and higher neutrophil counts in blood compared to healthy controls [3]. Specifically, CD8\textsuperscript{+} lymphocytes and NK cells were significantly reduced in severe infection compared to patients with mild infection and healthy controls [3].

\textbf{Hypothesis: Innate immunity is compromised by SAR-CoV-2, which could be overcome by Monalizumab treatment to restore the function of CD8\textsuperscript{+} T and NK cells}

We hypothesize that patients with severe COVID-19 have a severely compromised innate immune response and are therefore more prone to co-infections and opportunistic infections of the lung. In this context, we propose that COVID-19 severity could be treated via the following:

1) Interferon therapy to generate adequate immune response  
2) Use of chloroquine, broad-based antibiotics, and antivirals to limit viral replication and co-infections  
3) Use of Monalizumab to restore the function of CD8\textsuperscript{+} T and NK cells

\textbf{Evaluation of the hypothesis}

COVID-19 predominantly seems to affect most patients primarily in the lungs [5], with the primary mode of infection through droplets [6].
The virus has an asymptomatic incubation period of 2–14 days, during which transmission could occur [6]. 80% of patients studied have been asymptomatic, or mildly symptomatic, with the rest exhibiting severe symptoms [7]. Most patients initially present with flu-like symptoms progressing to a sore throat, cough, breathlessness, and chest pain. Most symptomatic patients develop lymphopenia and pneumonia with a characteristic ground glass appearance following a CT Scan [1,7,8]. Patients with severe COVID-19 exhibited high levels of an array of proinflammatory cytokines in their blood, including IL-2, IL-7, IL10, IP-10, TNF-α, G-CSF, MCP-1 and MIP-1α [1]. This concurrence of a “cytokine storm” with lymphopenia could underlie viral sepsis and inflammatory damage of the lung [9].

An effective innate immune response depends on the interferon type-1 responses and downstream cascades resulting in effective induction of an adaptive immune response. SARS-CoV and SARS-CoV-2 both enter cells through the ACE-2 receptor, which is expressed in a small set of Alveolar Type 2 epithelial cells [10]. Although the main pathogenesis of SARS-CoV is thought to be through direct infection of macrophages and T cells, whether SARS-CoV-2 infects immune cells is not known [11]. The proposed mechanism of injury by SARS-CoV-2 includes: 1) Infection of ACE-2 expressing target cells such as immune cells; 2) Suppression of interferon-responses leading to uncontrolled viral replication; 3) Increased influx of neutrophils and macrophages with release of proinflammatory cytokines leading to lung injury; and 4) Specific Th1/Th17 activation resulting in B cell activation and further inflammatory response via antibodies against SARS-CoV-2 [5].

Several studies indicate overtly elevated serum proinflammatory cytokine levels in COVID-19 patients [1], correlated with the severity of pneumonia as with MERS-COV and SARS infections [12,13]. Cytotoxic T-cells (CTLs) and Natural Killer (NK) cells are required to generate an effective immune response against viruses [3], functional exhaustion of which resulted in disease progression [4]. Indeed, patients with COVID-19 presented with significantly lower lymphocyte and higher neutrophil counts in blood compared to healthy controls [3].

The NK group 2 member A (NKG2A) heterodimeric receptor is one of the most prominent NK cell inhibitory receptors. Ligation by peptide-loaded HLA-E induces NKG2A to transduce inhibitory signalling through 2 inhibitory immune-receptor tyrosine-based inhibition motifs, suppressing NK cytokine secretion and cytotoxicity [12]. Over-expression of NKG2A (an inhibitory receptor) on CD8+ and NK cells of COVID-19 infected patients compared to healthy controls has been demonstrated recently [3]. NKG2A overexpression also functionally exhausts CD8+ and NK cells, severely compromising the innate immune response [14], while blocking NKG2A on CD8+ and NK cells in cancers diminished tumor growth in several studies [15]. Binding of NKG2A to its cognate ligands inhibits the effector function of CD8+ and NK cells, while blocking NKG2A restores CD8+ T and NK cell function [14]. A recently proposed mechanism via which SARS-CoV-2 overrides the innate immune response of the host is by over-expressing NKG2A on CD+ T and NK cells [3], culminating in functional exhaustion of the immune response against the viral pathogen.

Overexpression of NKG2A and subsequent functional exhaustion of T and NK cells has been demonstrated previously in several cancers leading to tumor growth [16,17]. In this context, Monalizumab an inhibiting antibody against NKG2A has been developed which has shown promise to restore the function of CD8+ and NK cells in cancers, limiting tumor growth [15]. In Phase-2 clinical trials Monalizumab treatment successfully ceased tumor progression, with no significant side effects [15].

Consequences of the hypothesis and discussion

We propose that COVID-19 infection severely compromises the hosts innate immune response, and ability to generate a sufficient adaptive immune response. We also propose that such suppression of the innate immune response occurs via over-expression of NKG2A (an inhibitory receptor) on CD8+ and NK cells, leading to their reduction and an increase in opportunistic and coinfections of the lung in COVID-19 patients with severe symptoms. We posit that such severe symptoms could perhaps be alleviated by treatment with Monalizumab, a drug that has successfully cleared Phase 2 clinical trials, by inhibiting NKG2A receptors and restoring CD8+ T and NK cell function as previously recorded in a number of cancers. Finally, perhaps a combination of Chloroquine, antivirals, interferons, and broad-based antibiotics can prevent co-infections and severe infections of the lung which culminates in Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure.

Recently, Multicenter Clinical trials have shown that use of chloroquine, an antimalarial drug, may show beneficial effects against COVID-19 [18,19]. Chloroquine is a weak base which gets trapped inside membrane bound organelles resulting in changes in their acidification process [20], potentially increasing lysosomal pH and inhibiting pH-dependent viral fusion with lysosomal enzymes and replication [20]. Furthermore, chloroquine can block clathrin-mediated endocytosis by SARS-CoV-2 [21].

Interferon-α is a broad-spectrum antiviral drug previously used for viral hepatitis, and could also potentially block viral replication of SARS-COV [22]. Lopinavir/ritonavir are antiviral agents used in treating Human Immunodeficiency Virus (HIV) infections [23], and are HIV protease enzyme inhibitors that result in the formation of non-infectious viral particles. Lopinavir/ritonavir have shown anti-SARS-CoV activity in clinical trials and in vitro studies [24]. Similarly, Ribavirin a guanosine analog, which inhibits viral RNA synthesis couldower the risk of ARDS in patients with SARS-CoV [24]. Furthermore, Favipiravir, an RNA-dependent RNA polymerase inhibitor has been shown to be more effective than lopinavir/ritonavir in treating COVID-19 patients with fewer side effects [25]. Another antiviral drug, Remdesivir, which has been used for treating Ebola virus infections has also been under investigation for treating SARS-CoV-2 infections [25], and can block viral replication of SARS-CoV-2 even at a very low concentrations [19].

Some other potential drugs which can be used in treating COVID-19 infection include Type II Transmembrane Serine Protease (TMSPSS2) inhibitor and imatinib (Tyrosine kinase inhibitor) [26,27]. The summary of the potential drugs which can be used in treating COVID-19 infection is provided in Table 1. In conclusion, we propose a combination of these drugs should be evaluated for their safety and efficacy in

| Sr | Drugs                  | Mode of Action                                                                 |
|----|------------------------|--------------------------------------------------------------------------------|
| 1  | Chloroquine            | 1. Alters the pH in the lysosomes and prevents viral fusion and replication.    |
|    |                        | 2. Prevents clathrin mediated endocytosis in the cells and viral entry.       |
| 2  | Lopinavir/ritonavir    | Viral protease inhibitor                                                       |
| 3  | Interferon-α           | Generation of adaptive immune response                                        |
| 4  | Ribavirin              | Stops viral RNA synthesis                                                      |
| 5  | Favipiravir            | RNA dependent RNA polymerase inhibitor                                        |
| 6  | Remdesivir             | Nucleoside analogue                                                           |
| 7  | TMSPSS2 inhibitor      | Serine protease inhibitor which is required for COVID-19 S2 protein priming and binding to ACE2 receptor |
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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