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

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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, $CD8^+$ 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 $CD8^+$ and NK cells of COVID-19 infected patients compared to healthy controls, while NKG2A overexpression also functionally exhausts $CD8^+$ cells and NK cells, resulting in a severely compromised innate immune response. Blocking NKG2A on $CD8^+$ cells and NK cells in cancers modulated tumor growth, restoring $CD8^+$ 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 CD^+ 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 + 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 α, chloroquine, and other antiviral agents.

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, $CD8^+$ lymphocytes and NK cells were significantly reduced in severe infection compared to patients with mild infection and healthy controls [3].

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

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].

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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-1A [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-I 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 + T 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 inhibtiing 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

Table 1
Potential drugs which can be used in treating COVID-19 infection.

Sr	Drugs	Mode of Action
1	Chloroquine	1. Alters the pH in the lysosomes and prevents viral fusion and replication. 2. Prevents clarithrin mediated endocytosis in the cells and viral entry.
2	Lopinavir/ritonavir	Viral protease inhibitor
3.	Interferon-α	Generation of adaptive of 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

a randomized, placebo-control, phase-2 clinical trial.

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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