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Forum

A Potential Role of Interleukin 10 in COVID-19 Pathogenesis

Ligong Lu,^{1,*} Hui Zhang,² Danielle J. Dauphars,³ and You-Wen He^{3,*}



A unique feature of the cytokine storm in coronavirus disease 2019 (COVID-19) is the dramatic elevation of interleukin 10 (IL-10). This was thought to be a negative feedback mechanism to suppress inflammation. However, several lines of clinical evidence suggest that dramatic early proinflammatory IL-10 elevation may play a pathological role in COVID-19 severity.

Cytokine Storm in COVID-19 Patients

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infection has caused >1.1 million deaths and >42 million COVID-19 cases globally as of 24 October 2020 (Johns Hopkins Coronavirus Resource Center, <https://coronavirus.jhu.edu>). Severe and critically ill COVID-19 patients develop a pathological state termed **cytokine release syndrome** (CRS; see [Glossary](#)) that is characterized by rapid and prolonged systemic elevation of >20 inflammatory cytokines and chemokines [1]. CRS induces **acute respiratory distress syndrome** (ARDS) and **secondary hemophagocytic lymphohistiocytosis**, which often lead to multiorgan failure and death. Chief among the >20 elevated inflammatory cytokines/chemokines is IL-6, a key cytokine in CRS-induced mortality in blood cancer patients receiving engineered T cell therapy. Previous clinical experience in treating cellular immunotherapy-induced CRS suggests that blocking IL-6/IL-6R signaling might potentially reduce COVID-19

mortality. However, a recent randomized, double-blind Phase III COVACTA trial (NCT04320615; clinicaltrials.gov) testing the clinical efficacy of the anti-IL-6R antibody tocilizumab in COVID-19 patients failed to demonstrate a significant reduction in mortality [2].

Although CRS in COVID-19 patients is similar to that previously seen in SARS patients infected by SARS-CoV, a unique feature of the COVID-19 cytokine storm is the dramatic elevation of **interleukin 10** (IL-10) in severe/critically ill patients [1,3–6]. Peripheral IL-10 concentrations were significantly higher in intensive care unit (ICU) COVID-19 patients compared to non-ICU patients [1,5]. Furthermore, IL-10 concentrations strongly correlated with those of IL-6 and other inflammatory markers such as C-reactive protein [3]. How SARS-CoV-2 infection differs from SARS-CoV in its capacity to stimulate IL-10 expression is currently unknown. However, the importance of IL-10 as a putative immune biomarker in surveying COVID-19 disease severity has emerged. Similarly to IL-6, high IL-10 expression can predict poor outcomes in COVID-19 patients [3,4]. Recent meta-analysis of 1242 non-severe and 915 severe COVID-19 patients from 18 clinical studies identified IL-6 and IL-10 as covariates that accurately predicted disease severity [7]. Furthermore, IL-10 is elevated earlier than IL-6 in COVID-19 patients [4]. Given the long-recognized pathological role of IL-6 in CRS and resulting mortality, many clinical trials have been undertaken to test the efficacy of blocking IL-6/IL-6R in potential COVID-19 treatments. By contrast, the clinical significance of highly elevated IL-10 amounts in the serum of COVID-19 patients has been generally regarded as an anti-inflammatory or immune-inhibitory mechanism (and hence biomarker), stimulated by the rapid accumulation of pro-inflammatory cytokines as a **negative feedback loop** [4,5]. Furthermore, recombinant IL-10 has been proposed by

Glossary

Acute respiratory distress syndrome (ARDS): a serious lung condition characterized by fluid accumulation in alveoli of the lung, accompanied by severe shortness of breath, low blood oxygen, and mortality.

Cytokine release syndrome (CRS): a clinical condition caused by the rapid release of inflammatory cytokines from different cell populations into the circulation; CRS can be triggered by infections and some therapeutic agents such as chimeric antigen receptor T cells.

Endotoxemia: exemplified by high levels of lipopolysaccharide (LPS) in the blood. LPS is a pathogen-associated molecular pattern that stimulates systemic inflammatory responses.

Exhausted effector PD-1⁺TIM3⁺LAG3⁺CD8⁺

T cells: these display functional deterioration and lack full capacity to execute cytotoxicity. They express the inhibitory receptors PD-1, TIM3, and LAG3, but can be reinvigorated by immune-activating cytokines or blockade of immune checkpoint signaling pathways.

Interleukin 10 (IL-10): has many different and sometimes contradictory functions. It suppresses and promotes inflammation as well as innate and adaptive immune responses in a context- and dose-dependent manner.

Negative feedback loop: a host reaction that causes a specific decrease in function. For example, induction of immunosuppressive cytokines during a cytokine storm might be necessary to prevent hyperinflammation.

Neopterin: catabolic product of GTP belonging to the chemical group of pteridines; it is synthesized by human macrophages and can be indicative of the proinflammatory status of the immune system.

Secondary hemophagocytic

lymphohistiocytosis: a clinical syndrome that includes fever, hepatosplenomegaly, cytopenia, and progressive organ failure; can be caused by infections, cancer, and autoimmune disease.

Viral sepsis: virus infection that causes life-threatening organ dysfunction produced by systemic host responses and direct pathogenic effects of viruses.

some investigators for treating ARDS in COVID-19 patients based on its immunoregulatory and antifibrotic functions [8]. However, several lines of clinical evidence from human studies suggest that the early and dramatic IL-10 elevation upon SARS-CoV-2 infection might instead play a detrimental pathological role in COVID-19 severity.

Proinflammatory and Immune-Activating Effects of IL-10 in Patients with Autoimmunity or Cancer

Frequently labeled as an immunosuppressive or anti-inflammatory cytokine, pleiotropic IL-10 can be an immune-activating and proinflammatory cytokine in some autoimmune diseases and cancers in human patients [9–11]. For example, in a placebo-controlled double-blind study, chronically active Crohn's disease patients treated with recombinant human IL-10 (rIL-10) exhibited enhanced serum production of proinflammatory **neopterin**, as well as *ex vivo* phytohemagglutinin (PHA)-induced IFN- γ production from whole blood cells [9], suggesting that IL-10 promotes inflammatory cytokine production in humans. Moreover, administration of polyethylene glycol-modified (PEGylated) rIL-10 at high dose (20 μ g/kg) into advanced solid tumor patients induced systemic immune activation, as evidenced by circulating cytokine production and T lymphocyte activation and proliferation, in addition to exhibiting encouraging clinical efficacy in tumor control [10,11]. In a Phase I, non-randomized, single group (51 participants), patients with advanced renal cell carcinoma (RCC) treated with rIL-10 alone achieved a 27% overall response (OR) (NCT02009449) [10]. Furthermore, the combination of rIL-10 and anti-PD-1 antibody treatment of patients with advanced melanoma, RCC, or non-small cell lung cancer exhibited an impressive 42% OR (eight of 42 patients) (NCT02009449, NCT03267732) [11]. Three major features of immune activation were observed in these cancer patients. First, rIL-10 induced sustained systemic elevation of inflammatory and immune-activating cytokines/mediators, including IL-2R α , IL-4, IL-7, IL-18, IFN- γ , GM-CSF, and TNF- α , in patient serum [10,11]. Second, rIL-10 stimulated extensive clonal expansion of effector IFN- γ ⁺CD8⁺ T cells in both the periphery and tumors of the treated cancer patients [11]. Third, rIL-10

drove peripheral **exhausted effector PD-1⁺TIM3⁺LAG3⁺CD8⁺ T cells** into active proliferation and expansion [11]. Consistent with these observations in cancer patients, an older study reported that administration of rIL-10 to healthy human subjects who were rendered **endotoxemic** via lipopolysaccharide (LPS) intravenous injection resulted in enhanced peripheral production of IFN- γ as well of IFN- γ -inducible protein 10 (IP10) and CXCL-9 chemokines [12]. These human studies have demonstrated that rIL-10 has a potent immune activating/proinflammatory effect and can amplify endotoxemia-induced inflammation (Figure 1).

Potential Roles of IL-10 in COVID-19 Pathogenesis

Mortality of COVID-19 patients is caused by severe pneumonia and vital organ damage with the involvement of many pro-inflammatory mediators. Thus far, no definitive efficacy of reduced mortality in COVID-19 patients has been established in randomized placebo-controlled clinical trials testing blockade of IL-6, IL-1, or GM-CSF [13]. We propose that IL-10 is a potential target for reducing COVID-19 mortality. As mentioned, severe/critically ill COVID-19 patients present dramatically

elevated serum IL-10 concentrations that correlate with disease severity [1,3–6]. Accordingly, recent studies also demonstrate immune activation and inflammation in COVID-19 patients [13], which supports the hypothesis that IL-10 may play a pro-inflammatory and immune-activating role in COVID-19 pathogenesis.

In support of this hypothesis, first, the inflammatory/immune-stimulating cytokines discussed in the previous text (IL-2R α , IL-4, IL-7, IL-18, IFN- γ , GM-CSF, TNF- α , and chemokines IP-10 and CXCL9) are elevated in peripheral blood in severe/critically ill COVID-19 patients [1,3,4]. Second, severe/critically ill COVID-19 patients bear circulating hyperactivated and proliferating cytotoxic CD8⁺ T cells despite a total reduction in peripheral CD8⁺ T-cell count [14]. Furthermore, correlating with high serum IL-10 in severe/critically ill COVID-19 patients, the percentages of IFN- γ -producing effector CD4⁺ and CD8⁺ T cells can be increased in peripheral blood [6]. Third, as COVID-19 disease progresses, exhausted PD-1⁺TIM3⁺CD8⁺ T cells in the peripheral blood of patients have been found to increase, and these correlate with serum IL-10 concentrations in COVID-19 patients, suggesting a role of

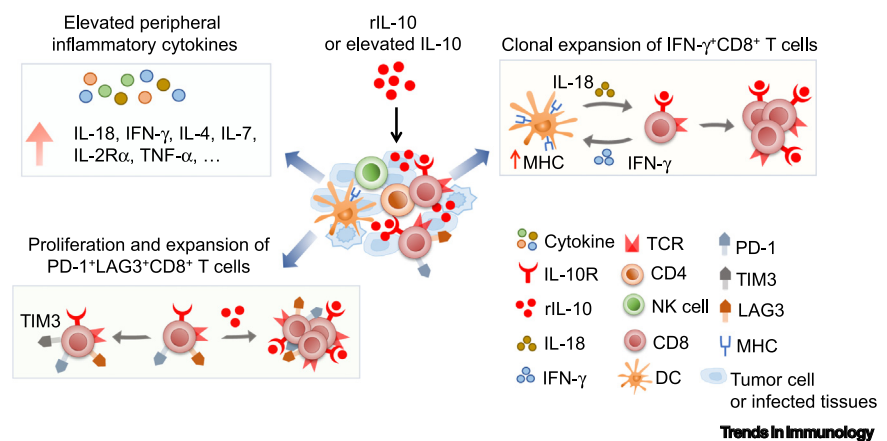


Figure 1. The Proinflammatory and Immune-Activating Role of IL-10 in Humans. Recombinant (r)IL-10 or elevated endogenous systemic IL-10 stimulates inflammatory cytokine production and directly activates and promotes effector CD8⁺ T cell proliferation. The combined effect may play a detrimental role in COVID-19 pathogenesis. Abbreviations: CD4/CD8, CD4⁺/CD8⁺ T cells; DC, dendritic cell; MHC, major histocompatibility complex; NK cell, natural killer cell; TCR, T cell receptor.

IL-10 in T cell exhaustion, presumably via overactivation and proliferation [5]. Such immune features in severe/critically ill COVID-19 patients with highly elevated systemic IL-10 have led us to speculate that IL-10 might play a pathological role in COVID-19 disease progression. However, this hypothesis remains to be rigorously tested.

In this scenario, how might elevated IL-10 contribute to COVID-19 mortality – if at all? The immunopathological pathway leading to patient death following SARS-CoV-2 infection can be divided into three stages: initiation, amplification, and consummation [13]. We propose that early induction of IL-10 upon SARS-CoV-2 infection during the initiation phase in the lung might indeed represent a negative feedback mechanism that serves as a countermeasure to inflammation caused by other proinflammatory mediators. However, as endogenous IL-10 production increases, we speculate that it might function as an immune activating/proinflammatory agent that stimulates the production of other mediators of the cytokine storm (Figure 1). As reported for human endotoxemia [12], IL-10 might also amplify the **viral sepsis**-related hyperinflammation observed in some severe/critically ill COVID-19 patients [15]. Because IL-10 directly expands cytotoxic effector CD8⁺ T cells in human studies, hyperactivation of adaptive immunity in COVID-19 patients might contribute to exacerbating disease severity. Although this possibility remains conjectural, we posit that the combined effects of IL-10 in promoting systemic inflammatory cytokine production and stimulating T cell activation and proliferation in COVID-19 patients might contribute to a lethal immunopathological process.

Currently, clinical trials are underway to test the therapeutic efficacy of blocking agents, either alone or in combination with more than ten inflammatory mediators reported as highly elevated in COVID-19 patients presenting a cytokine storm (discussed in [13]). Preliminary results from using blocking/neutralizing antibodies against IL-6/IL-6R, GM-CSF, and IL-1 suggest that further improvement is necessary to lower mortality in COVID-19 patients [13]. By attempting to block its pathological proinflammatory function, we suggest that IL-10 might constitute a potential target to reduce COVID-19 mortality. As such, the timing of blocking IL-10 activity in severe/critically ill COVID-19 patients might be crucial [13]. We propose that using a neutralizing antibody to block IL-10 to limit its potential immune-activating effects in the initiation phase of COVID-19 may be worth testing. Furthermore, we argue that combinatorial targeting of multiple proinflammatory mediators including IL-10, chemokines, IL-6, and IL-1 might be necessary to substantially reduce mortality in severe/critically ill COVID-19 patients. Evidently, the potential roles of systemically elevated IL-10 in COVID-19 pathogenesis and putative treatments warrant robust experimental validation, but certainly merit further attention.

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¹Zhuhai Interventional Medical Center, Zhuhai Precision Medical Center, Zhuhai People's Hospital, Zhuhai Hospital Affiliated with

Jinan University, Zhuhai, Guangdong Province, 519000, PR China

²First Affiliated Hospital, China Medical University, Shenyang, PR China

³Department of Immunology, Duke University Medical University Medical Center, Durham, NC 27710, USA

*Correspondence:

luligong1969@126.com (L. Lu) and
youwen.he@duke.edu (Y.-W. He).

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