COMMENT

IL-33 in COVID-19: friend or foe?

Yuejin Liang[1,2], Yiyue Ge[3] and Jiaren Sun[1,2]

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The COVID-19 pandemic, caused by the highly transmissible and pathogenic severe acute respiratory syndrome coronavirus 2 (SAR-CoV-2), has led to more than 2.7 million deaths worldwide as of March 2021. Although considerable efforts are underway to reveal the immunopathology of COVID-19, the key factors and processes that initiate hyperinflammatory responses and cause severe clinical outcomes in certain individuals remain unclear. The damage-associated molecular pattern (DAMP) molecule IL-33 belongs to the IL-1 family and has been recognized as an alarmin that indicates cellular damage or infection. Full-length IL-33 requires cleavage by proteases to generate its mature bioactive form, which can bind to the ST2 receptor (also known as IL-1RL1), leading to activation of the NF-κB pathway in various innate and adaptive immune cells. The relatively high abundance of IL-33 in epithelial and endothelial cells accounts for its proinflammatory role in respiratory diseases.[1] Recent observations have revealed that serum IL-33 is upregulated in elderly patients with COVID-19 and associated with adverse outcomes.[2,3] The increased IL-33 levels in severe infection could result from epithelial damage caused by strong interactions between the airway epithelium and activated immune cells. SARS-CoV-2-derived papain-like protease (PLpro), a powerful inducer of IL-33 in epithelial cells,[4] may also trigger epithelium-derived IL-33 to initiate inflammatory responses in the lungs. To test whether SARS-CoV-2 infection induces IL-33 expression in epithelial cells, we infected two human epithelial cell lines, Fadu and LS513, with SARS-CoV-2 (viral strain: BetaCoV/JS02/Human/2019, isolated by Jiangsu Provincial Center for Disease Control and Prevention, China) in vitro. Our results demonstrated significant increases in IL-33 transcript levels in both cell lines at 72 h postinfection (Fig. 1A). Therefore, we provide evidence for the first time that SARS-CoV-2 infection promotes IL-33 expression in human epithelial cells. Clinically, transcriptomic analysis of bronchoalveolar lavage fluid from COVID-19 patients demonstrated strong upregulation of IL-33,[5] indicating the induction of IL-33 in pulmonary recruited cells (e.g., macrophages) after infection. Moreover, a recent preprint reported that a SARS-CoV-2 spike peptide mixture could induce IL-33 secretion in the culture supernatant of PBMCs from seropositive individuals,[6] suggesting that immune cells may also be a source of IL-33 in COVID-19. However, it is not clear whether IL-33 is secreted by activated immune cells or is directly released due to cell death.

Upon binding with a receptor complex composed of ST2 and IL-1RAcP, mature IL-33 boosts type-2 immunity via the activation of eosinophils, mast cells, M2 macrophages, T helper 2 cells and group 2 innate lymphoid cells (ILC2s).[1] Compared with severe acute respiratory syndrome coronavirus (SAR-CoV), SAR-CoV-2 favors a cytokine storm composed of low levels of type 1 cytokines (IL-12p70 and IL-15) but high expression of Th2/9 cytokines (IL-4, IL-9, IL-10, IL-13 and TGF-β).[7] Elevated type 2 cytokine levels correlated with an increased number of ILC2s in COVID-19 patients[3] and may contribute to the differentiation of pathogenic γδ T cells (IFN-γ^{low} GM-CSF^{high}). Therefore, an elevation in IL-33 in the lungs following SAR-CoV-2 infection might be the driving force of type 2 immune cytokines and account for respiratory immune dysregulation. Moreover, IL-33-dependent lung-resident ILC2s can modulate NK cell innate immunity by suppressing IFN-γ production and cytotoxic functions,[8] leading to an impaired NK cell responses against SARS-CoV-2 infection. In addition to NK cells, IL-33 inhibits innate immunity in respiratory viral infection by degrading IL-1 receptor-associated kinase (IRAK1) and viperin in plasmacytoid dendritic cells, leading to TLR7 hyporesponsiveness.[9] As TLR7 may be necessary for recognition of the SARS-CoV-2 genome and production of antiviral type I interferon,[10] IL-33 may dampen innate antiviral immunity and delay viral clearance in COVID-19 patients.

Prominent neutrophil infiltration has been reported in severe COVID-19 patients, and a high neutrophil-to-lymphocyte ratio (NLR) is a predictor of in-hospital death.[11] Notably, IL-33 promotes rapid neutrophil migration via macrophage-derived CXCL1 and CXCL2, whereas neutrophil elastase and cathepsin G further contribute to IL-33 processing and maturation to exacerbate inflammatory responses. It is plausible that pathogenic γδ17 T cells may also accelerate neutrophil recruitment to the lungs via IL-17 production. Furthermore, immature neutrophils have been reported in severe COVID-19 cases.[12] Neutrophil dysregulation may be attributed to increased IL-33/ILC2 responses, since IL-33 can educate neutrophils towards a unique immunosuppressive phenotype via ILC2s and dampen the appropriate antiviral T cell immune response,[13] which is potentially involved in the control of SARS-CoV-2 infection. Importantly, elevated IL-33 levels and the associated type 2 immunity in chronic viral infection are considered potential inducers of pulmonary fibrosis, which is a recognized sequelae of acute respiratory distress syndrome (ARDS) observed in approximately 40% of COVID-19 patients.[14] Therefore, blockade of the IL-33/neutrophil feedback loop using IL-33- or ST2-neutralizing antibodies might be a novel therapeutic strategy for severe COVID-19 patients. Encouragingly, a phase II clinical trial of Astegolimab (anti-ST2) treatment in patients with severe COVID-19 pneumonia is close to completion (ClinicalTrials.gov Identifier: NCT04386616).

Although increased IL-33 levels are considered a predictor of severe COVID-19, their precise roles in different stages of disease are still unclear (Fig. 1B). Individuals with relatively mild COVID-19 symptoms exhibit increased numbers of Treg cells, which are associated with the resolution of inflammation. In addition to proinflammatory activity, IL-33 may drive $ST2^{+}$ regulatory T cell

[1]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA; [2]Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, USA and [3]NHC Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China
Correspondence: Yuejin Liang (yu2liang@utmb.edu) or Jiaren Sun (jisun@utmb.edu)
These authors contributed equally: Yuejin Liang, Yiyue Ge

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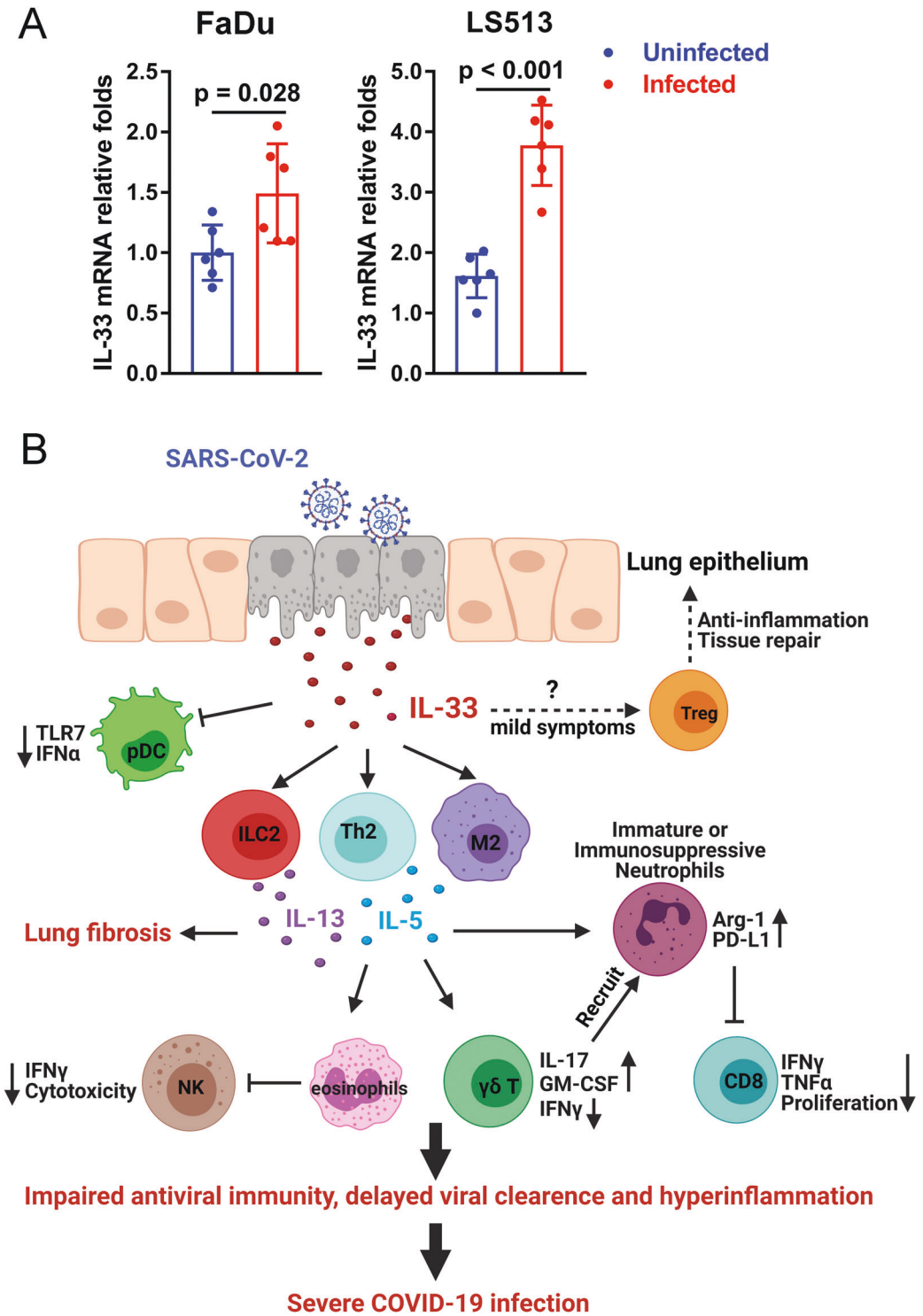

Fig. 1 The potential role of IL-33 in COVID-19. **A** Upregulation of IL-33 in human epithelial cells by SARS-CoV-2 infection. Human epithelial cell lines (FaDu and LS513) were infected with SARS-CoV-2 (0.01 $TCID_{50}$/cell, viral strain: BetaCoV/JS02/Human/2019). Total RNA was extracted at 72 h postinfection, and IL-33 transcript levels were analyzed by real-time PCR. Primers: IL-33 Forward-GTGACGGTGTTGATGGTAAGAT, IL-33 Reverse-AGCTCCACAGAGTGTTCCTTG; and β-actin Forward-ACCAACTGGGACGACATGGAGAA, β-actin reverse-GTGGTGGTGAAGCTGTAGCC. A two-tailed Student's *t* test was used for statistical analysis. **B** Immune modulation by IL-33 in COVID-19. SARS-CoV-2 infection triggers IL-33 release from damaged epithelial cells in the lung. IL-33 initiates type 2 immune responses via the activation of M2 macrophages, T helper 2 cells and type 2 innate lymphoid cells, leading to impaired antiviral activities of plasmacytoid dendritic cells, NK cells and CD8 T cells, as well as the dysregulation of neutrophils. Dampened antiviral immunity results in delayed viral clearance and hyperinflammation in patients with severe COVID-19

(Treg) expansion, inhibit innate γδ T cell responses, and restore respiratory tissue homoeostasis in patients who develop asymptomatic or mild disease. Notably, IL-33 is critical for antiviral CD8 T cell responses to persistent infection and may contribute to elimination of the virus.[15] Additional animal studies and clinical trials are essential for us to better understand the precise role of IL-33 and how manipulation of the IL-33/ST2 axis could be an effective therapeutic strategy for COVID-19 treatment.

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AUTHOR CONTRIBUTIONS

Y.G. designed and completed the in vitro experimental work. Y.L. and J.S. wrote this manuscript. All authors approved the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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