Can immunological manipulation defeat SARS-CoV-2? Why G-CSF induced neutrophil expansion is worth a clinical trial

G-CSF treatment against COVID-19

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INTRODUCTION

The rapid global spread of SARS-CoV-2 infection has caused the COVID-19 pandemic. According to the news release from Johns Hopkins Coronavirus Resource Center in mid-October 2020, the number of infected patients was above 37 million and that of the dead, above one million worldwide. Although various vaccines against SARS-CoV-2 are being produced or under clinical trials, they are not available yet. Likewise, there is a lack of effective drugs. Under such circumstances, immunity-boosting appears to be a relevant alternative. Some patients with this disease develop pneumonia, while others develop only symptoms of limited severity. Inter-individual differences in the immunological response to the virus may be responsible for the low severity of the disease in the latter group. Human beings combat pathogenic microbes through activation of innate immunity, followed by the development of adaptive immunity. Here, I present an immunological approach to eradicating SARS-CoV-2 infection.

NEUTROPHILS ARE CRUCIAL AGENTS IN THE BODY’S DEFENSE AGAINST VIRAL INFECTIONS

Neutrophils as well as dendritic cells are major components of the innate immune system and are at the forefront of the battle against pathogenic microbes. Since neutrophils predominantly target extracellular microbes via phagocytosis, their role in viral clearance has
not attracted attention, and the mechanism of this clearance remains largely unclear\(^{[1,12]}\). However, the following phenomena suggest the involvement of neutrophils in viral clearance. Neutropenia is associated with enhanced influenza viral replication in the respiratory tract and facilitates the progression of mild influenza to severe clinical disease.\(^{[13]}\) Approximately 80% of the cells infiltrating the lower respiratory tract infected with respiratory syncytial virus are neutrophils, which can limit viral replication and spread.\(^{[4]}\) The Influenza virus causes neutrophil dysfunction, which leads to insufficient clearance of secondary bacterial infections.\(^{[5]}\) In addition, neutrophil extracellular traps (NETs) were found to be a host defense strategy against invading pathogens. NETs, which were discovered in 2004, are the extrusions comprising chromatin (DNA and histones) in a net-like structure along with neutrophil-derived enzymes and mitochondria or their fragments. NETs trap viruses as well as bacteria and fungi and eradicate or inactivate them via myeloperoxidase and α-defensin.\(^{[8]}\) The viruses suppressed by NETs include dengue virus,\(^{[9]}\) coxsackievirus B3,\(^{[10]}\) chikungunya virus,\(^{[10]}\) human immunodeficiency virus,\(^{[8]}\) and SARS-CoV-2.\(^{[11,12]}\) Furthermore, NETs have been reported to promote Th17 induction.\(^{[13]}\) Th17 cells play a critical role in host defense against SARS-CoV-2, which is described in detail in this study. The facts described above may resolve the ambiguity concerning the involvement of neutrophils in viral clearance and alleviation of disease symptoms.

**IL-17A IS AN INDISPENSABLE PREREQUISITE FOR THE ERADICATION OF PATHOGENIC MICROBES**

Neutrophils\(^{[14]}\) and Th17 cells, as well as γδ T cells, group-3 innate lymphoid cells, CD3\(^+\) invariant natural killer cells, and NK cells, produce IL-17A.\(^{[15]}\) An important role of IL-17A in the early host defense against pathogenic microbes has been recognized by the fact that humans with genetic abnormalities in the synthesis of IL-17A, IL-17A receptor (IL-17AR), and STAT3 are particularly susceptible to bacterial or fungal infections.\(^{[15–18]}\) For example, patients with mutations in RAR-related orphan receptor C (RORC), which encodes ROR\(_{yt}\), a key transcriptional factor regulating IL-17 production, have increased susceptibility to *Mycobacterium tuberculosis* infection.\(^{[19]}\) Similarly, mice genetically deficient in IL-17AR and mice overexpressing soluble IL-17AR are sensitive to intranasal *Klebsiella pneumoniae* challenge, with 100% mortality.\(^{[20]}\)

Influenza virus infection induces IL-17A expression, and IL-17A-deficient mice showed lower survival rates and increased viral titers than did wild-type mice.\(^{[21]}\) Additionally, intranasally inoculated enterovirus caused airway inflammation effected mainly by neutrophils and monocytes in mice, and high levels of IL-17A mRNA expression and IL-17A protein were observed in the BAL inflammatory cells of these mice compared with those in the cells from the control mice.\(^{[22]}\) Inoculation with rhinovirus showed a similar result. These facts suggest that neutrophils produce IL-17A at virus-infected sites.

**TH17 CELLS PARTICIPATE IN INTRACELLULAR PATHOGEN KILLING AFTER CONVERSION INTO T\(_{\text{RM}}\) CELLS AND THEN EX-TH17 CELLS THAT PRODUCE IFN-γ**

To date, two distinct effector cell subsets, Th1 and Th2, have been recognized. Th1 cells, which produce IFN-γ, mediate protection against intracellular pathogens, and Th2 cells, which produce IL-4, IL-5, and IL-13, mediate the clearance of extracellular pathogens. Th17 cells represent the third subset of Th cells. In addition to producing IL-17, Th17 cells produce IL-21 and IL-22.\(^{[22]}\) Th17 cells appear as an early response to pathogens that are not eradicated by Th1- or Th2-type immunity.\(^{[24]}\)

While an important role of Th17 cells in the host defense against pathogenic microbes has been recognized, their pathogen-killing mechanism remains unclear. Many researchers reported that under certain conditions, Th17 cells, due to their plasticity, are converted into cells with the Th1 phenotype.\(^{[25–29]}\) Furthermore, Anezcuza Vesely et al.\(^{[31]}\) using IL-17A tracking-fate-mouse models, demonstrated that a significant fraction of lung CD4 tissue-resident memory T cells (T\(_{\text{RM}}\) cells) were derived from IL-17A-producing effector cells following immunization with heat-killed *K. pneumoniae*; this suggests that natural Th17 (nTh17) cells at the infection site are converted to T\(_{\text{RM}}\) cells upon encounter with microbes. These T\(_{\text{RM}}\) cells continue to exist as ex-Th17 cells and can express IFN-γ during a memory response. It is also known that T\(_{\text{RM}}\) cells play a pivotal role in mucosal immunity.\(^{[27,32–34]}\)

The scenario described above suggests that once nTh17 cells have been generated at the infection site, pathogenic microbes, whether intracellular or extracellular, can be removed by the Th1-type immunity as a natural progression.

**A SYSTEM TO GENERATE NTH17 CELLS AT SARS-COV-2 SITES OF INFECTION**

In psoriasis, nTh17 cells are produced continuously in the involved epidermis.\(^{[35]}\) This process can be a helpful reference when considering ways to generate nTh17 cells in lung tissue infected with SARS-CoV-2. The involvement of the IL-23/Th17 axis in the pathogenesis of psoriasis is widely recognized, and the administration of anti-IL-17A or anti-IL-23 antibodies drastically improves psoriasis. The mechanism of psoriasis is as follows: neutrophils are continuously attracted to leukotriene B\(_4\) present in the subcorneal portion; they are activated by the chemoattractant and by binding to the basement membrane when they infiltrate the epidermis; the infiltrated neutrophils in the epidermis secrete IL17A, which stimulates keratinocytes to express CCL20, a ligand for the chemokine receptor CCR6; nTh17 cells expressing CCR6 are attracted to CCL20 and expand in the presence of IL-23 and IL-17; keratinocytes perturbed by the neutrophil infiltration produce heat shock protein 70, which acts as an endogenous ligand for Toll-like receptor 4 (TLR4); accordingly, TLR4-mediated signaling stimulates keratinocytes to produce IL-23.
The process by which nTh17 cells are recruited to SARS-CoV-2 infection sites. Neutrophils, attracted by pathogen-derived chemoattractants and host chemokines expressed on the lung epithelium, secrete IL-17A. IL-17A stimulates lung epithelial cells to express CCL20, which in turn attracts nTh17 cells. nTh17 cells recruited to the infection site expand in the presence of IL-23 and IL-1β and secrete IL-17A, establishing a positive feedback loop, beginning with IL-17A production by neutrophils and ending in IL-17A production by Th17 cells. As Th17 cells recruit neutrophils, this loop can also be regarded as a neutrophil supply system.

Considering the above description, COVID-19 and psoriasis present opposite neutrophil dynamics; in psoriasis, excessive neutrophils infiltrate the lesion, whereas in COVID-19, neutrophil supply is insufficient.

G-CSF-INDUCED NEUTROPHIL EXPANSION MAY BE EFFECTIVE AGAINST COVID-19

Patients with severe COVID-19 may demonstrate an impaired ability to (A) generate nTh17 cells or (B) convert nTh17 cells into ex-Th17 cells. In case (A), administration of IL-17A or G-CSF to COVID-19 patients would compensate for this impaired ability. Ishikawa et al. reported that neutrophil dysfunction occurs in influenza virus infection—a situation attributed to insufficient G-CSF production. The mechanism by which IL-17A induces the accumulation of nTh17 cells at the infection site can be deduced from my previously published theory. Briefly, IL-17A stimulates bone marrow stromal cells to secrete G-CSF, and G-CSF promotes neutrophil expansion and egress from the bone marrow to the bloodstream, resulting in the accumulation of neutrophils at the infection site. IL-17A-mediated...
granulopoiesis has already been confirmed in mice.\(^{[40]}\) However, IL-17A cannot currently be used as a drug, but G-CSF is utilized clinically. To date, G-CSF has been used exclusively for chemotherapy-associated neutropenia in patients with malignancies,\(^{[41]}\) and also for a rare adverse reaction: chemotherapy-unrelated, drug-induced agranulocytosis.\(^{[42]}\) However, despite the beneficial effects of G-CSF on these diseases, it should be noted that G-CSF administration would exacerbate diseases in which neutrophil infiltration plays a key role. Therefore, although a robust immune response is required for efficient viral clearance, the response may also trigger or enhance chronic inflammation.

**CONCLUSION**

Here, I present the hypothesis that G-CSF administration may be effective against SARS-CoV-2 infection. While the important role of Th17 cells in the host defense against pathogenic microbes is recognized, the mechanism by which Th17 cells contribute to pathogen killing is unclear. Recently, however, many researchers have reported the conversion of Th17 cells into cells with a Th1-like phenotype. Furthermore, Amezcuca Vesely et al. demonstrated that Th17 cells are converted into a long-lasting ex-Th17 population with the ability to express IFN\(\gamma\). Therefore, it may be possible to eradicate SARS-CoV-2 by generating Th17 cells at the infection site via G-CSF administration. Treatment with G-CSF is unique in terms of utilizing neutrophils, epithelial cells, and nTh17 cells in combination. I believe that it is worth conducting a clinical trial for evaluating the effects of G-CSF administration on patients infected with SARS-CoV-2.

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I am the sole contributor of this manuscript.

**DATA AVAILABILITY STATEMENT**

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

**CONFLICT OF INTEREST**

I declare that I have no conflict of interest.

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APPENDIX

In this study, I used the term “IL-17A” instead of “IL-17” because IL-17A is the prototype family member, and the use of “IL-17” is justified to identify “IL-17A” in the literature.