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EDITORIAL

Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: A promising inhibitory strategy

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SARS-Cov-2 infection causes local and systemic inflammation mediated by pro-inflammatory cytokines and COX-2 eicosanoid products with metabolic dysfunction and tissue damage that can lead to patient death. These effects are primarily induced by interleukin (IL)-1 and IL-6, cytokines which are involved in the elevation of hepatic acute phase proteins and fever. IL-1 has a broad spectrum of biological activities and participates in both innate and acquired immunity. In infections, IL-1 induces gene expression and synthesis of several cytokines/chemokines in both macrophages and mast cells (MCs). The activation of MCs triggers the secretion of mediators stored in the granules, and the *de novo* synthesis of pro-inflammatory cytokines. In microorganism infections, the release of IL-1 macrophages acts on adhesion molecules and endothelial cells, leading to hypotension and septic shock syndrome. IL-1 activated by SARS-CoV-2 stimulates the secretion of IL-6 and TNF, a pro-inflammatory complex that can lead to cytokine storm and be deleterious both in the lung and systemically. In SARS-CoV-2 septic shock, severe metabolic cellular abnormalities occur which can lead to death. Here, we report that SARS-CoV-2 induces IL-1 in macrophages and MCs causing the induction of gene expression and activation of other pro-inflammatory cytokines. Since IL-1 is toxic, its production from ubiquitous MCs and macrophages activated by SARS-CoV-2 can also provoke both gastrointestinal and brain disorders. Furthermore, in these immune cells, IL-1 also elevates nitric oxide, and the release of inflammatory arachidonic acid products such as prostaglndins and thromboxane A2. All together these effects can generate cytokine storm and be the primary cause of severe inflammation with respiratory distress and death. Although IL-1 administered in low doses may be protective, when it is produced in high doses in infectious diseases it can be detrimental, therefore, IL-1 blockade has been studied in many human diseases including sepsis, resulting that blocking it is absolutely necessary. This definitely nurtures hope for a new effective therapeutic treatment. Recently, two interesting anti-IL-1 cytokines have been widely described: IL-37 and IL-1Ra. IL-37, by blocking IL-1, has been observed to have anti-inflammatory

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action in rodents *in vivo* and in transfected cells. It has been reported that IL-37 is a very powerful protein which inhibits inflammation and its inhibition can be a valid therapeutic strategy. IL-37 is a natural suppressor of inflammation that is generated through a caspase-1 that cleaves pro-IL-37 into mature IL-37 which translocates to the nucleus and inhibits the transcription of pro-inflammatory genes; while IL-1Ra inhibits inflammation by binding IL-1 to its IL-1R receptor. We firmly believe that blocking IL-1 with an anti-inflammatory cytokine such as IL-37 and/or IL-1Ra is an effective valid therapy in a wide spectrum of inflammatory disorders including SARS-CoV-2-induced COVID-19. Here, we propos for the first time that IL-37, by blocking IL-1, may have an important role in the therapy of COVID-19.

In December 2019, a new SARS-CoV-2 virus was found to be the causative agent of coronavirus disease-19 (COVID-19), an acute respiratory distress syndrome (1). Last February, the Food and Drug Administration (FDA) announced a state of emergency for the severe COVID-19 disease induced by SARS-CoV-2 coronavirus-2. This is a highly transmissible and pathogenic virus that caused the pandemic that threatens the world population with potential degree of mortality (2). The pandemic is a global health crisis, with a high morbidity that engages health systems in diagnosis and treatment. SARS-CoV-2 belongs to a family of heterogeneous microorganisms capable of infecting many animals including humans. It can cause severe infections of the airways including lungs, and provoking cough, systemic inflammation, muscle aches, headaches and other symptoms (3). Additionally, it has recently been reported in thousands of patients that brain fog afflicts Covid survivors by impeding their ability to work and function in daily life. This occurs mainly in people who are not seriously ill and had no previous illness. The cause of this dementia is still unclear, although it can be attributed to inflammation of the blood vessels of the brain induced by pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF). Today, it is well-known that COVID-19 patients who are sicker may die from breathing difficulties due to the accumulation of edematous fluid in the lungs, and fibrosis (4).

Therapies based on the reduction of pro-inflammatory cytokines in COVID-19 and reduction of viral load is very important. Furthermore, preventing coronavirus-19 from entering the cells is one of the first therapeutic approaches, but also targeting IL-1 in COVID-19 pneumonia represents a rational and strategic intervention. Moreover, it is important to remember that in ubiquitous mast cells IL-1 is a strong inducer of IL-6 which is also a pro-inflammatory cytokine (5). Therefore, antibodies that can bind IL-6 can have a plausible blocking mechanism of action by reducing the severity of an advanced COVID-19 episode, especially if they can be administrated at the initial symptoms (6). However, a combination therapy with a cocktail of anti-virus and anti-inflammatory cytokines may be able to fight to SARS-CoV-2 infection more effectively.

IL-1 is the main cytokine that plays an important role in the TH1 response and innate immunity, and has the ability to induce other pro-inflammatory cytokines such as IL-6 and TNF. The maturation of IL-1 occurs through the cleavage by caspase-1. By inhibiting IL-1 with the IL-1 converting enzyme (ICE), suppression of this cytokine is achieved, an effect that can provoke immunosuppressive innate response and inhibition of inflammation.

Macrophages and COVID-19

Macrophages are immune phagocytic cells derived from blood monocytes, that play an important role in innate and adaptive immunity. Macrophages are activated by microorganisms, including pathogenic coronavirus. Activated macrophages engulf and kill the virus; these activities cause the secretion of harmful

pro-inflammatory cytokines that can induce a cytokine storm that can lead to patient death. IL-1 is mainly produced by activated mononuclear phagocytes the principal function of which is to mediate host inflammatory responses (7). The IL-1 receptor (IL-1R) domain in innate immune cells and Toll-Like receptors (TLRs) respond to microorganisms, including SARS-CoV-2, triggering an immune response. Microorganisms are recognized by the 11 human TLRs via leucine enzyme, resulting in signal transmission within the cell (8). Both IL-1R and TLRs recruit MyD88 causing the initiation of signal transduction. Therefore, IL-1R and TLR share similar functions and properties. Hence, blocking the IL-1 receptor or neutralizing TLRs cause an inhibition of inflammation, including that provoked by SARS-CoV-2, with therapeutic activity. In addition, caspase-1 is the intracellular cysteine protease that cleaves the immature IL-1 inactive precursor to active mature cytokines; therefore, inhibiting caspase-1 may also be beneficial in SARS-CoV-2 infection and inflammation (9). Blocking the IL-1 receptor with the IL-1 receptor antagonist (IL-1Ra) can slow viral inflammation, and reduce signs, symptoms, and progression of COVID-19 disease (10).

Mast cells (MCs)

MCs are effector cells with innate immune responses, present in all vascularized tissues, and represent the sentinels of innate immunity (11). MCs are IgE-dependent and traditionally intervene in allergic diseases by producing stored chemical mediators, but they can also participate in the defense against invading pathogens, such as viruses, by generating pro-inflammatory cytokines (12). Therefore, MCs activated by coronavirus-19 release histamine, proteases, arachidonic acid products (thromboxane A2), and numerous cytokines, such as IL-1, IL-6 and others (13). During acute infectious attack induced by SARS-CoV-2, MCs also intervene among the immune cells which contribute to increasing inflammation of the tissues, both in the initial phase with the production of chemical mediators, and later with the generation of pro-inflammatory cytokines (4). These cytokines together with others, such as TNF, IL-18 and

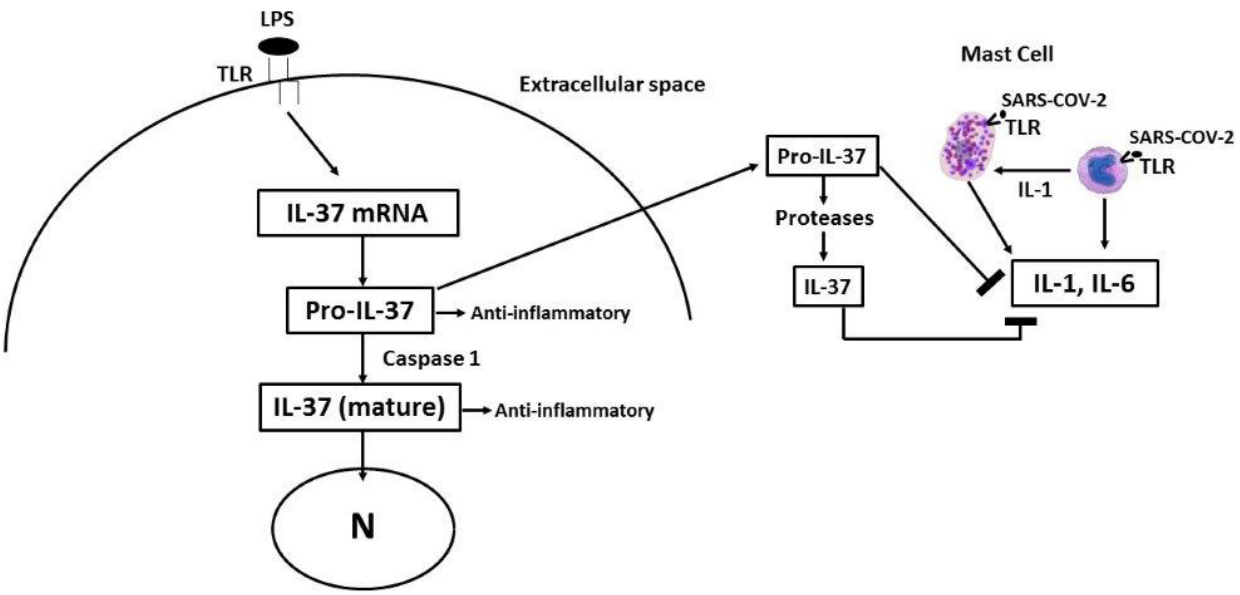

Fig. 1. *IL-1 released by mast cell and macrophage SARS-COV-2 activation induces IL-6 as well as causing cytokine storm which can be inhibited by IL-37.*

IL-33, can trigger strong reactions in the lungs attributable to the cytokine storm, resulting in toxic shock syndrome with falling blood pressure and patient death (14).

MCs also participate in septic shock where they produce TNF and IL-1 which act synergistically to induce hypotension in COVID-19 (9). Endotoxic shock, a hemodynamic decompensation, can occur after SARS-CoV-2 infection and then during COVID-19, with a systemic response and damage to microvascularization, reduced vascular flow, and increased leukocyte adhesiveness (14). However, the systemic inflammation triggered by SARS-CoV-2 causes tissue damage, cell death, and acute organ dysfunction, including lung.

Selective inhibition of MCs and their products can decrease SARS-CoV-2-induced acute tissue inflammation which can be one more tool to save the lives of COVID-19 patients (4). Activated MCs by multivalent antigen triggers early secretion of granule-stored chemical mediators, as well as late de novo synthesis of cytokines, including IL-6 (15). In COVID-19, IL-1 is one of the most important pro-inflammatory compounds which stimulate MCs to release newly synthesized IL-6 (in the absence of degranulation), aggravating inflammation. Inhibiting IL-1 and IL-6 would have a therapeutic benefit that could reduce not only the symptoms, but also the mortality of patients infected by SARS-CoV-2.

Interleukin-37

IL-37, formerly called IL-1 family member 7, is a natural cytokine with anti-inflammatory activity, whose overexpression in epithelial cells and macrophages suppresses the production of pro-inflammatory cytokines, including IL-1 (16). Therefore, IL-37 is a cytokine which can inhibit or even prevent a broad spectrum of inflammatory models (17). IL-37, one of the newest members of the IL-1 family cytokines, is a fundamental suppressor of innate and acquired immunities (18). In experimental models, IL-37 suppresses IL-1 and other related cytokines, an effect that can lead one to think that this cytokine may have clinical therapeutic implications (19). This anti-inflammatory cytokine has been reported to be generated by different tissues and cells such as macrophages, circulating monocytes, dendritic cells, B lymphocytes, and plasma cells (20). Activation of TLR or IL-1R with pro-inflammatory stimuli leads to the endogenous synthesis of the IL-37 precursor (pro-IL-37) and the activation of caspase-1 with consequent cleavage of the IL-37 precursor (21). From these reactions, mature IL-37 is formed which, associated with phosphorylated Smad-3, moves to the nucleus and is subsequently secreted by the cell together with the pro-IL37 form, both biologically active (22). In the exogenous synthesis of mature IL-37, cytoplasmic endocellular pro-IL-37 is secreted out of the cell and, through a still unknown protease, transforms into mature IL-37 with anti-inflammatory activity (22). IL-37 is applicable where inhibition of anti-inflammatory cytokines is ineffective; although this cytokine is used only in laboratory experiments and is not yet commercially available.

Here, we propose that IL-37, a strong inhibitor of IL-1, could have a therapeutic effect on COVID-19 patients through the inhibition of pro-inflammatory cytokines.

REFERENCES

1. Zou L, Ruan f, Huang MX, et al. sars-cov-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020 Mar 19; 382(12):1177-79.
2. Pavan K. Bhatraju, Bijan J, et al. Covid-19 in critically ill patients in the Seattle region - Case series. N Engl J Med 2020; NEJMoa2004500. Published online 2020 Mar 30. doi: 10.1056/NEJMoa2004500.
3. Kai L, Sirois P, Ai-Guo D, Liao DF. SARS-CoV-2 Infected cases with untraceable epidemic origin: its values, identification and tcm treatment in preventing recurrence. Digital Chinese Medicine 2020 Jun; 3(2):133-38.
4. Conti P, Gallenga CE, Tetè G, et al. How to reduce the likelihood of coronavirus-19 (CoV-19 or SARS-CoV-2) infection and lung inflammation mediated by IL-1. J

Biol Regul Homeost Agents 2020; 34(2):333-38.
5. Kandere-Grzybowska K, Letourneau R, Kempuraj D, et al. IL-1 induces vesicular secretion of IL-6 without degranulation from human mast cells. J Immunol 2003; 171(9):4830-36.
6. Saha A, Sharma AR, Bhattacharya M, Sharma G, Lee SS, Chakraborty C. Tocilizumab: A therapeutic option for the treatment of cytokine storm syndrome in COVID-19. Arch Med Res 2020; 51(6):595-97.
7. Wakabayashi G, Cannon JG, Gelfand JA, et al. Altered interleukin-1 and tumor necrosis factor production and secretion during pyrogenic tolerance to LPS in rabbits. Am J Physiol 1994; 267(1 Pt 2):R329-36.
8. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev 2018; 281(1):8-27.
9. Conti P, Caraffa A, Gallenga CE, et al. Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. J Biol Regul Homeost Agents 2020; 34(6).
10. Powers NE, Swartzwelter B, Marchetti C, et al. PASylation of IL-1 receptor antagonist (IL-1Ra) retains IL-1 blockade and extends its duration in mouse urate crystal-induced peritonitis. J Biol Chem 2020; 295(3):868-82.
11. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu Rev Immunol 2005; 23:749-86.
12. Barrett NA, Austen KF. Licensed to Ill: IL-9 Generation in immature mast cells permits food-elicited anaphylaxis. Immunity. 2015; 43(4):626-28.
13. Conti P, Caraffa A, Gallenga CE, et al. Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. J Biol Regul Homeost Agents 2020; 34(6).
14. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. J Infect Dis 1991; 163(6):1177-84.
15. Kandere-Grzybowska K, Kempuraj D, Cao J, Cetrulo CL, Theoharides TC. Regulation of IL-1-induced selective IL-6 release from human mast cells and inhibition by quercetin. Br J Pharmacol 2006; 148(2):208-15
16. Dinarello CA. The IL-1 family of cytokines and receptors in rheumatic diseases. Nat Rev Rheumatol 2019; 15(10):612-32.
17. Tsilioni I, Patel AB, Pantazopoulos H, Berretta S, Conti P, Leeman SE, Theoharides TC. IL-37 is increased in brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. Proc Natl Acad Sci U S A 2019; 116(43):21659-65.
18. Papathanasiou E, Conti P, Carinci F, Lauritano D, Theoharides TC. IL-1 Superfamily members and periodontal diseases. J Dent Res. 2020 Aug 6:22034520945209.
19. Cavalli G, Koenders M, Kalabokis V, et al. Treating experimental arthritis with the innate immune inhibitor interleukin-37 reduces joint and systemic inflammation. Rheumatology (Oxford); 55(12):2220-29.
20. Henry CJ, Casás-Selves M, Kim J, et al. Aging-associated inflammation promotes selection for adaptive oncogenic events in B cell progenitors. J Clin Invest. 2015; 125(12):4666-80.
21. Conti P, Lauritano D, Caraffa A, Gallenga CE, Kritas SK, Ronconi G, Martinotti S. Microglia and mast cells generate proinflammatory cytokines in the brain and worsen inflammatory state: Suppressor effect of IL-37. Eur J Pharmacol 2020; 875:173035.
22. Cavalli G, Dinarello CA. Suppression of inflammation and acquired immunity by IL-37. Immunol Rev 2018; 281(1):179-90.