EDITORIAL

INDUCTION OF PRO-INFLAMMATORY CYTOKINES (IL-1 and IL-6) AND LUNG INFLAMMATION BY CORONAVIRUS-19 (COVI-19 or SARS-CoV-2): ANTI-INFLAMMATORY STRATEGIES

P. CONTI¹, G. RONCONI², Al. CARAFFA³, C.E. GALLENGA⁴, R. ROSS⁵, I. FRYDAS⁶ and S.K. KRITAS⁷

¹Postgraduate Medical School, University of Chieti, Chieti, Italy; ²Clinica dei Pazienti del Territorio, Fondazione Policlinico Gemelli, Rome, Italy; ³School of Pharmacy, University of Camerino, Camerino, Italy; ⁴Department of Biomedical Sciences and Specialist Surgery, Section of Ophthalmology, University of Ferrara, Ferrara, Italy: ⁵University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA; ⁶Aristotelian University, Thessaloniki, Greece; ⁷Department of Microbiology and Infectious Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Macedonia, Greece

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Coronavirus-19 (COVI-19) involves humans as well as animals and may cause serious damage to the respiratory tract, including the lung: coronavirus disease (COVI-19). This pathogenic virus has been identified in swabs performed on the throat and nose of patients who suffer from or are suspected of the disease. When COVI-19 infect the upper and lower respiratory tract it can cause mild or highly acute respiratory syndrome with consequent release of pro-inflammatory cytokines, including interleukin (IL)-18 and IL-6. The binding of COVI-19 to the Toll Like Receptor (TLR) causes the release of pro-IL-18 which is cleaved by caspase-1, followed by inflammasome activation and production of active mature IL-1 β which is a mediator of lung inflammation, fever and fibrosis. Suppression of pro-inflammatory IL-1 family members and IL-6 have been shown to have a therapeutic effect in many inflammatory diseases, including viral infections. Cytokine IL-37 has the ability to suppress innate and acquired immune response and also has the capacity to inhibit inflammation by acting on IL-18Ra receptor. IL-37 performs its immunosuppressive activity by acting on mTOR and increasing the adenosine monophosphate (AMP) kinase. This cytokine inhibits class II histocompatibility complex (MHC) molecules and inflammation in inflammatory diseases by suppressing MyD88 and subsequently IL-1β, IL-6, TNF and CCL2. The suppression of IL-1β by IL-37 in inflammatory state induced by coronavirus-19 can have a new therapeutic effect previously unknown. Another inhibitory cytokine is IL-38, the newest cytokine of the IL-1 family members, produced by several immune cells including B cells and macrophages. IL-38 is also a suppressor cytokine which inhibits IL-1ß and other pro-inflammatory IL-family members. IL-38 is a potential therapeutic cytokine which inhibits inflammation in viral infections including that caused by coronavirus-19, providing a new relevant strategy.

The virus that causes severe acute respiratory syndrome, as many people now know, is called CoV-19 (coronavirus disease-19 or SARS-CoV-2), which

compares with the flu (1). CoV-19 potentially has a pandemic power that involves humans as well as animals and causes serious damage to the respiratory

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tract including the lung. The cases of infections that occurred a few months ago are all related to the Wuhan seafood wholesale market, which trades fish and a variety of live animals including poultry, bats and snakes, and not to the creation of CoV-19 in the laboratory, as someone imaginatively suggested. The pathogen has been identified in swabs performed on the throat and nose of patients who suffer from or are suspected of the disease (2). The factors that influence the survival of the virus on surfaces depend on various conditions such as the number of viruses deposited (viral load), the type of surface, suspension medium, temperature, relative humidity among others (3). Therefore, the exact survival time of CoV-19 on non-biological material is not known. The transmission by indirect contact, that is with the contamination of inanimate surfaces, is also uncertain, while direct contact transmission and air transport between persons close to each other is certain. Once CoV-19 contaminates the environment, it can penetrate the mucous membranes of the nose, eyes and/or mouth and then reach the vital organs including the lung (4). COV-19 in the open air does not survive more than approximately 9 days, although influenza viruses in general can remain active for months (5).

High temperature certainly reduces the replication of any species of virus. Currently, the inactivation temperature of CoV-19 is not entirely known, but some studies report that it can be inactivated at about 27° C, but resists the cold even below 0°C.

Human COV-19 are divided into low pathogenic and highly pathogenic; however, infection is not necessarily followed by the characteristic symptoms, but can be silent (6). Low pathogenic CoV-19 infect the upper respiratory tract and cause mild respiratory diseases, similar to colds; on the contrary, highly pathogenic ones. such as CoV-19. cause severe acute respiratory syndrome, mainly by infecting the lower airways with consequent pro-inflammatory cytokine release and pneumonia, which can be fatal, especially in debilitated subjects or those suffering from relevant pathologies (7). Furthermore, it is not excluded that several CoV-19 strains may exist, but this will be studied in the near future. The average age of the deceased patients is around 70, with greater morbidity and mortality among older subjects. CoV-19 affects more than 50% of male patients, while the female gender is less affected, perhaps because it is more immunologically covered since many immune cell genes reside on the X chromosome (8).

Severe pneumonia caused by coronavirus is often associated with rapid virus replication, infiltration of inflammatory cells and elevated responses to inflammatory proteins such as cytokines, resulting in damage of internal organs and acute respiratory stress syndrome. Children are less likely to become infected, however if they are, the effect is only slight. It is interesting that there have been very few cases of infection in children under the age of 15, probably because they are more protected from acquired maternal immunity and also because they have a larger and more efficient thymus than older people (9). The mortality rate due to CoV-19 is currently reported around 2-3%, slightly higher than a severe seasonal flu, while SARS had a mortality rate between 9% and 10%. On average, each virus carrier can potentially spread the infection to 2-3 or more other people; until this number drops below 1 it is likely that the epidemic will continue to spread. The prevention and control of infections include hand hygiene and protective devices of the mucous membranes and respiratory tract, as well as better cleansing and disinfection of environmental surfaces (10).

Therefore, in order to attack the efficiency of viral transmission, infected people must be placed in quarantine, implementing a containment strategy, which could include, where possible, isolation of the sick, including voluntary isolation at home, closing of schools, the use of collective means of transport and the frequency of crowded places. The new coronavirus is structurally related to the virus that causes severe acute respiratory syndrome (SARS) and currently there are no effective therapeutic elements that can block it, but only supportive drugs (11). Constant surveillance, timely diagnosis and intense research are important to understand the biology of the virus and its pathogenetic power as these elements allow to develop defense strategies. Many research studies are currently underway around the world to develop a CoV-19 vaccine that is expected in 3-9 months. Therapy today is carried

out with non-specific anti-viral drugs, including interferon and monoclonal antibodies (12). The antibodies taken from the serum of healed subjects have also proved to be helpful, although the efficacy was very mild.

Despite the rigorous global containment and the quarantine adopted in all the affected countries, the incidence of CoV-19 continues to increase all over the world, even if the percentage of deaths has been partially stemmed and is more related to individuals with previous diseases. To date, the countries affected are over 100 and the most affected are probably because the virus was prematurely imported from the infected countries. It is also probable that in some countries there were more carriers who transmitted the infection, and the percentage of deaths compared to the number of infected subjects was higher than in other countries, because the deaths that would have occurred in any case with normal seasonal flu were not separated in those percentages. The governments of the affected countries are preventing a possible pandemic by sensibly isolating the subjects positive to CoV-19. Certainly, in a few months the number of patients will decrease and with the passage of time the virulence will significantly diminish or die out, as happened with previous viral infections. In addition to the many negative effects related to this infection, a positive effect of the CoV-19 virus, as for other flu viruses, is to cause the increase of interferons (IFNs) in immune cells, which reinforces protection against cancer and autoimmune diseases (13).

CoV-19 induces pro-inflammatory cytokine generation and secretion: Proposed mechanisms

Pro-inflammatory cytokines, and in particular interleukin (IL)-1, are important mediators in local and systemic inflammation (14). Stimulated IL-1 in viral infections mediates lung and tissue inflammation, fever and fibrosis (15). Macrophages activated by CoV-19 are crucial for the pathogenesis of fibrosis, since macrophages perform phagocytic activity on debris of dead cells and tissues, releasing inflammatory substances. These reactions can be related to danger-associated molecular patterns (DAMPs) which have receptors called "pattern recognition receptors" (PRRs) which also include toll-like receptors (TLRs). Activation of TLR2, TLR3 or TLR4 by COV-19 leads to the release of inflammatory cytokines, including IL-1 β . Suppression of IL-1 has been shown to be effective in many inflammatory diseases including rheumatoid arthritis (16-17).

CoV-19 infection is catered for by a large replication of the virus in respiratory epithelial cells provoking acute inflammation and violent respiratory disease. In the place of infection proinflammatory cytokines are produced which worsen the clinical picture of the disease. Among these cytokines interferon-alpha (IFN α), tumor necrosis factor (TNF) and IL-1 are of considerable importance (2, 18-19)

Inflammation is mediated by pro-inflammatory cytokines including IL-1, IL-6, TNF and IL-8. IL-1 is the most studied cytokine with properties that are relevant to several inflammatory diseases including viral infections (20-21). The complex synthesis and release of inflammatory IL-1 occurs after the binding of CoV-19 to the Toll Like Receptor (TLR). Activation of this receptor causes a biochemical cascade that begins with the formation of pro-IL-1 cleaved by caspase-1, and followed by activation of inflammasome (22). High levels of adenosine triphosphate (ATP) (over 100 µM) are correlated with the activation of the P2X7 receptor, which belongs to the P2 (purinergic 2) receptor family, with pharmacological activity, cellular toxicity, and autoinflammatory mediation. The P2X7 receptor causes the activation of the inflammasome with the production of mature interleukins. Through procaspase-1 and Ca++ flow, there is the synthesis of IL- 1β in the lysosome (23). IL- 1β is then secreted outside the macrophage, mediating lung inflammation, fever and fibrosis, and provoking severe respiratory problems. Immune cells are attracted to the place of infection by IL-8, a chemokine that is generated at the inflammatory site. Pro-inflammatory cytokine levels are correlated with CoV-19 replication and disease. It is intuitive to think that anti-inflammatory cytokines, such as IL-1Ra, IL-37 or IL-38, can provide relief in both systemic inflammation and fever that occur after infection (23).

Inflammation occurs to restore the homeostasis

after CoV-19 infection and can be very harmful if not controlled. IL-1 generated during inflammation by immune cells, fibroblasts and endothelial cells is a response to the pathogenic virus and plays an important role in the pathogenesis of both acute and chronic obstructive respiratory disease and in the progression of pulmonary fibrosis (24). In CoV-19 infection, IFN type I is essentially produced, which has various effects on both innate and acquired immunity, but above all against viral infections. The generation of IFN, however, can cause side effects. such as inflammation, and also in some cases suppression of the immune system (25).

Therefore, in addition to anti-viral functions, IFNs can have harmful effects, such as induction of apoptosis, suppression of cell proliferation, tissue damage and inflammation. CoV-19 infection can be followed by a bacterial infection to aggravate the state of the patient and, in particular, lung function (26).

IL-37

IL-37 is a member of the IL-1 family with the ability to suppress innate and acquired immune response (27). IL-37 carries out its suppressive inflammatory activity by acting on the IL-R5 or IL-18R α receptor. IL-37 orchestrates several mechanisms that suppress inflammation and immunity and performs its immunosuppressive activity by acting on mTOR and increasing the AMP kinase of IL-37. This cytokine inhibits histocompatibility complex (MHC) molecules and inflammation in rheumatic diseases by suppressing IL-1 β , IL-6, TNF and CCL2 (28).

Since IL-37 inhibits IL-1, which induces other pro-inflammatory cytokines and inflammatory response, it is reasonable to think that IL-37 could inhibit inflammation induced by CoV-19 which provokes pro-inflammatory IL-1 family member release. The suppression with IL-37 of CoV-19induced inflammation opens a new interesting pathway for the use of this inhibitory cytokine in the treatment of viral and other inflammatory diseases.

IL-38

IL-38 is the newest member of the IL-1 family and is a cytokine of the IL-36 subfamily (29). IL-38 binds to the IL-1R6 receptor and causes suppression of inflammation. In vitro cultures of activated peripheral blood mononuclear cells (PBMCs) are inhibited by IL-38 in the production of several cytokines, including IL-1, IL-17 and IL-22. Circulating levels of IL-38 are elevated in many inflammatory diseases, as a natural response to inflammation, and mice that do not produce IL-38 are more sensitive to inflammation. IL-38 produced by various cells, including B cells and macrophages, is capable of reducing virus-induced inflammation and other inflammatory diseases. This reduction is due to its ability to inhibit pro-inflammatory cytokines such as IL-1, IL-6 and TNF. These cytokines are stimulated by CoV-19 and mediate lung inflammation, fever and fibrosis, therefore, the use of IL-38 as a potential therapeutic cytokine may be relevant and will provide a theoretical basis for developing of new strategies.

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