EDITORIAL

IL-1 induces throboxane-A2 (TxA2) in COVID-19 causing inflammation and micro-thrombi: inhibitory effect of the IL-1 receptor antagonist (IL-1Ra)

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IL-1 induces a significant number of metabolic and hematological changes. In experimental animals, IL-1 treatments cause hypotension due to rapid reduction of systemic blood pressure, reduced vascular resistance, increased heart rate and leukocyte aggregations. IL-1 causes endothelial dysfunction, the triggering factor of which may be of a different nature including pathogen infection. This dysfunction, which includes macrophage intervention and increased protein permeability, can be mediated by several factors including cytokines and arachidonic acid products. These effects are caused by the induction of IL-1 in various pathologies, including those caused by pathogenic viral infections, including SARS-CoV-2 which provokes COVID-19. Activation of macrophages by coronavirus-19 leads to the release of pro-inflammatory cytokines, metalloproteinases and other proteolytic enzymes that can cause thrombi formation and severe respiratory dysfunction. Patients with COVID-19, seriously ill and hospitalized in intensive care, present systemic inflammation, intravascular coagulopathy with high risk of thrombotic complications, and venous thromboembolism, effects mostly mediated by IL-1. In these patients the lungs are the most critical target organ as it can present an increase in the degradation products of fibrin, fibrinogen and D-dimer, with organ lesions and respiratory failure. It is well known that IL-1 induces itself and another very important pro-inflammatory cytokine, TNF, which also participates in hemodynamic states, including shock syndrome in COVID-19. Both IL-1 and TNF cause pulmonary edema, thrombosis and bleeding. In addition to hypotension and resistance of systemic blood pressure, IL-1 causes leukopenia and thrombocytopenia. The formation of thrombi is the main complication of the circulatory system and functionality of the organ, and represents an important cause of morbidity and mortality. IL-1 causes platelet vascular thrombogenicity also on non-endothelial cells by stimulating the formation of thromboxane A2 which is released into the inflamed environment. IL-1 is the most important immune molecule in inducing fever, since it is involved in the metabolism of arachidonic acid which increases from vascular endothelial organs of the hypothalamus. The pathogenesis of thrombosis,

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vascular inflammation and angiogenesis involves the mediation of the activation of the prostanoid thromboxane A2 receptor. In 1988, in an interesting article we reported for the first time that IL-1 induces thromboxane B2 (TxB2) releases in activated neutrophils and macrophages. An increase in thromboxane can induce leukocyte aggregation and systemic inflammation, which would account for the dramatic thrombi formation and organ dysfunction. Hence, IL-1 stimulates endothelial cell-leukocyte adhesion, and TXB2 production. All these events are supported by the large increase in neutrophils that adhere to the lung and the decrease in lymphocytes. Therefore, eicosanoids such as TxA2 (detected as TxB2) have a powerful action on vascular inflammation and platelet aggregation, mediating the formation of thrombi. The thrombogenesis that occurs in COVID-19 includes platelet and cell aggregation with clotting abnormalities, and anti-clotting inhibitor agents are used in the prevention and therapy of thrombotic diseases. Prevention of or induction of TXA2 avoids thrombi formation induced by IL-1. However, in some serious vascular events where TxA2 increases significantly, it is difficult to inhibit, therefore, it would be much better to prevent its induction and generation by blocking its inductors including IL-1. The inhibition or lack of formation of IL-1 avoids all the above pathological events which can lead to death of the patient. The treatment of innate immune cells producing IL-1 with IL-1 receptor antagonist (IL-1Ra) can avoid hemodynamic changes, septic shock and organ inflammation by carrying out a new therapeutic efficacy on COVID-19 induced by SARS-CoV-2.

Key words: COVID-19; IL-1; SARS-CoV-2; inflammation; thrombosis; thromboxane

It is now known to all that the new coronavirus SARS-CoV-2 infecting humans causes the COVID-19 disease (1). There are thousands of coronaviruses on our planet, some of which are responsible for the common cold and others which are harmless. Virus can be very aggressive and resistant to therapy, as can many bacteria, therefore, today there is substantial interest in viral pathogenesis and how to create new drugs that kill or prevent the spread of the microrganisms into the environment. SARS (severe acute respiratory syndrome) in 2002 caused hundreds of deaths (770) worldwide, but it could have caused many more if precautions had not been taken.

The novel coronavirus SARS-CoV-2 has created a worldwide pandemic with millions of infected and hundreds of thousands of deaths in many nations. Infection with SARS-CoV-2, an RNA virus which is about 29,900 bases long, is transmitted by carriers with symptoms such as fever and dry cough, but can also be transmitted by asymptomatic patients. Coronavirus-19 is constituted by RNA and N-protein inside, while the outside features present a membrane with E and M proteins, lipids and the spike (S) glycoprotein (2). The virus measures approximately 88 nm in diameter plus 50 nm spike protein, visible under electron microscope. SARS-CoV-2, which uses cellular metabolism to live, after entering the body through the nose and/or mouth, travels in the upper and lower airways by binding to the ACE2 receptor of lung cells; while it binds to the toll like receptor (TLR) on innate immune cells, including macrophages (3-4). The entry of the virus into the host cell stimulates the genes to the cell's ribosomes which cause the translation of proteins that create protective vesicles in the endoplasmic reticulum where, through the viral polymerase RNA, the virus can replicate. An infected cell can release hundreds of copies of the virus as well as interferon, an immune cytokine that alerts and stimulates B cells, T cells, neutrophils and macrophages to protect the host. B cells assisted by T helper cells generate neutralizing antibodies which, when they are efficient and numerous, block the spike protein of the virus (5). The activation of the immune cells that rush to the host's defense causes inflammation.

IL-1 can be generated by innate immune cells but also by many other non-immune cells. IL-1 is mainly a macrophage cytokine emerged as being pivotal, promoting and mediating inflammation. IL-1 is a member of the IL-1 family and its synthesis occurs after a microorganism activates the TLR expressed

on innate immune cells, including macrophages (6). The activation causes pro-IL-1 to accumulate in the cytosol which is secreted in many different ways. The NLRP3 inflammasome plays an important role in the generation of IL-1 causing inflammation, therefore its inhibition could help in thrombosis-mediated diseases which occur in COVID-19. The activation of NLRP3 inflammasome begins with the cleavage of pro-IL-1 by caspase-1 and ends with the release of mature IL-1 which is associated with inflammation. The active metabolites, known as eicosanoids which collectively include PGs, Txs, prostacyclins (PGI_2), LTs and lipoxins, are derived from membrane phospholipids and have biological activities.

In 1986, we showed for the first time that human recombinant IL-1 enhances TxA2, monitored as TxB2, in polymorphonuclear leukocytes and macrophages *in vitro*, demonstrating that not only IL-1 is capable of inducing inflammation by the activation of eicosanoids, but also causes neutrophil, macrophage and platelet aggregation *in vitro*, mediating thrombosis and inflammation (7). IL-1 induces COX-2 which causes an increases of arachidonic acid products, including PGE2 and thromboxane A2 (TxA2), detected as TxB2 (as TxA2 is unstable). SARS-CoV-2 infection causes neutrophilia and lymphopenia (4). The numerous neutrophils are activated both by coronavirus-19 and IL-1 secreted by mast cells and macrophages. Activation of neutrophil granulocytes by SARS-CoV-2 and IL-1 leads to the release of prostaglandins (PGs), leukotrienes (LTs) and Txs, which are highly inflammatory substances that contribute to the pathogenesis of COVID-19. These effects alone or in combination with others can be lethal for the patient. In addition, people affected by COVID-19 can have the so-called "cytokine storm" which causes excessive inflammation in the lungs leading to severe respiratory syndrome necessitating life-

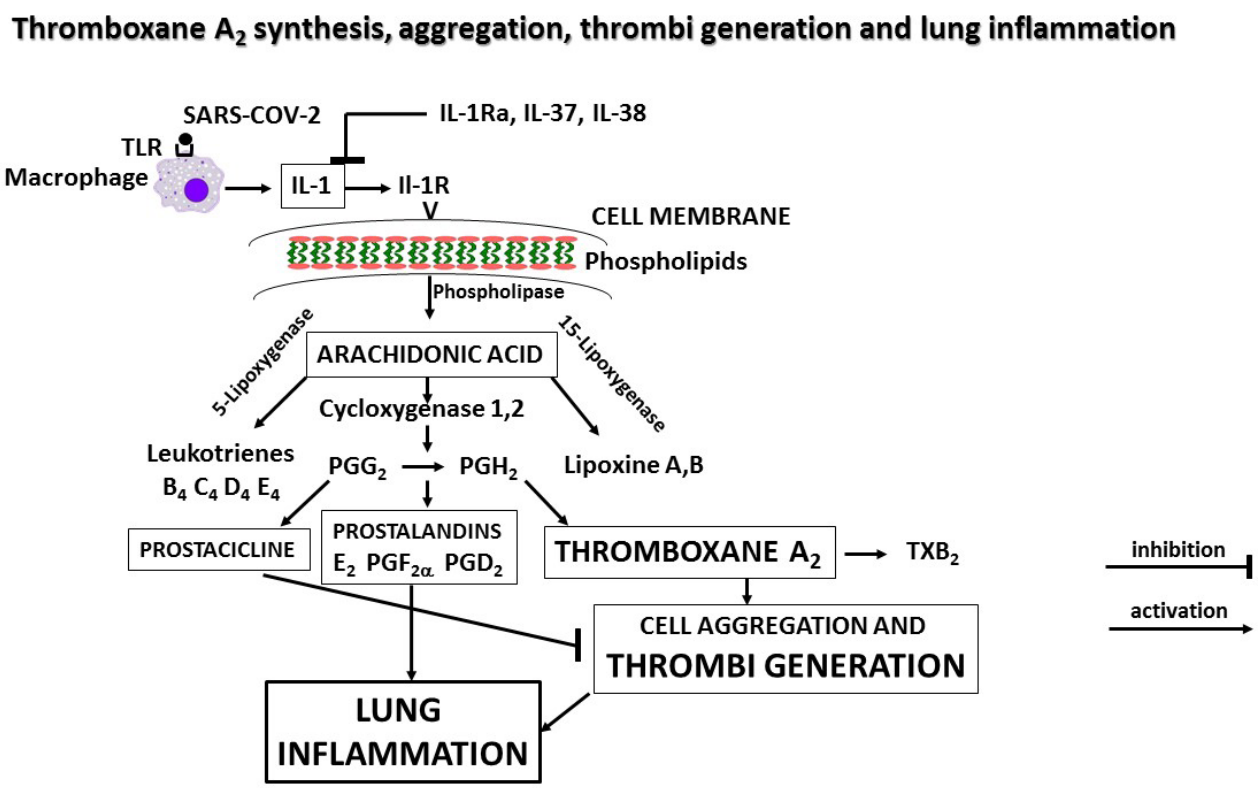

Fig. 1. *Generation of thrombi and lung inflammation by macrophage-secreted IL-1, through induction of thromboxane A2*

saving treatment (8). Thus, affected patients have severe disease and lower levels of protecting Treg cells, a phenomenon that occurs less in children who have better-functioning Treg cells.

Endotoxin released by microorganisms activates macrophages and neutrophils to produce inflammatory cytokines and eicosanoids such as TxA2 (9). TxA2 belongs to the family of arachidonic acid metabolites produced by the action of enzymes such as phospholipase A2, cyclooxygenase (COX)-1 and 2, and thromboxane-synthetase. TxA2 is not only generated by platelets, but also by immune cells such as macrophages, endothelial cells and neutrophils. Platelets can be activated by coronavirus-19 through the TLR, such as TLR4, resulting in the release of neurotransmitters such as substance P, histamine, epinephrine, etc. and they can also produce a series of proinflammatory mediators of both cytokine and chemokine types, such as IL-1, TGFβ1 and CXCL1, CXCL4 CXCL5, CCL3, respectively. In addition, platelets play a role in the regulation of tissue inflammation and may generate platelet-activating factor (PAF) and lipid pro-inflammatory mediators such as PGs and TxA2. In humans, activation of platelet alpha granules by SARS-CoV-2 induces the synthesis of IL-1β and TGFβ1, promoting pro-inflammatory action.

TxA2, after being released, is strongly involved in the formation of thrombi by acting on platelet and neutrophil granulocyte aggregation. TxA2 is a pro-inflammatory eicosanoid acting as a vasoconstrictor involved in various diseases such as myocardial infarction, stroke, bronchial asthma, allergies, kidney damage, angiogenesis, pulmonary hypertension and multi-organ failure (10). An alteration of TxA2 has been noted in various diseases including diabetes, coronary heart diseases, hypertension, inflammation, auto-immune disorders, atopic eczema, Alzheimer dementia, cancer, and in tissue infections.

SARS-CoV-2-induced COVID-19 disease causes a systemic inflammatory response mediated by IL-1 with severe pathological consequences that can lead to the death of the patient. It has recently been reported that several cytokines are capable of inhibiting IL-1, such as IL-37, IL-38 and IL-1 receptor antagonist (IL-1Ra), an effect that could aid in microorganism-induced inflammation. In fact, in viral infections, such as COVID-19 where IL-1 is synthesized, there is a high inflammation that is difficult to keep in check (Fig. 1).

In COVID-19, the inflammatory phenomenon can be countered in different ways by: i) preventing the entry of the coronavirus-19; ii) preventing the replication of the virus; iii) reducing the inflammatory reaction; iv) developing a vaccine with different types of viruses; v) weakening or inactivating SARS-CoV-2 with various pieces of the virus.

The action of coronavirus-19 on tissues triggers an inflammatory response mediated by the cytokine storm. The virus activates immune cells such as macrophages which essentially produce two highly inflammatory cytokines, such as IL-1 and IL-6, and cause lymphopenia. IL-1 is the main cytokine of inflammation and after being generated it induces other cytokines and itself in an autocrine–manner. Moreover, the administration in the liver of IL-1 and IL-6 in combination causes a synergistic pro-inflammatory stimulus on the release of serum amyloid A (SAA) (11). IL-1 provokes the activation of other pro-inflammatory compounds such arachidonic acid compounds including TxA2. In SARS-CoV-2 infection, in addition to the cytokine storm, there is also a storm of pro-inflammatory eicosanoids which includes the release of PGs, LTs and Txs that can mediate pain, fever, pulmonary fibrosis, thrombosis, and acute respiratory distress syndrome (12). Pro-inflammatory eicosanoids predominantly generated in the endothelium act in the vascular tone and mediate inflammation. Eicosanoid inhibitors could influence the inflammatory cascade and constitute an effective therapeutic approach also in the inflammation that occurs in COVID-19.

Viral infection causes cell death (apoptosis) and necrosis of T helper cells which should aid in the production of antibodies by B cells which should stem the pathogenesis and aid the survival of the host.

This editorial introduces new functions, including thrombus formation, due to TxA2 released by the neutrophils stimulated by IL-1-derived macrophage induced by SARS-CoV-2 in COVID-19. Furthermore, it proposes inhibition of inflammation through the use of anti-inflammatory cytokines as a new therapy.

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