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COVID-19: Pathogenesis and Role of IL-10 and TNF-α in Severe Cases

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Abstract:- COVID-19 remains as a health concern all around the world since World Health Organization (WHO) declared it as a pandemic in March 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is the pathogen responsible for COVID-19. It belongs to family Coronaviridae, human-to-human transmission happens through droplets and aerosols.

As of December 2021 there were around 278.5 million reported cases around the world, majority of which had recovered. However there were some cases in which progression of the disease became uncontrollable resulting in severe pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure, even death.

In mild or moderate cases human innate and adaptive immune responses are enough to fight the virus. Still in some patients immune system runs wild, producing enormous cytokines and other markers resulting in a condition of hyperinflammation and hypercytokinemia called the cytokine storm. Cytokine storm is regarded as the main reason for disease progression to severe COVID-19. It involves many pro-inflammatory markers, some of the more notable ones are interleukins and tumor necrosis factors. Interleukin 10 (IL-10) is suggested to have a role as an anti-inflammatory cytokine by a negative feedback mechanism while tumor necrosis factor-α (TNF- α) as a pro-inflammatory cytokine released by mainly monocytes or macrophages. This review highlights cytokine storm as well as IL-10 and TNF- α role in pathogenesis of severe COVID-19.

Keywords:- *COVID-19, Severe Case, IL-10, TNF- α, Cytokine Storm, Pathogenesis.*

I. INTRODUCTION

Several acute respiratory tract infection cases arose in December of 2019 in Wuhan City, Hubei, China which rapidly spread and became an epidemic. Pathogen responsible for this epidemic then recognized as a novel coronavirus which belongs to family of Coronaviridae and genus of Betacoronavirus.[1,2] This virus has about 80% of similarity with severe acute respiratory syndrome coronavirus (SARS-CoV) responsible for the pandemic in 2002—2003. World Health Organization (WHO) then named it as SARS-CoV2 at 12th of January 2020 and the disease caused by it as coronavirus disease 2019 (COVID-19) at 11th of February 2020.[1,3,4]

Initial spread of SARS-CoV2 was from a zoonotic transmission in a local seafood market in Wuhan City in 2019. Human-to-human transmission later recognized as the reason of community spread to around 200 countries around the world. WHO declared COVID-19 as a pandemic at 11th of March 2020.[1] Preliminary data from the Chinese Center for Disease Control and Prevention showed about 81% of all the COVID-19 cases were mild, 14% of those became severe pneumonia and 5% became acute respiratory distress syndrome (ARDS), sepsis, and/or multisystem organ failure (MOF).[2] As of December 2021 there were around 278.5 million reported cases around the world, 249.3 of which already recovered and 5.4 million reported dead.[5]

II. VIRAL INVASION INTO HOST CELLS

SARS-CoV2 comes into human body through droplets and aerosols. Droplet transmission occurs when a person is at a close distance to patients who is coughing or sneezing. Aerosol transmission reported to occur when aerosol producing procedures are carried out. These procedures are endotracheal intubation, bronchoscopy, open suctioning, nebulization, bag and mask ventilation, tracheostomy, and during cardiopulmonary resuscitation.[1] This virus has four structural proteins, the spike protein (S), the membrane protein (M), the envelope protein (E), and the nucleocapsid protein (N). The S protein located on the surface of the virus and protrudes to the outside. It has two functional subunits namely the S1 subunit which has a role in adhering to the host's cell by binding to a receptor and the S2 subunit which is responsible for penetration into the cell by fusing the virus and host's cell membranes.[1,2]

Angiotensin converting enzyme 2 (ACE-2) is an important enzyme in cardio-renal function to regulate blood pressure. ACE-2 catalyzes formation of angiotensin II from the angiotensin I.[3] It is known as the receptor which SARS-CoV2 binds to when the virus invades the host's cell. SARS-CoV2 attacks the upper and lower respiratory tracts partly because ACE-2 is highly expressed on the nasal ciliated and alveolar epithelial cells. However it is not impossible for other organs to be infected when viremia occurs because ACE-2 also found on tissues of small intestine, kidneys, heart, thyroid, testicles, and adipose.[2]

After binding to ACE-2, SARS-CoV2 will undergo two process of protease cleavage. They are cleavage on S1/S2 for priming and cleavage on S2 for activation of membrane fusion.[4] The virus then gets into the cell, releases its contents, and replicates its RNA. The N protein binds to the new RNA and the M protein facilitates integration with the cell's

endoplasmic reticulum. The new nucleocapsids then transferred to the cell's membrane through golgi vesicles and taken out of the infected cell through exocytosis. The new virus particle is now ready to infect other adjacent cells.[1]

III. HOST RESPONSE

After the virus invades the epithelial cells, local replication and propagation of the virus take place. In this stage the immune response is limited, although the viral load is not that high the patient is highly infectious. This occurs for several days and the virus can be detected by the nasal swab test. Virus then infects the upper respiratory tract by the conducting airways. In this stage symptoms like fever, malaise, and dry cough begin to appear. Immune response in this stage involves release of C-X-C motive chemokine ligand 10 (CXCL-10) and interferons. This response is enough to retain spread in most of the patients.[1]

SARS-CoV2 infection induces cell death and release of various damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Receptors on alveolar macrophages and endothelial cells like Toll-like receptors (TLRs) recognize PAMPs in the extracellular space, trigger induction of proinflammatory cytokines, and activate interferon regulatory factors. Nucleotide-binding domain leucine-rich repeat (NLR) proteins recognize DAMPs in the intracellular space and trigger inflammatory responses and conversion of proIL-1β to active IL-1β which represents activation of local inflammation without involving systemic inflammation. All of these responses increase secretion of proinflammatory cytokines and chemokines, such as IL-6, type II interferon (IFNγ), monocyte chemoattractant protein 1 (MCP1), interferon gamma-induced protein 10 (IP-10), and recruitment of macrophages and dendritic cells. Cytokine release, immune cell recruitment, and antibody neutralization contribute to successful viral clearance.[2]

Innate immune response to SARS-CoV2 involves neutrophils, eosinophils, basophils, monocytes, macrophages, lung epithelial cells, mast cells, and natural killer cells (NK cells). Dendritic cells (DCs) located in the airway epithelium, alveolar septa, pulmonary capillaries, and airway spaces will be activated and change to antigen-presenting cells (APCs) upon viral infection. APCs captures the virus and moves it to the lymph node where T helper cells become activated, differentiated to helper T cells (CD4), and cytotoxic T cells (CD8). CD4 undergoes further differentiation to Th1 and Th2. Th1 cells induce cellular immunity involving release of pro-inflammatory cytokines like IFN-γ, IL-1β, IL-12, and TNF-α. IFN-γ is reported to have benefits such as inhibition of viral replication and enhancement of antigen presentation. Th2 cells activate humoral immunity and production of antibodies, they induce release of anti-inflammatory cytokines like TGF-β, IL-4, IL-5, IL-9, IL-10 and IL-13.[6]

IV. CYTOKINE STORM AND PROGRESSION TO SEVERE COVID-19

Progression to a more severe disease like severe pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure happened to some cases in which immune system running wild with defective regulation that brought about different cytokines and inflammatory markers being released beyond necessary. This condition called cytokine storm involves pro-inflammatory cytokines and chemokines such as interleukins (IL-1β, IL-6, IL-12, IL-17, IL-18, IL-33), tumor necrosis factor-α (TNF- α), interferons (IFN-α, IFN-γ), transforming growth factor β (TGF β), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophages colony-stimulating factor (GM-CSF), CCL-2, CCL-3, CCL-5, CXCL-8, CXCL-9, CXCL-10, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α).[1,6] Other cytokines found elevated in severe cases are IL-2, IL-7, IL-8, and IL-10.[7]

Cytokine storm attracts neutrophils, CD4, and CD8 to come to the lung tissue and worsen the inflammation and tissue injury. Furthermore CD4 differentiates into pathogenic Th1, secrets GM-CSF, and induces CD14 and CD16 accelerating the inflammation.[7] Apoptosis of cells accompanied by release of viral particles that are ready to infect adjacent cells. Death of both type 1 and type 2 of pneumocytes leads to diffuse alveolar damage and become an acute respiratory distress syndrome.[1]

Secondary hemophagocytic lymphohistiocytosis (sHLH) is also induced by cytokine storm and could be fatal as it often leads to multi-organ failure.[8] sHLH is a hyperinflammatory syndrome with hypercytokinemia that is fulminant and fatal. Cytokines involved are IL-2, IL-7, GCSF, IP-10, MCP-1, and MIP-α.[3] Antibody-dependent enhancement (ADE) is also suspected as a factor induces severe inflammatory response as a study found that titers of IgG and total antibodies were higher in patient with severe disease.[9]

Lymphopenia is a clinical characteristic found in severe COVID19 cases but not in the mild ones. The numbers of CD4, CD8, NK cells, and B cells are remarkably reduced. Moreover there is sign of exhaustion that is up-regulation of exhaustion markers such as NKG2A receptors of NK cells and CD8. Findings from histochemical staining are decreased CD4 and CD8 in spleen and lymph nodes, infiltration of a lot of monocytes and macrophages but really few lymphocytes in the diffusely damaged lung.[6,7,9]

Complement system is an immune surveillance system that links the innate and adaptive immunity. Over activation of the complement system could be harmful as it induces inflammation both acute and chronic, endothelial cell dysfunction, and intravascular coagulation.[10] In COVID-19 complement system activation is likely via multiple pathways such as the infection of SARS-CoV2 itself, tissue damage, and apoptosis of cells. The protein S of SARS-CoV2 binds with mannose-binding lectin (MBL), induce activation of MBL-associated serine protease-2 (MASP2). MASP2 activates the complement and clotting pathways. The endothelium is

destroyed, procoagulant von Willebrand factor and FVIII are released resulting in a procoagulant state. Clinical manifestations of this state are increased D-dimer and thrombocytopenia which could progress to dangerous and life-threatening disseminated intravascular coagulation (DIC).[11]

V. ROLE OF IL-10 AND TNF-A IN SEVERE COVID-19

As SARS-CoV2 highly resemble SARS-CoV, cytokine storm in COVID-19 patients is similar to SARS-CoV infected patients. One thing that is unique to cytokine storm of COVID-19 is the remarkably elevated interleukin 10 (IL-10) level in severe COVID-19 patients. Recent studies showed that IL-10 concentrations were significantly higher in COVID-19 patients admitted in intensive care unit (ICU) compared to patients admitted to normal COVID-19 ward and correlated with other markers such as IL-6 and C-reactive protein (CRP). IL-10 was also associated with COVID-19 severity. A meta-analysis study involving more than 2000 COVID-19 patients showed that IL-6 and IL-10 could be used as a predictor for COVID-19 severity.[8,12,13,14]

IL-10 seemed to be elevated earlier than IL-6 in COVID-19 patients and only in severe cases but not in mild ones. IL-10 elevation was observed in the first two weeks of the disease. On the other hand increased IL-6 was found mostly in the fourth week of the disease.[12,13]

As to why increased IL-10 in COVID-19 patients differs from SARS-CoV infected patients is yet to be explained. Elevation of IL-10 is suggested as an anti-inflammatory or immune-inhibitory response of marked release of pro-inflammatory cytokine. It is regarded a negative feedback loop.[8,14] On the contrary IL-10 was also found to have an immune-stimulatory effect as administration of recombinant IL-10 resulted in increased level of LPS-induced IFN-γ in healthy volunteers. Current hypothesis is at the early stage of the disease IL-10 acts as an anti-inflammatory cytokine to counter elevation of pro-inflammatory cytokines in a negative feedback mechanism. However at later stage of the disease IL-10 acts as a pro-inflammatory cytokine and worsen the cytokine storm.[15]

TNF-α is a transmembrane precursor produced by monocytes or macrophages. Other immune cells such as T cells, B cells, mast cells, neutrophils, fibroblasts, and airway epithelial cells also produce it. TNF-α-converting enzyme (TACE) cleaves the TNF-α precursor and liberates TNF-α by binding to TNFR1 and TNDR2 that are membrane receptors on target cells. TNFR1 is expressed in the lymphoid system and almost all cells of the body whereas TNFR2 is expressed by a certain lymphocyte populations for example the T-regulatory cells. Binding of TNF-α to TNFR1 induces apoptosis while binding to TNFR2 induces cell survival. Significantly increased level of serum TNFR1 were found in patients with severe COVID-19.[16]

A study showed that TNF-α level was found significantly higher in COVID-19 patients compared to healthy controls. However unlike IL-10, increased TNF-α level did not correlate with disease severity.[13] On the other hand another study showed that TNF-α contributed to organ damage and was a predictor of disease severity and poor outcome.[17]

TNF level was found higher in critical and deceased group compared to the mild and moderately ill groups. It was twofold or higher in deceased patients in comparison to recovered patients, its level gradually increased as the disease severity progressed. Severe COVID-19 patients with progressive disease also shows significantly higher TNF compared to the stable one. TNF along with IL-6 as a pro-inflammatory factors highly contributes to the cytokine storm.[18]

VI. CONCLUSION

Human innate and adaptive immunity are initially enough to fight SARS-CoV2 infection resulting in successful viral clearance in most cases. However in some cases cytokine storm, a condition where immune system runs uncontrollably wild happens and could lead to more severe disease like severe pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure, or even death. IL-10 was found significantly increased in severe cases and could be used as a predictor for disease severity. Studies suggested that IL-10 acts as an anti-inflammatory cytokine to counter elevation of pro-inflammatory cytokines in a negative feedback mechanism at early stage and as a pro-inflammatory cytokine at later stage. TNF-α is a pro-inflammatory cytokine that contributes to organ damage in cytokine storm.

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