Acta Scientific Biotechnology

Volume 3 Issue 1 April 2022

Review Article

Cytokine Storm in COVID-19

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Received: February 14, 2022

Published: March 19, 2022

Abstract

SARS-CoV-2 is the viral pathogen that is abbreviated as "Severe Acute Respiratory Syndrome Coronavirus 2" and responsible for the disease known as Corona Virus Disease 2019 or COVID-19. What started as a few individual cases linking to a Fish Market situated in Wuhan, China, quickly took the form of a global pandemic in the span of upcoming 6 months with around 4 million cases of COVID-19 along with 7million death worldwide as of in June 2021. Through various studies done by multiple medical institutes a relation between COVID-19 progression and resulting cytokine storm has been established. This further enables us to develop treatments that decreases the disease severity.

Keywords: COVID-19; SARS-CoV-2; Pathophysiology; Cytokine Storm; Antibody; Therapeutic Strategies

Introduction

COVID-19 a short abbreviation of "Coronavirus Disease 2019" which is a respiratory disease caused by a strain of SARS corona virus which originally begin sometime around early December 2019 in Wuhan, China. Despite of initial signs of human-to-human infection reported by officials in Taiwan, medical research organizations along with WHO weren't totally assured about the symptoms and contingency of the viral strain due to lack of clinical data availability which added further delay in taking early steps to curve the spread. However, what started as a few isolated cases [2].

SARS-CoV-2 is capable of spreading faster than that of other members in SARS family due to its high pathogenicity and high transmission rate. The virus is able to survive on metallic surfaces and can stay air-born for hours. After about one and half years of constant medical research and restless works of many scientists and medical officials we have a somewhat clear picture on the workings of this virus.

It turns out that the viral infection causes Cytokine Storm which is a crucial factor leading to the cellular damage and a cause in the death of patients suffering from COVID-19. This infection also blocks the working of ACEII enzyme which ultimately leads to the painful coughing which is common feature of the infection. Here we have reviewed the current understanding on role of cytokine storm in SARS-CoV-2 symptoms, its pathophysiological mechanisms and their potential treatments.

COVID-19 and cytokine storm

Cytokine storm could be a lethal consequence of COVID-19 infection, which could ultimately lead to Multiorgan failure. The elevated amount of cytokine in COVID-19 patients include IL-1β, IL-6, TNF, macrophage inflammatory proteins (MIPs). The higher Interleukin 6 (IL-6) levels are related with the severity of the disease [22]. Relative amount of CD4, CD8 and plasma-blasts are also increased during a COVID-19 infection [22].

It is observed that the correlation between nasopharyngeal viral load and cytokine levels is significant in the patients with severe COVID-19 infection, which further indicate that the cytokine storm is proportional to the virus burden [17]. Host immune responses and immunity related symptoms are extremely variable between

asymptomatic patients and severely affected patients, which suggests that immune-dysregulation is responsible for the progression of the disease. A hypothesized mechanism involves auto-immunity due to the molecular mimicry between SARS-CoV-2 and some self-antigens. This involves sub groups of patients such as children with pre medical history of multisystem inflammatory syndrome, a condition that seems to be ameliorated by immunoregulatory therapies such as glucocorticoids, anti-IL-1 and anti-IL-6 therapies. This is further unclear whether the cytokine storm is a driver of the COVID-19 infection or this is a complete secondary process. However, it is very clear that COVID-19 patients can be asymptomatic or can have an acute level of infection with heterogenous severity.

Pathogenesis of COVID-19 infection

Dry cough, fever, shortness of breath, fatigue leucopenia, signs of pneumonia are the common symptoms of COVID-19 and SARS-CoV infections. This may relate their pathogenesis to be similar [2]. A hypothesis explaining the high serum levels of ferritin and D-dimer disproportionation along with the severity of the infection and tendencies of monocytosis, rather than lymphocytosis including a low number of natural killer cells and cytotoxic T-cells is believed to be the indication of a cytokine storm.

Spike glycoproteins are the most immunogenic parts of the corona virus, which are very likely to bind with the angiotensin converting enzyme-2 (ACE-2) receptors in order to enter the cell. Similarities can be seen between spike glycoproteins of SARS-CoV and SARS-CoV-2 in a greater degree. The distribution of ACE-2 receptor expression mainly on the surface of alveolar epithelial type 2 cells, cardiac and renal cells is very consistent with the target organ involved in COVID-19 [4].

We know that SARS-CoV-2 spreads primarily with direct contact through droplets of saliva or respiratory discharge as through the cough and sneeze of an infected person [1]. After binding with the cell surface receptor of ACE-2 by the spike glycoprotein, the virus enters the cell cytoplasm where it releases the viral RNA genome and replicates the viral particles. This results in the disintegration of the cell and the virus is released, infecting surrounding healthy cells.

The immune system recognizes the viral agents, antigen-representing cells analyze these antigens and present them to the CD8 cytotoxic T-cells and natural killer cells in the context of tissue histocompatibility (MHC). This activates both innate and adaptive immunity causing the production of large amount of pro-inflammatory cytokines. In some cases, the activation becomes so massive that it rapidly develops a thrombotic tendency which can cause multiple organ failure and eventually lead to death of the patient [5,6].

Another pathogenic mechanism not involving ACE-2 receptors were also hypnotized, claiming that the virus might bind to the beta chain of porphyrins inside the erythrocytes, resulting in distribution of heme metabolism and release of iron. However, this peculation requires further studies [7,8].

Significance of cytokine storm

Unregulated Hyper-inflammatory responses or Hypercytokinemia results from the systemic spread of a localized inflammatory response to viral or bacterial infection. Endothelial dysfunctions can be caused by elevated levels of cytokine along with vascular damage and metabolic dysfunction which can cause damages to multiple organs.

The levels of immediate response cytokines such as TNF-γ and IL-1 along with chemotactic cytokines such as IL-8 can rise in early stages of Hypercytokinemia, directing a sustained release of IL-6 which binds to either membrane receptors or soluble receptors forming a complex that acts on gp130.

IL-6 along with other pleiotropic cytokines can mediate an acute response, various blood tests can measure the rising levels of serum ferritin, complement and pro-coagulant factors. Response involved in the acute phase of cytokine storm is relatively extra-exaggerated as high levels of cytokine are inversely proportional to the total lymphocyte count causing may contribute to the reduced viral clearance by cytotoxic T cells. Blocking forward mechanisms related to proper cytokine responses, such as JAK-STAT signaling of macrophages to reduce the levels of IL-1 and IL-6 production, this could be considered a potential therapeutic target of cytokine storm. Cellular strategies can be considered however the effective time of anti-B lymphocyte directed therapies such as use of rituximab are too long, rendering them clinically irrelevant.

Macrophages and dendritic cells in reaction to SARS-CoV2 infection triggers an initial immune response which includes the release of cytokines. However, the inflammatory response results in the degeneration of lymphocytes while attempting to stop the pro-

gression of SARS-CoV2 infection. Lymphopenia has been observed for the severely affected patients that required ICU admission. Here, cytokine production becomes rapidly unregulated, damaging healthy cells and tissues primarily in the lungs and then it further spreads in to other organs including blood-vessels, kidneys, heart tissue and brain. The primary cascade of induced cytokine storm generally disrupts the epithelial barrier in the lungs.

Relation between cytokine storm and disease progression in patients

Higher expression levels of IL-6, IL-1B, TNF-α and monocyte chemoattractant protein have been detected in patients with COVID-19. These inflammatory cytokines activate the T-helper type 1 cells as the response. In activation of specific immunity Th-1 activity is known to have a key significance. Whoever, unlike COVID-19, SARS patients also have the higher levels of Th-2 cytokines (IL-10, IL-4), which inhibit the inflammatory response. The serum levels of IL-6 in the patients suffering from the COVID-19 is established to be corelated with the severity of the disease.

A report on the severe new-type coronavirus-infected pneumonia showed that 71.2% patients required ventilation and 67.30% suffered with ARDS. Moreover, the mortality of the elderly patients with ARDS was significantly elevated [22].

The core pathological change in ARDS is the pulmonary and interstitial tissue damage caused by nonspecific inflammatory cell inflation [23]. Local excessive release of cytokines in the decisive factor that includes pathological change and clinical manifestation [24]. In COVID-19, the inflammatory cytokines storm is closely related to the development and progression of ARDS. The serum levels of cytokines are significantly increased in the patients with ARDS, and the degree of the increase is positively corelated with the mortality rate [25]. The cytokine storm is also a key factor in determining the clinical course of extra-pulmonary multiple-organ failure [16].

In summary, new types of coronavirus infection causes an inflammatory cytokine storm in the patients. The cytokine storm leads to ARDS or extrapulmonary multiple organ failure is an important factor that causes COVID-19 exacerbation or even death.

Normal Interactions between innate system and viruses

A normal immune system involves Macrophages, Monocytes, Neutrophils and Dendritic Cells to form various Pattern Recognition Receptor (PRR) systems which can detect their specific pathogen-associated molecular patterns (PAMPs), associated various infectious agents. Among PRRs, the membrane bound Toll-like receptors (TLRs) help in the recognition of such PAMPs present in the extracellular environment. This produces a signal which leads to the expression of pre-inflammatory cytokine inducing transcription factors along with factors for immediate interferon dependent antiviral response [10]. The second group of pathogen recognition sensors is located in the cytosol and includes another group of nucleotide-binding domain leucine rich repeat (NLR) proteins. These sensors are crucial for the detection of endogenous danger associated molecular patterns (DAMPs) expressed inside the cell. Binding of DAMPs activates the NLRs, triggering the creation of multiprotein cytoplasmic complexes called inflammasomes, which usually convert procaspase-1 to caspase-1. This further activates the IL-1β which is a very crucial immune protein [10,11]. It should also be noted that if these signaling activation process is controlled, they tend to be beneficial for the human body.

For the viruses the PAMPs are generally their nucleic acids. The viral RNA tends to bind with endosomal TLR-3, TLR-7 and cytosolic receptors including RIG-I like receptors (RLRs). These RLRs are mainly 3 types, retinoic acid-induced gene 1 (RIG-1), melanoma differentiation associated gene 5 (MDA5) and LGP2 [12,13]. Upon binding of viral RNA with RLR, it interacts with the mitochondrial adaptor antiviral signal (MAVS) proteins. Leading to the activation of Type-1 interferons (IFNs), these play a major role in directing the cellular responses to viral infections, contributing to the normal antiviral response of immune system [14].

In normal circumstances, cells infected by viruses are terminated by Natural Killer (NK) cells and CD8 positive T-cells, utilizing perforin-mediated granulysin secretion. This leads to the cellular apoptosis of the antigen presenting cell (APC) and relevant cytotoxic cells to void unwanted activity of the immune system after the infection is over. However, in case of inactivity of NK cells or CD8 T-cells to lyse infected cells and APC due to acquired or genetic conditions, the interactions between innate and adaptive systems are prolonged. In this case many pro-inflammatory cytokines such as

TFN-γ, IL- 1, IL-6, IL-18 and IL-33 are secreted in an uncontrolled manner resulting a Cytokine Storm. The entire pathologic activity starts with errors in the cytolytic activity, leading to increase macrophage activity and rapid activation of the whole immune system, which further assists the cytokine storm. ARDS and multiple organ failure (MAS) [3,15,16]. These situations often lead to death of COVID-19 patients.

Treatment for complications in COVID-19 infections

Along with various anti-viral agents, treatment of immunological conditions including cytokine storm using appropriate immune-suppressive and immune-regulatory drugs is very much important [19]. Currently, there are many drugs available for Rheumatoid Arthritis (RA) which have excellent immune-suppressive properties. These are notably Chloroquine (CQ) and its derivative Hydroxy Chloroquine (HCQ), IL-6 receptor inhibitors like Tocilizumab (TCZ), IL-1 antagonist like anakinra, TNF inhibitors are few agents that are used for these purpose [5,9]. While various non-steroidal anti- inflammatory (NSAIDs) agents like Ibuprofen are not recommended in these cases as they tend to further worsen the condition by increasing the ACE-2 expressions.

Hydroxychloroquine in COVID-19

Chloroquine and its derivative Hydroxychloroquine are commonly used in the treatment of rheumatoid arthritis (RA), Sjogren's syndrome and systemic lupus erythematosus (SLE) for their well-known immunomodulatory properties. Besides these obvious anti- inflammatory CQ and HCQ are also known to have antiviral activities against several viruses like Ebola, SARS, H5N1 and dengue. Recently they are also reported to be useful for COVID-19 and were primarily included in the guidelines of China and Italy [20,21]. In India, Japan, USA these drugs were made exclusive for the health workers as a preventive measure of the infection.

The working of CQ and HCQ is believed to be through the accumulation on the cellular lysosomes which increased the pH of the endosome, this interferes in the viral entry and exit of the host cells [21]. Also, the fact that these two drugs are known to have interactions with the ACE-2 receptors which is responsible for the entry of virus in the host cell. This is achieved by the reduced glycosylation of ACE-2 receptors, therefore preventing SARS-CoV-2 from binding with the cell surface [8]. This might also prevent the production of pro-inflammatory cytokines including IL-6 thereby blocking various pathways that eventually lead to ARDS. Although there are multiple reports of adverse effects induced with the use of CQ and HCQ, it is likely to remain as a suggestive drug in treatment of COVID-19.

There is also a speculation that RA patients already prescribed with these drugs for a long time might be protected from the SARS-CoV-2 infections. However, reports of various cases where patients die from COVID-19, having prior medical history of RA and were consuming HCQ already, seems to point otherwise. Hence the initial suggestions of using HCQ as a preventive measure of COVID-19 is not recommended at all.

Interleukin-6 inhibitors in COVID-19

As discussed before SARS-CoV-2 can turn deadly by causing multiple organ failure (MAS) through rapid cytokine storm. T-lymphocytes are hyper activated along with the enormous amount of IL-6 and IL-1, which highly contribute to the further vascular permeability, plasma leakage, thereby causing pulmonary damage and ARDS, as well as MAS [15]. Cytokine storm and related issues are also observed after the chimeric antigen receptor T-cell treatment (CAR-T) [3].

Tocilizumab (TCZ) is a humanized version of anti-IL-6 receptor antibody, which is currently used for the treatment of RA, temporal arteritis and other major auto immune disorders [26]. This is also proposed to be used in the treatment of cytokine storms, which might be induced by CAR-T treatment.

Based on these previously known speculations, TCZ treatment was carried out for the COVID-19 patients which severe cytokine storm and ARDS. Retrospective studies from worldwide reported the resolution of fever and hypoxemia and further improvement in serum CRP levels and pulmonary CT reports [18-20,22].

Interleukin-1 inhibitors in COVID-19

IL-1 is another well-known pro-inflammatory cytokine that plays a major role in a cytokine storm, which is very much relevant in COVID-19 infection [5]. Anakinra is a recombinant IL-1 receptor antagonist, which is also the first IL-1 blocking agent produced with biotechnology. Anakinra blocks the binding of IL-1α and IL-1β to the subsequent IL-1 receptors, resulting reduced pro-inflammatory activity [27]. Anakinra was found to be highly beneficial in pa-

tients with severe sepsis without significant adverse effects based on the randomized phase 3 trials [28]. Unlike TCZ, Anakinra does not inhibit CRP synthesis directly, enabling the serum CRP levels to follow up systemic acute phase response [27]. Another molecule made for IL-1 inhibition is canakinumab, which is a high affinity, fully humanized, monoclonal anti IL-1β antibody that is an IgG isotope. The use of canakinumab for severe COVID-19 infections has not been tested well.

TNF-α inhibitors in COVID-19

TNF-α is known to be a key proinflammatory cytokine leading to various chronic and acute inflammatory pathologies and septic shock. Anti-TNF compounds are mostly used for the treatment of RA, psoriatic arthritis and ankylosing spondylitis. The serum level of TNF-α is reported to be much higher in COVID-19 patients than SARS patients which relates with the disease severity. TNF-α inhibitors are promising treatment for COVID- 19 but it lacks sufficient data to prove that [18,19].

Conclusion

Through this short review on the relation between COVID-19 infection and its immunological consequences was briefly described. The mechanism involved in the infection induced cytokine storm and why many complications occur in the patients during the viral infection ware also discussed and some possible therapies were reviewed. As the information about the viral disease if changing quite fast as new strains or variants are arriving in short intervals as for the rapid mutations of the viral strain, it is quite hard for a certain characteristic of a strain to remain same for a longer time making a definitive review obsolete for longer term.

Although cases of COVID-19 could be largely asymptomatic or cause mild symptoms which can be mistaken as other immunological conditions, the presence of cytokine and RT-PCR tests are dependable methods for determination.

It is recommended that longitudinal follow-up of COVID-19 patients with and without the traces of cytokine storm to further comprehend the specific natures of immune- pathological mechanisms and biomarkers for severe disease. As the cytokine storm resolves and immunologic memory of the SARS-Cov2 infection is likely to persist. This rises the further possibility of reinfection in previously positive patients that have recovered from the infection. Understanding the various pathophysiological mechanisms linked to cytokine storm could be used to develop targeted diagnostic and therapeutic strategies for critically affected COVID-19 patients.

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