Immunomodulation: a broad perspective for patients' survival of COVID-19 infection

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ABSTRACT: The pathogenesis of the SARS-CoV-2 virus is yet to be well understood. However, patients with the virus show clinical manifestations which are very similar to those of SARS-CoV and MERS-CoV. This and other scientific findings reveal that acute respiratory distress syndrome (ARDS) is the main cause of death in most COVID-19 patients. A vital mechanism for the development of the ARDS is cytokine storm which arises from an aggressive uncontrolled systemic inflammatory response that results from the release of large numbers of pro-inflammatory cytokines. This review seeks to draw the attention of the scientific community to the possibilities of improving the clinical outcome of COVID-19 patients based on the knowledge of altering the development of this hyper-inflammatory process by suggesting drugs that targets the implicated immune cells, receptors, cytokines and inflammatory pathways without having generalized effect on the entire immune system.

Keywords: Coronavirus; COVID-19; Inflammation; Cytokines; Immunomodulation.

1. INTRODUCTION

The World Health Organization (WHO) has currently declared COVID-19, a global pandemic. The disease is caused by a new coronavirus referred to as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [1, 2]. This virus differs from the severe acute respiratory syndrome coronavirus (SARS-CoV), which was first identified in 2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV) [2]. The SARS-CoV was thought to be an animal virus, perhaps from bats which spread it to other animals and had its first infected human case in the Guangdong province of Southern China in 2002 [3]. This new virus had its origin from Wuhan, which is the capital city of Hubei province of China in December 2019. The virus has currently sporadically spread all across the regions of the world with about 3 million cases and about 250,000 deaths reported in 213 countries and territories around the world [4]. This is a great concern for the whole world and has led to the search of answers through research for both therapeutic and prophylactic measures against the virus.

Coronaviruses are enveloped viruses. They are non-segmented positive-sense single stranded RNA viruses. Their genome size ranges from 26-32 kilobases. Hence they are the largest known viral RNA genome. They have a nucleocapsid that is composed of both the RNA genome and phosphorylated nucleocapsid (N)

protein. Externally, this component is buried inside phospholipid bilayers which is covered by two different types of spike proteins. They are the spike glycoprotein trimmer (S) and the hemagglutinin-esterase (HE) [2].

The COVID-19 spreads through person to person contact through respiratory droplets produced when an infected person coughs or sneezes within a proximity to an uninfected individual majorly when within a distance of about 6 feet from each other. Another significant way of spreading the virus is by touching the mouth, nose or eyes after contact with a surface or object that has the virus [1]. Clinical manifestations of the infection include fever, fatigue, nonproductive cough, dyspnea, normal or decreased leucocyte counts, and radiographic evidence of pneumonia which are very similar to the presented symptoms of SARS-CoV and MERS-CoV [5]. However, not much is known about the pathogenesis of COVID-19. A report in Lancet reveals that acute respiratory distress syndrome (ARDS) is the main cause of death in most COVID-19 patients. According to the report, in an early survey of the 41 SARS- CoV-2 infected patients in admission during the outbreak, six of them died from ARDS [6]. ARDS is majorly experienced as shortness of breath and it's a common immunopathological event in SARS-CoV and MERS-CoV infections [7].

Several literature reviews revealed that one of the vital mechanism for the development of the ARDS is Cytokine storm and sepsis [5, 8, 9]. Cytokine storm is a deadly uncontrolled systemic inflammatory response that results from the release of large numbers of Pro-inflammatory cytokines such as IFN-alpha, IFN- gamma, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-alpha, TGF-beta, etc., and also some chemokines like CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc., by immune effector cells in SARS-CoV infection [5]. This cytokine storm triggers a violent attack by the immune system on the body, which in the case of COVID-19 is on healthy lung tissues of the patients. This leads to ARDS and in some cases multiple organ failure eventually leading to death in severe cases of SARS-CoV-2 infection [8]. In severe cases, a cytokine profile resembling Secondary haemaphagocytic lymphohistiocytosis (sHLH) is associated and it is characterized by increased production of IL-2, IL-7, granulocyte-colony stimulating factor, interferon-gamma, tumour necrosis factor-alpha, inducible protein 10 and macrophage inflammatory protein 1-α [9]. Secondary haemaphagocytic lymphohistiocytosis (sHLH) is an inflammatory syndrome that is recognized by a fulminant and fatal hypercytokinaemia along with multiple organ failure. In most adults, sHLH is most commonly triggered by infections of viral origin and occurs in about 3.7-4.3% cases of sepsis [9]. The Italian medical society reports that COVID-19 infection may be very well associated with the classical syndrome named disseminated intravascular coagulation (DIC) i.e. thrombosis. These findings consistently demonstrate close connection between thrombosis and inflammation of which cytokine storm is uniquely implicated [7, 10]. Therefore, the hypothesis of improving the clinical outcome of COVID-19 patients should be based on the knowledge of altering the development of this hyper-inflammatory process. This could be very helpful in offering novel insights and potential therapeutic targets for combating the COVID-19 infection.

Generally, there is yet to be any approved vaccine or treatment for the COVID-19. However, evidence of supportive treatments through immunomodulatory approach and immunosuppression have been recently seen and used to prevent fatality of the disease [9, 11]. Most worthy of note is the development of specific drugs that target immune cells or cytokines without generalized suppression of the entire immune system which often occurs in the use of steroids and corticosteroids. For instance, Mehta et al. [9] reported significant reduction in mortality rates among patients that were treated via immunomodulation i.e. drug targeting mechanism. Although, this approach may not clear the virus (SARS-CoV-2), it can however aid the survival of patients until a successful adaptive immune response is mounted by the body. Since most patients eventually recover, this various immune targeting approach could help sustain survival of patients till they are fully recovered.

2. IMMUNOMODULATION

Immunomodulation involves a broad scope of all therapeutic measures and interventions that are aimed at modifying the response of the body's immune system. In the development of an infection, inflammation begins when the cells of the innate immune system recognizes a pathogen-associated molecular pattern (PAMP) possessed by the invading organism [12]. This receptors on the host phagocytic cells that recognize PAMPs are known as pattern recognition receptors (PRRs) [13, 14]. They are of several different categories, namely Toll-like receptors (TLR) and soluble PRRs such as mannose binding lectins (MBL). Intracellular PRRs, like the Nod-like receptors (NLRs) are found in the cytosol for the detection of intracellular pathogens and are responsible for viral detection [12]. Once the PRR is activated and ligand binding occurs, a signaling cascade is immediately triggered. This cascade results in expression of specific pro-inflammatory cytokines. For instance, stimulated TLRs causes the releases of some pro-inflammatory cytokines [12].

Generally, cytokines play a vital role all through the various stages of inflammation. During an infection, these special protein signaling molecules signals the immune system. This is done in order to regulate the duration and gravity of the immune response to damage or infection. Based on the specific secreted cytokine, their function can be either to activate (pro-inflammatory) or down regulate (anti-inflammatory) the host response [14]. Stimulated TLRs induce pro-inflammatory cytokines, while the production of the anti-inflammatory cytokine IL-10 is very important during the later stages of infection so as to control disease-induced tissue pathology [15]. The activation of inflammatory response must be regulated to prevent a damaging systemic inflammation as in the case of cytokine storm [16]. Each cytokine acts on a different part of the inflammatory response. Based on the understanding of this progression of the disease process, D'Elia et al. [12] suggested various immunomodulatory approach to altering the process. This could be done either by targeting the overactive immune response, or targeting stimulation of anti-inflammatory pathways, or drug targets like prostaglandins and cyclooxygenase inhibitors or Chemokine manipulation and T- regulatory cells manipulation. This would be effective in preventing cytokine storm, ARDS or secondary haemophagocytic lymphohistiocytosis, and disseminated intravascular coagulation [thrombosis] shown in some other reports which all arises from inflammatory or cytokine overload.

2.1. Immune system targets

Although, targeting the overactive immune response such as the use of steroids and corticosteroids could help to block immune overcrowding, they however have generalized effect on the immune system. This could have deleterious effects on the adaptive immunity of the host and cause fatality in the case of COVID-19 infection. Therefore specific immune target mechanism without generalized immunosuppression is most desirable. A novel discovery of a therapeutic has shown the ability to control tissue damage without disrupting the general beneficial inflammatory response. These are called resolvins. Resolvins are newly identified lipid-based mediators derived from omega-3 polyunsaturated fatty acid (EPA) and docosahexaenoic acid (DHA) [12]. Their ability to promote resolution without necessarily affecting the inflammatory response is a unique property of these agents [17]. This means these molecules can be a promising therapeutic strategy for treating infections and is worthy of investigating further for its possibilities in the COVID-19 cases. The two types of resolvins were demonstrated to have *in vivo* therapeutic efficacy in many mouse models of diseases. They infact, halt neutrophil recruitment in peritonitis [18, 19]. Arita et al. [20] showed that resolvin E1 increases host survival in models of colitis. Other target mechanisms aimed at the immune system should therefore be explored as a therapeutic for this ongoing pandemic.

2.2. Modulating the inflammatory pathway

Understanding and modifying the various inflammation pathways is an important immunomodulatory approach to be considered. Various pathways that could be targeted for modulation includes the Cyclooxygenase pathway, Chemokine network and the cholinergic anti-inflammatory pathway [12]. Chen et al. [14] also suggested some inflammatory pathways that are extremely important in the development of inflammation. They are NF-κB, MAPK, and JAK-STAT pathways, and have all been reported as possible agents in the development of the cytokine storm in COVID-19 patients. In targeting the anti-inflammatory pathway, one of the most important mechanisms is the cholinergic anti-inflammatory pathway. It is a neural mechanism that inhibits pro-inflammatory cytokine release via signals that require the vagus nerve and α7 receptors [21]. Bernik et al. [22] affirmed that an efferent or motor vagus neural mechanism uses acetylcholine; the principal vagus nerve neurotransmitter to inhibit the release of cytokine from resident tissue macrophages. Nicotine and acetylcholine which are cholinergic agonists, have been shown to significantly inhibit the release of TNF and other cytokines from endotoxin-stimulated human macrophages [21]. They do this by interacting with the acetylcholine receptor thereby inhibiting the synthesis of pro-inflammatory cytokines, but not anti-inflammatory cytokines. According to their experiment, CNI-1493: a therapeutic agent that was used is a tetravalent guanylhydrazone inhibitor of macrophage activation which prevents the phosphorylation of p38 mitogen-activated protein kinase. Bernik et al. [22] showed that the administration of CNI-1493 inhibits the systemic TNF release and synthesis of TNF in tissues during endotoxemia by a mechanism that is dependent upon an intact cholinergic anti-inflammatory pathway. This is very useful as TNF-alpha represents one of the major cytokines released during the COVID-19 infection [5]. Tocilizumab which is an IL-6 receptor inhibitors also leads to the potential suppression of the JAK/STAT signaling [10].

Currently, a group of antimalarial drugs, including hydroxychloroquine sulfate (HCQ), chloroquine phosphate (CQ), and quinacrine (or mepacrine) which are classified as small molecule inhibitors (SMI), have been used widely in the treatment of some cases of COVID-19 with some desirable results. They were first used to treat autoimmune diseases (arthritis and SLE) after World War II [23]. Recently, their mechanisms of action on endosomal TLR signaling (TLR7/8/9) was identified. The modulation of pH can lead to suppression of autoantigen presentation, blockade of endosomal TLR signaling, and decrease in cytokine production with other specific mechanisms including inhibition of MAPK pathway signaling and phospholipase A2, antiproliferation, photoprotection as well as reduction of matrix metalloproteinase-9 (MMP-9) activity [23]. New immunomodulatory therapeutics should be explored in this regard in order to block the development and release of other COVID-19 mediator pro-inflammatory cytokines. There is much possibilities that a breakthrough could arise from modulating other pathways involved in either the release of pro-inflammatory and anti-inflammatory cytokines.

2.3. Finding drug targets

Another important immunomodulatory mechanism is via drugs that target specific immune cells, immune receptors or the cytokines themselves. Since the key receptors in the innate immune system which causes the cytokine release are the Toll-like receptors (TLRs), of which there are about ten in humans (TLR 1-10) [13]. They are therefore important target sites. Generally, TLR inhibition can be achieved by two major means: (a) blocking the binding of TLR ligands to the respective receptor and (b) interfering with the intracellular signaling pathways to stop the signal transduction [23]. TAK-242 (Resatorvid), which is an anti-sepsis SMI have been shown to successfully target TLR4 signaling pathways [25].
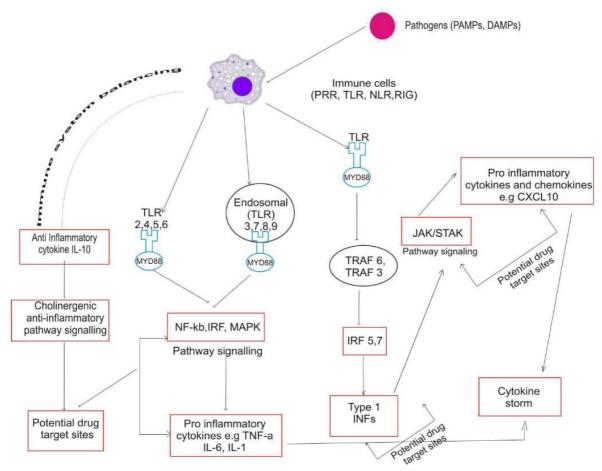
Figure 1. Schematic representation of the various potential drug target sites for immunomodulation and control of hyper-inflammation and cytokine storm. These various target sites include the signaling pathways such as the nuclear factor kappa-high-chain enhancer of activated B-cells (NF-kB), mitogen associated protein kinase (MAPK), JAK/STAT, IRF, cholinergic anti-inflammatory pathway and various cytokines and Toll-like receptors (TLR).

Imidazole quinoline molecule have been recorded to target TLR 7 and TLR 8 successfully, and cpG-carbon compound shown to target TLR 9 [24]. As earlier stated, targeting ligand binding sites of various receptors might also yield new therapeutic drugs. Ulevitch [24] suggested that leucine-rich repeats that are present in TLRs and the Toll/interleukin-1 receptor [TIR] domains of TLRs could be exploited in search for new drug targets. Experimentally, they have been shown to yield positive trial results. In fact, a multicenter, randomised and controlled trial of tocilizumab which is an IL-6 receptor blockade has been approved in patients with COVID-19 pneumonia and elevated IL-6 in China to block the build-up of cytokine storm [10]. Also, another report from a phase 3 randomized controlled trial of IL-1 blockade (anakinra) in sepsis, indicated significant survival benefit in patients with hyper-inflammation, without having increased adverse effects on the patients [11]. Eritoran (E5564), is another TLR4 antagonist (by Eisai Research Institute of Boston Inc.) [26]. Eritoran is the 2nd generation of its own kind derived from the initially developed E5531. The mechanism of action these compounds is competitive binding to the MD2 pocket by mimicry of the structure of the lipid A. This eventually prevents the LPS binding and the consecutive induction of TLR4 signaling [23]. Preclinically, eritoran is shown to significantly reduce LPS-induced NF-κB activation and and the eventual production of pro-inflammatory cytokine such as TNF-α, IL-1β, IL-6, and IL-8 both in vitro and in animal models [23]. Therefore, further investigative efforts should be taken to find and target more receptors, immune cells and cytokines that are implicated in the disease progression of COVID-19.

Table 1. Some therapeutic drugs used for Immunomodulation and resolution of hyperinflammation and their drug target mechanism [23].

Compound	Target	Drug Class	Clinical Phase
Eritoran (E5564)	TLR 4 Antagonist	Synthetic lipodisaccharide	Phase III
Tocillumab	Inhibition of IL-6 Receptor (suppression of the JAK-STAT pathway)	Recombinant monoclonal antibody	Approved
Chloroquine and Hydroxylchloroquine sulphate	Blockade of endosomal acidification (endosomal TLR and MAPK signaling inhibition)	Small molecule inhibitor	Approved
TAK-242 (Resartovid)	TLR 4 Antagonist	Small molecule inhibitor	Phase III
Anakinra	IL-1 Inhibitor	Recombinant human IL-1 receptor antagonist	Approved
Imiquimod	TLR 7 Agonist	Small molecule ssRNA	Approved
Rintatolimod	TLR 3 Agonist	ds RNA molecule	Phase II
CNI-1493 (Semapimod)	Inhibition of phosphorylation of p38 MAPK (cholinergic anti-inflammatory pathway)	Synthentic guanylhydrazone	Phase I
IMO-2125	TLR9 Agonist	CpG oligonucleotide	Phase I

Some of these drugs and their various developmental phases are shown in Table 1. The drugs can generally be classified into classes such as antibodies, lipid A analogs and oligonucleotides which primarily targets the ligand-receptor binding, whereas the small molecule inhibitors (SMIs) can function by both acting on the intracellular signaling cascades of TLR pathways and targeting the ligand receptor binding [23]. Tocilizumab, anakinra, chloroquine and hydroxychloroquine derivatives have been approved and used in several regions of the world for the treatment of COVID-19 patients with some positive results already recorded [1, 10, 11].

3. CONCLUSION

The perspective of immunomodulation in enhancing the survival of COVID-19 patients is exceptionally broad. However it promises quite novel and fascinating discovery of potential therapeutic agents for treatment of the disease. A better understanding of how to regulate the innate immune system, ligand binding site receptors and cytokine pathways would allow for more accurate selection and identification of agent-mediated inflammation and drug targets which in this case can be of great benefit to the treatment of COVID-19 patients. In view of this, further experiments, investigations and clinical trials based on immunomodulatory approach against the novel SARS-CoV-2 virus should be considered so that better survival opportunity would be made available for COVID-19 patients. This could be very helpful in offering novel insights and potential therapeutic agents for combating the COVID-19 infection.

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