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SPOTLIGHT ON IMMUNITY AGAINST COVID-19 WITH EMPHASIS ON CYTOKINE STORM

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ABSTRACT

This report provides a study on the ongoing infection, pathogenesis, and immunity of COVID-19. The study will also investigate the cytokine storm and the role of both dendritic cells and Natural Killer T cells, and will provide ample references for researchers who need immunological data on SARS-CoV-2. The review collects data from 60 different review and research articles. Viral antigens are predictable by the B cells or introduced to the T cells by MHC complexes, leading to the assembly of antibodies, magnified production of cytokines, and lysis during the acute stage of infection. In MHC, genetic polymorphism helps it to show a number of T lymphocyte epitopes over different MHCs. Their gene association and downregulated expression are related to the seriousness of COVID-19 and evaluated by special markers such as IL-1ra, MCP-3, IL-17, IP-10, GM-CSF, TNF, IL-10, IL-1β, and IL-6. Clinical researches have pointed out that both severe and mild forms of COVID-19 lead to changes in leukocyte subtypes and cytokine secretion. Not unexpectedly, treatments that concern immune response and curb the 2019 coronavirus cytokine storm (COVID-19) will increase the patients' chance to survive. Cytokine storm characteristics, coagulopathy, and alternative inflammatory consequences remain vague. New accurate medication techniques are often advanced by distinguishing shared or population-specific triggering and amplifying cytokines storm at a certain stage of COVID-19.

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Introduction

In December 2019, in China, a novel coronavirus (n-CoV-2) appeared causing global panic and called COVID-19 or SARS-CoV-2. It has spread to more than 210 counties and is considered a serious pandemic disease that WHO advised for isolation strategies, decrease human contact, and rapid identification of the virus [1]. The early researches were about how to identify, studying its symptoms, and structure. Then researches were directed to reveal the similarity to SARS-CoV and coronavirus of bats [2]. Accelerated researches usually communicate to the public to minimize the social and economic impact. At this stage, it is crucial to develop suitable drugs and vaccines [3]. Virus dynamic spread and epidemiology have varied intensely. First, the outbreak affected Asia mainly China but soon, it covered America and Europe especially Italy and many other countries as well [4]. However, the mortality rate varied in each country according to health condition and susceptibility also many countries show low efficient testing strategies and emergency response. COVID-19 transmission is by droplets during coughing, sneezing, and even speaking within a two-meter distance. The disease also can be transmitted during exposure to infected surfaces or asymptomatic persons. Moreover, infected persons can transmit the disease up to two weeks after recovering from symptoms. COVID-19's incubation period ranged between 2-14 days and the development of severe symptoms usually appear within 7-10 days. COVID-19 signs are generally flu-like symptoms such as fatigue, dry cough, fever, and in some cases diarrhea. 20% of infected cases, especially the elderly and chronic cases need hospitalization as the symptoms may progress into a well-known featured glass patterned pneumonia and severe chest pain [5]. Spain showed a high percentage of home recovery while China showed a much lower percentage and mild symptoms. 23% of people developed severe complicated pneumonia in Spain compared to 13.8 in China. Moreover, critical cases (respiratory failure, multiple organ failure, and shock) in Spain were 7%, while in China they were 4.7%. Few cases in children were recorded to

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be with much fewer symptoms. The high risk lies in elder age of above 50 years but the percentage differs from one country to another according to health services and number of ICU units. Italy and Spain recorded 67% casualties in patients above 80 years, 20% aged 70-79, and 8% aged 60-69. Besides, age, chronic illnesses such as hypertension, diabetes mellitus, and obesity, and patients with cardiovascular and lung diseases may be at high risk to develop severe illness [6].

Symptoms and Course of the Disease

The novel coronavirus became famous on January 10, 2020, and the mortality report has since risen at an unprecedented and speeding up pace. The fatal virus appeared in Wuhan, China and it causes severe acute respiratory symptoms, such as thrombocytopenia, pneumonia, asthenia, and fever, dyspnoea, and elevated levels of C-reactive protein (CRP) and dehydrogenase lactate [7]. According to studies, mildly affected individuals of COVID-19 showed an increased titer of highly expressed proinflammatory chemokines and cytokines such as IFN-alpha, IL-1β, IP-10, and MCP-1, while severely affected patients showed high levels of highly expressed MIP-1A, MCP-1, G-CSF, TNF-alpha, IL-10, IL-8, and IP-10, leading to cytokine storm syndrome followed by severe pulmonary damage and death from respiratory failure [8]. In addition, in extreme cases of COVID-19, all blood cells except neutrophils have been documented to decrease including B cells, NK cells, and T cells. Invasive lesions in both lungs are seen by chest radiographs, with defects to different degrees in the lungs [9].

Furthermore, in COVID-19 patients, bilateral subsegmental multilobular consolidation, ground-glass opacity with multiple mottling was noted [10]. Myalgia and exhaustion have recently been reported in rhabdomyolysis patients indicating the urge for rapid diagnosis with appropriate hydration treatment to minimize the risk of serious consequences due to rhabdomyolysis [11]. In addition, neurological symptoms such as nausea, headache [12], occasionally diarrhea and vomiting [13], and also dyspepsia and anosmia [14] can also occur in COVID-19 patients. Moreover, records of SARS-CoV infecting the nervous system of both animals and patients with serious brain stem involvement have been documented. Acute lung failure in patients with COVID-19 indicates a possible SARS-CoV-2 attack on the brain [15]. The neurotropic capacity of the SARS-CoV-2 virus was recently confirmed by a study, as about 36.5% of the COVID-19 patients had neurological signs [16]. The condition diverts seriously to acquired pulmonary edema that progresses to serious pneumonia and consequently into hypoxemic respiratory failure. It may be accompanied by multiple organ failure, acute respiratory distress syndrome (ARDS), or gastrointestinal infections [17]. Furthermore, hepatic and pancreatic damage and renal failure, due to SARS-CoV-19 have been recorded. One of the pathogenic mechanisms of COVID-19 is believed to be the destruction of microvasculature in different organs [18].

Pathogenesis and Immunity

The human testicles, brain, bowels, heart, lungs, nose, and kidneys are known to express ACE2. Since these target cells express ACE2 and TMPRSS2, which help combine viral Spike protein, respiratory cells are susceptible to coronavirus [19]. Data from several investigations have shown that the greater the amount of ACE2, the greater the risk of COVID-19. COVID-19 is not just a respiratory disease but can infect the gastrointestinal tract, nervous system, etc. in the body, too. Its complication can be serious as far as life-threatening due to strokes, blood thrombosis, hypoxia, and complication of the cardiovascular system, which is confirmed by recent examinations of the datasets of single-cell sequencing [20]. SARS-CoV-2 is primarily transmitted via respiratory droplets between individuals where certain particular cell types have been established as likely primary sites of infection. In one study, RNA sequencing stated that TMPRSS2 and ACE2 are most commonly expressed in bronchial transient secretory cells among different types of cells in the respiratory system [21]. Likewise, another research analysed the expression of the TMPRSS2 and ACE2 genes associated with viral entry and reported high expression in nasal epithelial cells, particularly goblet and nasal ciliated cells [22] further study supported that other than cells in the respiratory system, the essential viral-entry proteins, are also contained by cells in the eye and other organs. Gastrointestinal cells, for instance, are filled with ACE2 [23]. 3D models in advanced research showed that SARS-CoV-2 was not only able to infect organoid cells from the airway, but also from the stomach [24]. It remains uncertain, however, whether intestinal cells can become infected and produce viral particles. Nevertheless, long after respiratory symptoms were resolved, SARS-CoV-2 was found in human feces, and gastrointestinal symptoms are prevalent in patients with COVID-19, implying that the intestine might be a possible reservoir, representing the potential for fecal-oral transmission [25]. Notably, studies have also shown that the density of ACE2 receptors in bronchial cells increases with age and in males as opposed to females, which is associated with the higher rate of infection in males and seniors as demonstrated in epidemiological research. Moreover, ACE2 plasma is much higher in males than females. In particular, the gene encoding the ACE2 protein is X-linked and is expressed at high levels in the studies, which explains the gender difference found in mortality and infection [26]. In addition, the age-dependent expression of the ACE2 gene in the nasal epithelium is substantially fewer in young children than in adults, which explains why COVID-19 is much less dominant in infants [27]. Small epithelial lung cells in the airway of heavy smokers and those with a chronic respiratory disease also increase ACE2 levels, indicating that refraining from smoking can reduce the risk of serious COVID-19 disease [28]. Other genetic variations in the ACE2 sequence, allegedly associated with chronic diseases of the cardiovascular system and pulmonary conditions, may dispose infected people to more serious diseases [29]. According to recent investigations, the genes encoding amino acids that interact with TLR7 and ACE2 (SLC6A20) are also associated with COVID-19 severity. Many investigations have shown that the blood type and ABO gene may be involved in COVID-19 severity and

susceptibility [30]. In California, a private biotechnology and genomics company reported in their preliminary findings that O blood type seems to be protective against SARS-CoV-2 relative to other blood groups [31, 32]. However, another opinion from a group of hematologists proposed that the serum IgG anti-A, which is present in the O group is more essential than the blood type itself as a biomarker for COVID-19 defense [33]. Extreme or fatal COVID-19 is usually associated with peripheral blood cytokines, including C-X-C Motif Chemokine Ligand 10 (CXCL10) or interleukin 1, 6, and 8. However, inadequate information specifically that evaluating CD8+ and CD4+ T cells, and Ab produced in the same patients impedes the role of acquired immunity in the defense or pathogenesis of acute COVID-19 [34]. The reasons for this are still under investigation and it is very fundamental for finding the proper handling and treatment of this disease, as well as developing a proper vaccination that produces efficient immunity and adequate immune response. In an antigen-specific manner, the acquired immunity responds to pathogens to develop protective immunity. There are three main types of lymphocytes in the immune system: B cells that function the production of antibody, killer or cytotoxic, T cells (CD8+), and helper T cells (CD4+). In protecting against viral infections, all three types of adaptive immunity can be essential. Most approved vaccines operate on the concept of defensive antibody response, with the most common mechanism of action being neutralizing antibodies [35]. Therefore, most COVID-19 vaccine efforts concentrate on the release of antibodies that neutralize virus particles, with additional focus on stimulation of the immune system to produce CD4+ or CD8+ T cells [36]. Nearly all antibody reactions, enduring memory of matured definite B cells, are influenced by $CD4^+$ T cells to aid [37]. As such, responses from $CD4^+$ T cells are very important to the efficacy of most of the vaccines. In fact, CD4+ T cells pose various features that can be beneficial in the sense of antiviral immunity, beyond promoting antibody responses [38]. A mouse model of SARS showed that CD4+ alone, in the absence of antibodies or CD8+, may protect against lethal SARS-CoV challenges [39]. The role of CD8+ in maintaining immunity against viral infections has been evaluated in vivo in laboratory animals [40]. In order to gain insight into SARS-CoV-2 protection by adaptive immunity and potential immuno-pathogenesis, it is therefore important to evaluate the progression of the virus-infected patients across the spectrum of the severity of COVID-19 in a coordinated manner. Recent researches suggest that antigen-specific adaptive immune responses (ADIMs) reduces the severity of COVID-19 disease, also organized responses with prominent roles for CD4+ associated with lower severity of COVID-19. Moreover, CXCL10 is a plasma marker of impaired T cell responses in acute COVID-19; and aging and naive T cell scarcity may be correlated with risk factors for failure to produce organized ADIM, leading to enhanced vulnerability to serious prognosis COVID-19 [41]. The virus Spike (S) RBD domain is strongly divergent from other CoVs. The primary target of SARS-CoV-2-neutralizing antibodies is the RBD domain [42]. Therefore, SARS-CoV-2 RBD immunoglobulin IgA, IgM, and IgG titers have been tested. In almost all COVID-19 patients, RBD IgG is observable. RBD IgA was also reliably detected and well associated with RBD IgG. Distinguishable RBD IgM was less common [43]. Also, the full-length SARS-CoV-2 S IgA, IgM, and IgG titers were assessed. S IgA and IgG responses are robust in most COVID-19 situations. S IgM has been found less commonly, similar to RBD IgM. The low frequency of naive T cells is an immunological risk factor associated with extreme COVID-19, such as old age. A repertoire of less naive T cells is a risk factor for severe COVID-19 [44], may be aggravated, since the escape of SARS-CoV-2 from the innate immune system may restrict T cell priming [34]. As shown in small animal SARS models, there is also the risk of less advanced-age professional antigen-presenting cells in the lung [39]. The well-characterized total lymphopenia and general cytopenia of T cells found in severe COVID-19 can also intensify the harmful effects of less naive T cells [45]. This can explain the issues that arise in elderly cases. The capacity of the adaptive immune system for immunopathogenesis is present. No evidence was found supporting the hypothesis that the pathogenesis of COVID-19 causally involves pathogenic immune adaptive cells. In hospitalized COVID-19 cases, there were no TH17 or TH2 cytokine biased CD4+ responses [46]; and the cytokine CD8+ response profile was comparable between non-hospitalized and hospitalized instances. Although the probability of any functional T cell defects is not precluded, the data here generally support a model in which a sluggish or uncoordinated ADIM was correlated with serious disease [41].

The Role of NK T cells and Dendritic Cells (DC)

Few data on Natural Killer (NK) cell responses in COVID-19 is published. In SARS, NK is helpful as a biomarker for evaluating the severity of the disease as CD158b+ NK cells were found in association with anti-SARS-CoV-2 specific antibodies [47]. Several studies showed that NK cells in PB of COVID-19 patients are decreased, particularly in severe cases. Nevertheless, there was no difference in a separate analysis in the number of CD16+CD56+ NK cells in mild vs. extreme situations [48]. So, whether NK cells can be a COVID-19 predictor is unknown. Further research should conclude whether NKs can influence viral control or contribute to the release of cytokine during the COVID-19 [49]. No evidence is currently available to correlate between SARS-CoV2 infection and DC (plasmacytoid or myeloid) modulation, or to report how T cells can affect the disease. The DC percentage in the blood in COVID-19 cases did not improve with the seriousness of the disease but showed an extreme decrease in CD86 expression relative to mild disease [50]. For DC subsets, variance has been noted, with disease severity, during ARDS CD1c+ DCs decreased and recruited into the lungs [51]. The complement system plays an important role during body response to infection. It rapidly activates the host's immune system and attracts cells to the site of infection. Its activation is closely controlled to prevent detrimental injury. C5a and C3a have pre-inflammatory properties by attracting inflammatory cells and inducing PMN. Complementary mediated micro-thrombotic disease with Mannan-binding lectin serine protease 2 (MASP2), C4d, and C5b-9 deposits have been shown in recent histological examination of cutaneous and pulmonary autopsy/biopsy samples of five seriously ill SARS-CoV-2

infected patients. Therefore, certain atypical ARDS characteristics of severe respiratory distress related to COVID-19 could be caused by microvascular injuries following the activation of complement pathways [52]. By limiting the recruitment of inflammatory cells or microthrombopathy linked to direct complement activation, the complement system appears to be a suitable candidate for a therapeutic target to decrease the severity of the disease [52]. NKs with CD8+, the main cells responsible for killing infected host cells in order to avoid viral replication. A reduction of cell-mediated cytotoxicity with a decrease in CD158+NK and NK cells has been observed in previous investigations on SARS-CoV. No aggravation of the disease has been found in animal models of SARS-CoV infection with no NK depletion indicating that NKs are not crucial to the immune response to the virus infection [30]. On the other hand, clinical investigations during COVID-19 exhibited a reduction in the number of NKs in serious, but not mild, diseases possibly associated with viral propagation. The NK cell count dynamic profile showed no variations between the survivor or dead classes of the most serious cases [53]. Parallel clinical amelioration was the time to restore the normal cell count of NK cells. Extreme SARS-CoV disease, however, was characterized by a functionally depleted profile with elevated membrane expression of NKG2a and a decrease in CD107a+, IL-2+ IFN+, and TNF-α + NKs. Wilk *et al.* showed the reduction of NK $CD56^-$ and NK $CD56^+$ cells in ARDS and COVID-19 cases, respectively [48].

Cytokine Storm

It is a phenomenon in which the body launches compensatory-repair procedures with immune activation to renovate tissue and sustain organs. "Cytokine storm" was first created in 1993 to describe a host vs. graft disease [18]. The concept has since been used to identify any case of a sudden release of autoimmune-associated cytokines, hemophagocytic lymphohistiocytosis, sepsis, tumors, cancers that lead to infectious diseases and acute immunotherapy. Cytokine storm occurs when the immune system is over-activated by infection, medication, and other causes, resulting in elevated levels of releasing cytokines such as TNF, CSF, chemokines, IL, IFN, etc., into circulation that affect several organs, To date, it is unclear what factors are released, which are responsible for activating the inflammatory sequence and aid to the clinical syndrome associated with the high release of the cytokine in blood. Generally, an imbalance in the control of the immune system is assumed to happen i.e., increased activation of immune cells via TLR and other mechanisms, decreased anti-inflammatory response, etc. Cytokines are released by NK cells, activated T cells, macrophages, and humoral responses to help overcome the infection, followed by effector mechanisms e.g. cell-mediated antibody-dependent cytotoxicity [54]. They are activated in order to hold the pathogen progression. For instance, IL-1β, IFN-alpha, and INF-β and which are produced locally by epithelial cells may protect adjacent cells by fuelling IFN-stimulated gene expression and simultaneously activating immune-competent cells including NKs. This increases IFN-γ release and NKs' lytic ability. It amplifies subsequent TLR-mediated stimulation along with NK and myeloid cells to macrophages by IFN-γ. Moreover, high IL-6, IL-12, and TNF levels are secreted, which are capable of further modulating NKs in turn [55]. While IL-12 usually increases NK IFN-γ secretions, high levels of IL-6 may reduce the immune response by reducing the concentrations of intracellular perforin and granzyme B by influencing the cytotoxic effect of NKs. As the infection progresses, T-cell and antibody responses lead to further cytokine defense, which results in sustained or greater antigen release and more TLR ligands of cytotoxicity induced by the virus. Other pathogen- or host-related factors (including genetics, anti-inflammatory responses, and pathogen load reduction) act together once these responses are in motion to prevent an unchecked response or a CRS that could cause organ failure and tissue damage on its own if allowed to develop. For obvious reasons, it is predicted that the lack of negative feedback mechanisms for IL-4 and IL-10 increases the strength of cytokine responses to a cytokine storm or pathogenic CRS. Thereby, targeting therapy to interrupt the development of cytokine storms through the use of pharmacological agents like tocilizumab (anti-IL-6) will stabilize advanced cases from transit to a more extreme decline [56]. As a consequence of viral transfer based on droplets, there is usually a disadvantageous respiratory tract infection at the onset of SARS-CoV-2 infection. Nevertheless, a study [57], supports the hypothesis that SARS-CoV-2 via ACE2 can also directly infect intestinal enterocytes. On differentiated enterocytes, ACE2 is abundantly expressed and can help explain why both acute infection and reported diarrhea occur and fecal forfeiture. Therefore, having larger infection traces can affect the source of inflammatory cascades to include tissues other than the lung. In patients with moderate to severe symptoms, cytokine storming in COVID-19 disease is normal; meanwhile, the number of NKs and lymphocytes sharply decreases with increasing procalcitonin, ferritin, CRP, and D-dimer levels [57]. Diffuse alveolar damage marked by the formation of hyaline membrane and interstitial lymphocyte infiltration is demonstrated by the effects of a deadly storm of cytokines. In organ failure, collateral tissue damages, and poor consequences of COVID-19 patients and its associated unregulated inflammatory response, also associations with MERS and SARS. Serum levels of IL-8, MCP-1, TGF-β, IL-12, IL-6, IL-1, and IFN-γ were higher in extreme cases of SARS than in those with moderate to mild symptoms. In patients moderately infected with MERS-CoV, serum levels of IL-8, IL-6, and IL-1β were also increased. It is important to develop criteria for the prediction and diagnosis of a cytokine storm in patients with COVID-19 surrogate biomarkers because the peak levels of circulating cytokines are not regularly monitored for kinetic changes. A higher risk of continuing or forming cytokine storms in COVID-19 patients in addition to CRP and ferritin was also associated with increased procalcitonin and D-dimer [58]. Worth note, elevated CRP and ferritin levels in patients under treatment with chimeric antigen receptor T cells are associated with the initiation of cytokine storms. Macrophages and monocytes express ACE2 and may therefore be infected with SARS-CoV-2, activating and transcribing pre-inflammatory genes [38]. SARS-CoV-2 infection appears to significantly reduce per-cell expression of ACE2 on PB monocytes, which can be a secondary consequence of viral binding [59]. Whether

the down-regulation of ACE2 receptors is a substitute for viremia has not been determined yet. Besides, in patients with COVID-19, in spleen and lymph nodes, ACE2 expression was observed on CD169+ and CD68+ macrophages and provided additional confirmation that infection with SARS-CoV-2 can target ACE2-positive myeloid cells, including the lymph nodes and spleen, and the whole body [57]. The red pulp part of the spleens primarily contains contaminated macrophages of CD169+. Furthermore, at the margins of lymph nodes, macrophage-rich areas were more probable to test positive for viral nucleocapsid protein antigens. Published investigations have shown that, as a consequence of a type I IFN-dependent activation refractory state, CD169+ macrophages control the replication level of viral in support of immunity development. This shows that macrophage infection with CD169+ could be a conduit to translocate SARS-CoV-2 to lymph nodes and spleen. This may be responsible for additional systemic viral replication, resulting in reduced immunity. Human monocyte cell lines U939 and THP-1 express ACE2, in the investigation of myeloid modulation and the role of TLR interactions in triggering activation before and after infection, and may therefore be useful [59]. Similarly, hACE2 transgenic mice can be used to study both pharmacology and physiology of SARS-CoV-2 infection [46]. Monocytes from COVID-19 cases show an active but normal number of phenotypes, as shown by their morphology (FSChigh) and capability to produce IL-10, IL-6, and TNF [59]. The surface expression of CD206, CD163, CD80, CD68, CD16, CD14, and CD11b in patients with COVID-19 is the product of activated monocytes present in peripheral blood (PB). The PB monocytes' activation was chiefly associated with the severity of the disease and poor prognosis [59]. In patients with COVID-19, PB monocyte expression of CD206 and CD163 shows a propensity to an M2 or regulatory phenotype that can affect the responses of adaptive antiviral effector T cell. In addition to immune symptoms, the CD163 expression was also associated with activated monocyte/macrophage hemophagocytic lympho-histo-cytosis syndrome, which potentially contributes to increased development of cytokine and immunological pathogenesis in COVID-19 cases [48]. Wen *et al.* reported that the PB of COVID-19 patients had an overflow of IL1β+ and IFN-activated monocytes of inflammatory CD14+T cells [60]. Furthermore, SARS-CoV-2 activates macrophages via ACE2, inducing the expression of IL-6 in the lymph nodes and spleen and alveolar macrophage expression of PD-1, IL-10, TNF, and IL-6. This applied mechanism will increase lymphocytopenia and lead to a storm of cytokines, starting with an increase in lung viral levels [57]. Autopsy findings indicated the accumulation of inflammatory macrophages in COVID-19 patients' lungs. Inflammatory macrophages have also been shown to accumulate in the lung of COVID-19 cases. RNA sequencing confirmed that FCN1+ macrophages derived from monocytes that may be indicative of high inflammatory cells and chemokine development are the predominant subset in the broncho-alveolar lavage fluid (BALF) of COVID-19 patients with ARDS. In line with autopsy records, transcriptional examination of mononuclear PB cells and BALF of COVID-19 cases showed high IFN-induced MCP-1 and IP-10 levels that were likely to attract macrophage trafficking to the infection site [61]. Inflammatory monocyte/macrophage activation and aggregation can result in disordered T cell inflammatory response and apoptosis, caused by delayed signaling of Type I IFN. Analysis on lung tissue found that SARS-CoV-2 produced 3 times more infectious virus particles after 48 hours than SARS-CoV did. Nevertheless, SARS-CoV-2 caused less IFN and pro-inflammatory mediators than SARS-CoV [55]. This may be due to the ability of virus to prevent inherent innate responses during initial infection. The early response of type I IFN is principally important. Initial findings revealed that IF-alpha2b dramatically shortened the detection time of the virus in the upper respiratory tract, which decreased the length of the elevated levels of CRP and IL-6 as inflammatory markers in blood [38]. Further study, however, still needs the mechanism underlying the ability of SARS-CoV-2 to reduce initial innate response to acute infections and suppress Type-I IFN to maximize viral release. Zhang *et al.* suggested that the neutrophil/lymphocyte ratio coupled with IgG more strongly predict the strength of COVID-19 compared to neutrophil count alone [59]. It is also recognized that neutrophil extracellular traps (NETs) that are DNA/histone extracellular webs released for infection control by neutrophils, worsen inflammation. Previous studies have shown that cytokine storm (IL-1β), excessive thrombosis, cystic fibrosis, and ARDS may result from aberrant NETs. In severe COVID-19 cases, increased levels of myeloperoxidase (MPO)-DNA NETosis and cell-free DNA have been documented frequently. The initiation of arterial and venous thrombosis in COVID-19 cases is also likely to be caused by increased NETosis, in addition to contributing to the cytokine storm. This finding indicates that NETs may be a central pathological dysregulated mechanism that drives the release of cytokines and multiorgan damages, resulting eventually in coagulopathy and respiratory failure. Furthermore, in COVID-19 patients, transcriptional examination of PB and BALF mononuclear cells showed that elevated levels of CXCL-8 and CXCL-2 may be due to the recruitment of neutrophils at the infection site, further exacerbating the pulmonary inflammatory responses [62]. Other significant areas of neutrophil response remain unstudied, such as humoral or myeloid interactions, including the function of CD89 and IgA, CD16 and IgG, etc. **Figure 1** sums up what happiness during SARS-CoV-2 infection including cytokine storm and gives a short summary of this review article.

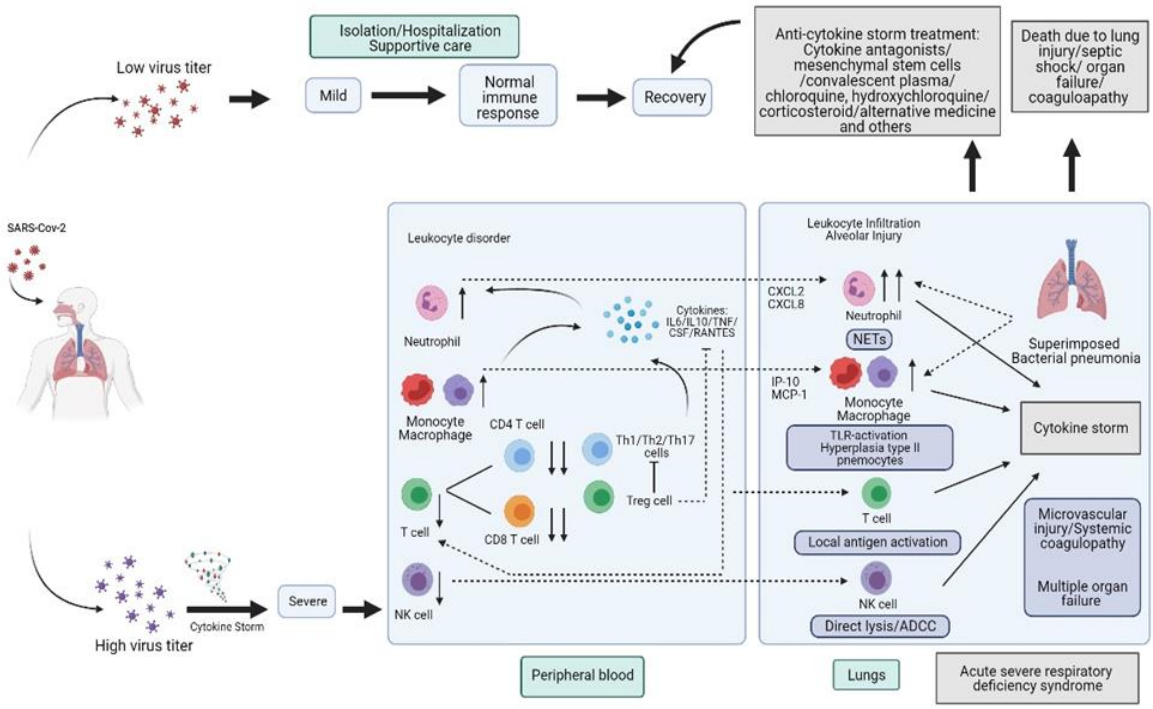

Figure 1. Conceptual model of observations related to SARS-CoV-2 infection. In addition to the penetration of leukocytes into lung tissue, the activation of macrophages/monocytes and lymphocyte subsets in the blood are probably major sources of the release of cytokines. It has been shown that alveolar injury is correlated with cell infiltrates, NETs release, type II pneumocyte hyperplasia, among others, all of which may lead to cytokine storm, lung failure, and ARDS. The figure has been designed using BioRender (https://app.biorender.com/).

Conclusion

In extreme or serious COVID-19, SARS-CoV-2 infection triggers a syndrome called cytokine storm, which is generally thought to be a central mediator of pulmonary injury and consequent ARDS. The current review provides explanations of leukocytes and cytokines that may contribute to cytokine storming from the early to the late stages in both severe and mild symptoms of COVID-19. However, more necessities to be learned about how increased levels could be used in particular cytokine(s) mixtures and reactions to explain what happens in each stage of the inflammatory reaction as the course of COVID-19 progresses (utilizing defined ranges and standardized methods). In addition to widely used cytokine-based biomarkers for disease development surrogates (procalcitonin, ferritin, CRP, and D-dimer), with an emphasis on ferritin and D-dimer, there is still no consensus on +cut-off values for the disease progress and evaluation. With the incorporation of cellular (decreased lymphocytes, neutrophilia, etc.) soluble plasma markers (e.g. D-dimer) along with cytokine shifts, disease staging is also essential to follow. The immunoregulatory cascade, coagulopathy, cytokine storm features, and other inflammatory effects are impaired by each of these conditions. By detecting shared or population-based of what triggers and amplifying such cytokine storm at a certain point of the disease stages, new precise medicinal strategies can be advanced.

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