Review Article

Immune Responses in SARS-CoV-2, SARS-CoV, and MERS-CoV Infections: A Comparative Review

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Abstract

Coronavirus, discovered in the 1960s, is able to infect human hosts and causes mild to serious respiratory problems. In the last two decades, the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been recognized. It has long been demonstrated that MERS-CoV binds to dipeptidyl peptidase 4 and SARS-CoV binds to angiotensin-converting enzyme 2. A "cytokine storm" is the main pathophysiology of aforementioned viruses. Infiltration of neutrophils at the site of the infection is a risk factor for the development of acute respiratory distress syndrome and death. The new coronavirus, SARS-CoV-2, has infected more people than SARS-Cov and MERS-CoV as it can easily be transmitted from person to person. Epidemiological studies indicate that majority of individuals are asymptomatic; therefore, an effective and an efficient tool is required for rapid testing. Identification of various cytokine and inflammatory factor expression levels can help in outcome prediction. In this study we reviewed immune responses in SARS-CoV, Mers-CoV, and SARS-COV-2 infections and the role of inflammatory cells.

Keywords: *Adaptive immunity, coronavirus; cytokine storm, SARS-CoV-2*

Introduction

Coronavirus, discovered in the 1960s, is a RNA virus. Six species have been known to infect human hosts. They cause mild to serious respiratory problems. In the last two decades, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have been recognized. These zoonotic and pathogenic coronaviruses have caused global or regional outbreaks. Recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified. It causes COVID-19, a respiratory illness. In a very short time, COVID-19 became the greatest health challenge worldwide.[1]

COVID-19 disease has some similarities with other viral diseases such as SARS and MERS. For example, all are initiated by zoonotic transmission and spread rapidly among humans. In addition, they cause severe respiratory illness and death, and all can develop a cytokine storm.[2,3] However, when they enter host cells, MERS-CoV binds to dipeptidyl peptidase 4 and SARS-CoV and SARS CoV-2 bind to angiotensin-converting enzyme 2 (ACE2).[4] SARS-CoV-2 has more affinity for ACE2 receptors makes it spread so easily.[5] Additionally, SARS-CoV-2 infects and replicates within endothelial cells. SARS-CoV-2 can infect both alveolar epithelium and pulmonary microvascular endothelium.[6] During SARS-coV-2 infection, a part of pathogenesis is caused by thrombin activation which results in thrombotic complications.[7]

The immune responses have essential roles in the resolution of a viral infection, but they can also result in immune pathogenesis. If the immune system functions properly and in the absence of any basic disease, the virus can be effectively suppressed in the acute phase. Nonetheless, when the body fails to produce an adequate adaptive response, a cytokine storm and diffuse organ involvement occur.[4] Increasing evidence shows that the development of an adaptive immunity is controlled by the invading microorganisms and innate immune systems.[8] The aim of this review was to gain improved understanding of the mechanisms of immune responses against coronaviruses, which are essential

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in the development of a safe prophylactic vaccine. Besides, studying the immune responses in SARS patients and MERS patients can improve the treatment strategies of SARS-Cov-2-infected patients.[9,10]

The Possible Transmission Routes

Although the mentioned viruses have a zoonotic nature, they rapidly spread from human to human. The transmission route of SARS-CoV-2 is controversial. It is suggested that virus is transmitted from person to person through droplets.[11] Saliva has a great impact on the transmission of infection. Coronaviruses, including SARS-Cov-2, have been demonstrated in saliva. SARS-Cov-2 virus has been detected in both salivary gland ducts and gingival crevicular fluid (GCF).[12] Besides, ocular surface is a potential infection route for both the entry and exit of the virus.[13] Of note, SARS-Cov-2 can be found in sputum, stools, urine, blood/serum, tear, and cerumen samples.[14,15] SARS-CoV binds to the host cells through its target receptor, the ACE2 protein. It is also believed that SARS-CoV might pass via the mucous membranes, particularly nasal and larynx mucosa then enters the lungs.[16] DPP4, the receptor for MERS-CoV, is expressed in type I and type II alveolar cells, bronchial epithelium, endothelial cells, alveolar macrophages, and leukocytes. Like the virus of SARS, MERS-CoV can infect and replicate in the human airway epithelial cells.[17]

Hypothetical Pathogenesis of SARS-CoV-2, SARS-CoV, and MERS-CoV

SARS-CoV-2 enters alveolar cells expressing ACE2 and transmembrane protease serine 2. Replication of the virus results in releasing viral nucleic acids which are recognized by other cells including epithelial cells, endothelial cells, and alveolar macrophages. Consequently, proinflammatory cytokines and chemokines including IL-6, IP-10, macrophage inflammatory protein 1 alpha (MIP-1α), MIP1β, and monocyte chemoattractant protein-1 (MCP-1/CCL2) are generated. Then, T cells, monocytes, and macrophages infiltrate to the site of infection which enhance more inflammation. Accumulation of immune cells in the lungs causes a "cytokine storm" which circulates in other organs.[18] A cytokine storm in response to type 1 and type 2 T helper (Th) cells has been proposed as the main pathophysiology of COVID-19 infection.[19] It has also been suggested that proinflammatory cytokine/chemokine responses result in apoptosis of lung epithelial cells and endothelial cells.[20]

ACE2 is also expressed on oral epithelial cells. Higher expression of ACE2 has been indicated in the tongue mucosa suggesting the susceptibility of oral cavity to SARS-CoV-2 infection. ACE2 also distributes in the salivary gland tissues. ACE2 receptors can be detected on salivary duct epithelial cells, early targets of SARS-CoV-2 infection. It is suggested that SARS-Cov-2 fuses into the salivary duct epithelial cells, replicates in them, and be released into saliva.[21] Vimentin, an intermediate filament protein, has a great impact on different cellular processes such as cell division and migration.[22,23] Besides, vimentin contributes to pathological conditions such as immune responses and autoimmune diseases. Vimentin is a cytoplasmic protein; however, it can be found at extracellular locations or at the surface of different cell types.[24] Interestingly, at the cell surface, it functions as a receptor for bacterial and viral pathogens. Regarding SARS-CoV, vimentin has a key role in virus attachment and entry. As vimentin is produced by activated macrophages, it can act as a proinflammatory factor. It can also induce the proinflammatory cytokines and tissue damage. On the other hand, vimentin presents in M2 macrophages; hence, it may accelerate lung repair.[25] A recently published study has shown the potential interactions between vimentin and several SARS-CoV-2 proteins including S protein. The authors have suggested that extracellular vimentin might act as an important component of the SARS-CoV-2 spike protein-ACE2 complex, which mediates SARS-CoV-2 cell entry. Therefore, vimentin may increase SARS-CoV-2 host cell invasion by functioning as an attachment factor.[26] Accumulating evidence suggests that SARS-CoV-2 infection promotes the innate and adaptive immune cell activation in infected host. An impaired immune system combined with a basic disease cannot effectively control the virus in the acute phase; hence, the patient enters a critical and severe phase.[27] Some of SARS proteins such as replicase, spike, Orf3, Orf4, Orf13, envelope, membrane, and nucleocapsid can stimulate T cell responses, which are correlated with higher neutralizing antibody activity.[28] The pathophysiology of SARS- CoV-2 infection depends on aggressive inflammatory responses causing damage to the airways. Thus, disease severity depends on both the viral infection and the host response.[29,30] Higher number of neutrophils and monocytes in the lung tissue and peripheral blood, serum cytokines, and chemokine levels are higher in severe cases compared to those in mild to moderate cases.[20] SARS-CoV virus can avoid innate antiviral type I interferon (IFN) responses of host cells in order to prolong viral replication and survival.[8] MERS-CoV is also able to manipulate the innate and acquired immune responses, block, or prevent IFN production pathways.[31]

Clinical Features

According to the clinical investigations, the mean incubation period of SARS-CoV-2 is 7.8 days. However, the clinicians recommend a quarantine period of 14 days.[32] The incubation periods of MERS-CoV and SARS-CoV are about 4.5–5.2 days and 4.0 days, respectively.[33] The clinical features vary from asymptomatic and mild clinical symptoms to acute respiratory-distress syndrome and death.[34] In addition to respiratory tract, coronavirus infections may involve other organs/systems including

heart, gastrointestinal tract, liver, kidney, skin, and eyes.[35] SARS-CoV and SARS-CoV-2 have similar clinical features including fever, pneumonia, dyspnea myalgia, dry cough, and fatigue.[36] Due to excessive viral immunoreaction and SARS-CoV-2 entry into salivary glands, chiefly parotid and submandibular glands, salivary tissue damage may happen. Consequently, chronic obstructive sialadenitis may develop which is associated with swelling, pain, and discomfort in affected salivary glands.[21] Advanced age, male gender, cardiovascular disease, and risk factors such as hypertension, diabetes, and obesity are associated with poor prognosis.[37] Fever, dyspnea, cough, generalized fatigue, vomiting or diarrhea, abdominal pain, confusion, and myalgia or arthralgia are common in patients with MERS-CoV infection.[38]

Immune Responses in MERS-CoV, SARS-CoV, and SARS-Cov-2

Due to the cytokine storm, different types of coronaviruses can cause severe pathogenic responses resulting in severe pneumonia, pulmonary edema, multiple organ failure, and even death. Both MERS-CoV and SARS-CoV are able to infect monocytes, macrophages, dendritic cells (DCs), and activated T cells,[39] however, only MERS-CoV can replicate in the infected macrophages and DCs to induce inflammatory responses and impaired apoptosis in T cells. These findings may explain higher mortality rate of MERS-CoV infected patients.[40] Besides, MERS-CoV can infect human airway epithelial cells. MERS-CoV infection results in increased expression of IFN-β, CXCL10 (interferon-γ-inducible protein; IP-10), and MxA.[39,40] MERS-CoV infection also triggers the production of IFN-α by infected cells, which causes the release of some chemokines including MCP-1, CXCL10, and IL-10 to mediate T cell recruitment.[31] In addition, remarkable upregulation of TNF-α, IFN-ɣ, CCL2, CCL3, CCL5, IL-12, and IL-17 has been indicated.[41,42] Nevertheless, some studies did not detect TNF-α expression in most patients infected with MERS-CoV.[43,44] IFNγ has a critical role in early immunity by stimulating both $CD8^+$ and natural killer (NK) cells to induce apoptosis of infected cells.[45] Downregulation of IFNγ and Th1-associated cytokines has been demonstrated in the lower respiratory tract of MERS-CoV-infected patients.[44] Elevated plasma concentrations of IL-6, IL-1RA, IP-10, and MCP-1 are detectable at the acute phase of MERS-CoV infection.[43] IL-6 is produced by T-cells, macrophages, DCs, and B-cells and promotes the proliferation of T-cells.[46] IL-6 is involved in trafficking of leukocytes, especially neutrophils which are important in pathogenesis of inflammatory reactions.[47] IL-6 inhibits the secretion of IFNγ by blocking $CD8^+$ cells.[34] SARS-CoV-2 infection is associated with a proinflammatory phase, which is characterized by elevated levels of cytokines including IL-1β, IL-1Rα, IL-2, IL-6, IL-10, fibroblast growth factor, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), IP-10, MCP-1, MIP-1α, platelet-derived growth factor, and vascular endothelial growth factor (VEGF). Among them, the expression of IL-6 has a crucial role across the immune system and is mainly correlated with increased mortality. Increased IL-6 signaling promotes the maturing naïve T cells into effector T cells, induces VEGF expression in epithelial cells, increases vessel permeability, and decreases myocardium contractility.[48] SARS-CoV-2 infects human lung tissues more effectively than SARS-CoV; however, SARS-CoV can trigger higher levels of proinflammatory cytokines and IFNs.[36] Interestingly, elevated IL-6 level in SARS-CoV-2-infected patients is higher than the patients with SARS-CoV or MERS.[49] A previously published paper has indicated that excessive amounts of inflammatory cytokines (IL-6, IL-10, IL-2, and IFN-γ) are correlated with COVID-19 severity.[50] CXCL10 (IP-10) has also been evaluated in viral infections. Neutrophils, endothelial cells, keratinocytes, fibroblasts, DCs, astrocytes, and hepatocytes are able to produce IP-10. CXCL10 binds to chemokine receptor 3 (CXCR3) to recruit T cells, monocytes, and NK cells. Enhanced serum level of IP-10 has been observed in patients with COVID-19 especially in the most severe cases.[45] Additionally, increased expression levels of IP-10, monocyte-chemotactic protein 3 (MCP-3), hepatocyte growth factor, monokine-induced gamma IFN (MIG), and MIP-1α are highly associated with disease severity during disease progression. Among them, IP-10 and MCP-3 are excellent predictors for progression of COVID-19.[51] In addition, upregulation of IP-10 in airway epithelial cells and lung fibroblasts is associated with severity of MERS-CoV infection.[52] Increased serum levels of IL-6 and IP-10 within 2 weeks can be indicated in severe cases with MERS infection.[53] Elevated CXCL10 expression levels have been detected in lung tissues during early stages of SARS-CoV infection; however, some of the inflammatory factors such as CCL3, CCL27, CXCL2, and CXCL8 are increased in lung tissues during late stages of the disease.[54] Elevated expression of proinflammatory cytokines including MCP-1, TGF-β1, TNFα, IL-1β, and IL-6 has been indicated in autopsy tissues from patients who died of SARS-CoV.[55] Besides, enhanced expression levels of IL-1α, IL-1β, and IL-8 can be measured in the lower respiratory tracts of patients infected with MERS-CoV and are associated with tissue damage and acute inflammatory responses, which lead to severe pathogenesis and mortality.[44] IL-8 has a great impact on neutrophil recruitment and activation. It is hypothesized that high IL-8 expression levels may cause the formation of neutrophil extracellular traps (NETs), web-like structures of DNA, and proteins; therefore, this results in severe MERS-CoV infection.[44] In addition, increased expression of IL-8 in MERS-CoV-infected patients may upregulate CD4 molecules to enhance helper T cell infection.[44] Elevated expression levels of IL-7 and IL-15 were also

recorded in most patients with MERS infection.[56] IL-7 plays a key role in T-cell homeostasis. Increased serum IL-7 level in severe cases of COVID-19 patients may indicate a homeostatic mechanism.[46] MERS-CoV infection also induces Th17 cytokines which recruit neutrophils and monocytes to the site of infection or inflammation.[42] Th2 cytokines have a great impact on the humoral immune responses. MERS-CoV infection is able to downregulate the Th2 responses.[44] Increased serum level of IL-12 has also been found in SRAS-CoV-2- and SARS-CoV-infected patients.[57]

Antibody response to MERS-CoV can be found in the second and third week after the onset of infection. In patients with pneumonia, antibodies can be detected 13 months after infection.[58] In SARS-CoV infection, IgM and IgG are produced and can be detected in patient's blood 3–6 and 8 days after infection, respectively.[59] Regarding SARS-CoV-2 infection, IgM can be idetected in early-stage infection (peaks after 2 weeks); however, IgG peaks in 3 weeks and maintains at a high level for over 48 days.[60] Higher IgG level can be observed during late stages or postrecovery.[61]

Toll-like receptors (TLRs) signaling pathways control the MERS-CoV infection. TLRs are the key mediators of innate immune response, and the spike protein of MERS-CoV is able to inhibit the TLR signaling pathways.[31] Stimulation of TLRs activates the nuclear factor-κB (NF-κB) signaling pathway, which results in the production of inflammatory factors from monocytes including IL-1, TNF-α, and IL-6 to control virus infections. The innate immune reaction signaling initiates by TLR2 through NF-kB in macrophages, monocytes, and epithelial cells.[62] The binding of SARS-CoV-2 to TLRs results in the release of pro-IL-1β, which is a mediator of lung inflammation, fever, and fibrosis.[63] During infection with SARS-CoV, TLR2 expression allows for IL-8 production in response to S protein.[64] Activation of TLR4 signaling increases cell surface expression of ACE2 to facilitate virus entry. It has also been shown that after entry to host cells, SARS-CoV-2 is recognized chiefly by TLR7 in endosomes.[65] NETs formation by neutrophils in the lungs of patients with SARS-CoV-2 may be induced *via* TLR7 signaling.[66] Also, activation of TLR7 results in the production of TNF-α, IFN-α, IL-12, and IL-6 to generate specific cytotoxic CD8+ T cells.[67] The Notch pathway has a functional role during the activity of innate and adaptive immune cells. In patients with COVID 19. Notch1 binds to IL-6 promoter in macrophages in response to IFN-γ resulting in IL-6 production. In turn, IL-6 increases the expression of delta-like ligand-1 to amplify the Notch signaling.[68] JAK1 and JAK3 also promote several cytokines function involving in antiviral responses such as IFNγ, IL-2, IL-15, and IL-21. Therefore, it is proposed that JAK1/JAK3 enhances the clearance of SARS- CoV-2. However, JAK2 facilitates SARS- CoV-2 entrance. Besides, JAK2 signaling induces the production of IL-6 and GM-CSF.[69] The transcription factor NFκB induces the transcription of proinflammatory cytokines. Activation of NFκB has been recorded in infections with both SARS-CoV and MERS-CoV. In MERS-CoV-infected patients, Th17 cells are able to produce IL-17 via the STAT3 and NF-κB signaling pathways.[31] Also, increased expression level of IL-10 in MERS-CoV-infected patients has been indicated. IL-10 has an antiinflammatory effect mediated through JAK-STAT pathway.[42]

The Role of Inflammatory Cells

In acute phase of MERS-CoV infection, proinflammatory Th1 and Th17 responses can be found with increased concentrations of IFN-γ, TNF-α, IL-15, and IL-17.[42] Th2 cells stimulate antibody production by secreting IL-4, IL-5, IL-9, IL-10, and IL-13.[70] Overactivation of CD8+ T cells can also be detected in acute phase of MERS-CoV infection, though $CD4^+$ T-cells play a minor role in acute phase.[43] Neutrophils are the most abundant immune cells in the innate immune system. Neutrophils play a critical role in the clearance of pathogens and debris through phagocytosis. It has been suggested that neutrophils promote antiviral defenses via different mechanisms and cytokine release.[62] Neutrophils release NETs for viral inactivation and cytokine production to restrict virus replication. On the other hand, neutrophils are highly immunogenic and toxic to the host tissue and cause inflammation, and epithelial and endothelial cell death. Increased neutrophil and monocyte counts have been observed in more severe and fatal cases of MERS infection.[56,71] Migration and recruitment of neutrophils have been induced by the proinflammatory cytokines such as IL-8, TNF-α, and IL-6.[72] CD8+ T cells contribute to MERS-CoV clearance by producing IFN-γ and granzyme.[31]

A significant increase of Th-1-related cytokines, interferons, and IL-2, IL-12, IFN-γ, and TNF-α has been indicated in mild cases of SARS-CoV patients.[72] Increased expression level of IL-2 has also been found in SARS-CoV patients. IL-2 is produced by CD4+ T cells and $CD8^+$ T cells.[73] Decreased number of $CD4^+$ and $CD8^+$ T cells has been shown at the early phase of SARS-CoV infection and is associated with adverse outcomes. It is believed that activation of IP-10 results in a significant decrease in peripheral $CD4^+$, $CD8^+$ T lymphocytes, and NK cells.[74] Th-1 cell phenotype stimulates the proliferation and activation of cytotoxic T lymphocytes and promotes the phagocytic activity of macrophages in SARS infections.[72] In addition, a marked elevated of Th2 cytokines (IL-4, IL-5, IL-10) has been reported in fatal SARS cases.[28] A previous *in vivo* study has shown the production of IP-10, CCL-2, CXCL-1, and CXCL-3 by neutrophils. The authors have suggested that neutrophils may induce other cell types to synthesize these chemokines.[75] DCs and macrophages are attracted to the site of infection via

increased expression levels of CXCL-10/IP-10, CCL-2/MCP-1, CXCL-5/RANTES, and CCL-3/MIP-1α. Infected DCs during SARS-CoV infection induce the expression of CCL2, CCL3, CCL5 and CXCL10.[76] In addition, the monocyte differentiation to macrophages is activated by proinflammatory cytokines, including GM-CSF, IFN-α, IL-6, and TNF-α.[77]

SARS-CoV-2 infection is characterized by lower total lymphocyte count ($CD4^+$ and $CD8^+$ T-cells, NK cells, and B cells) in circulation, higher neutrophil and monocyte counts, and an increased production of inflammatory cytokines which are correlated with disease severity and death.[46,78,79] Lymphopenia might be caused by increased serum cortisol level.[46] Also, it has been shown that IL-6, produced by infected macrophages, promotes lymphocyte necrosis.[74] Lower T-cell count is because of increased apoptosis and/or reduced proliferation rates.[46] Higher neutrophil-to-lymphocyte ratio, a well-known marker of infection and systemic inflammation, is suggestive for poor prognosis.[79] Besides, a decreased number of circulating $CD4^+$ cells, $CD8^+$ cells, B cells, NK cells, monocytes, eosinophils, and basophils can be indicated.[80] A significant increase in the proportion of naïve helper T cells and reduction in memory helper T cells and regulatory T cells can be detected in SARS CoV-2-infected patients. Rapid reduction of lymphocytes mainly T lymphocytes (both CD4+ and CD8+ T lymphocytes) in peripheral blood has been found in the acute phase of infection.[4] Higher serum levels of IL2, IL7, IL10, GSCF, IP-10, MCP1, CCL3 (MIP1A), and TNF-α in severe cases of COVID-19 patients reflect the activation of T-helper 1 (Th1) cells.[74] It is suggested that the number of $CD4^+$ T and $CD8^+$ T cells is negatively correlated with the levels of TNF-α, IL-6, and IL-10, respectively. This finding may suggest that aforementioned cytokines are involved in a decrease in T cell counts.[81] In patients with COVID-19, overactivation of $CD8^+$ T cells has been documented in COVID-19 cases.[82] However, the ratio of CD4:CD8 remain normal and stable.[83] Overactivation of $CD4^+$ and $CD8^+$ T-cells in the early phase of COVID-19 results in the production of GM-CSF.[74] Nonetheless, CD8+ T cells are cytotoxic killing virus-infected cells via producing the cytotoxic molecules such as perforin and granzyme B.[84] Cell-mediated immunity including T-cells (T helper and cytotoxic) has a great impact on efficient antiviral responses. T cells also have critical roles against viral infections. For instance, CD4+ T cells facilitate virus-specific antibody synthesis via the T-dependent activation of B cells[85] and in SARS-CoV-2 infections; $CD4^+$ T-cells (especially T_H1 cells) react to S-protein.[46] Memory $CD4^+$ T cells and $CD8^+$ T cells have been found in 100 and 70% of recovered patients, respectively. It has been suggested that memory T cell reactions are for different SARS-CoV-2 proteins such as spike protein, nucleoprotein, and membrane protein.[86] Additionally, it has been believed that disease severity in COVID-19 may be associated with low IFN-γ production by CD4+ T-cells. The proportion of Th17 cells is augmented in the peripheral blood. Th17 cells are mainly stimulated by IL-6 and IL-23.[50] GM-CSF synthesis is increased during the acute phase of COVID 19 patients by Th17 cells.[45] Additionally, reduced number of NK cells and B cells has been reported in patients with severe COVID-19 patients. A previously published paper has shown that overexpression of inhibitory signals can suppress T-cell and NK cytokine secretion in COVID-19 patients.[87] Reduced functional markers of NK cells (CD107a, IFN-γ, IL-2, and TNF-α) have been consistently reported in COVIC-19 patients compared to healthy controls. Also, a decreased expression levels of granzyme B in the NK cells of the patients have been detected.[78]

Two types of pulmonary macrophages can be indicated: alveolar macrophages, residing close to type I and type II epithelial alveolar cells, and interstitial macrophages which are predominantly found between the endothelial layer and alveolar epithelium zone.[88] Functionally, both macrophages are divided into two types: First, M1 macrophages which are activated by pathogens including viruses. Later, their activation is promoted by Th1 cells. M1 macrophages attract immune cells into the lung parenchyma. Second, M2 macrophages activated by Th2 cells (IL-4, IL-13). Increased activation of M1 macrophages in SARS-Cov-2-infected patients results in the production of inflammatory cytokines. Among the produced cytokines, IP-10 leads to cytokine storm.[89] GM-CSF stimulates monocytes/macrophages to produce IL-6 and some other inflammatory factors.[74] In the upper respiratory tract, macrophages express different chemokines and proinflammatory cytokines such as IL-1B, IL-8, IL-18, and TNF-α.[74] Also, it has been reported that monocytes stimulated NK cells to produce IFN-γ through IL-8.[90]

The production of chemokines and mediators by infected cells results in the infiltration of neutrophils at the site of infection. Neutrophilia may be a risk factor for the development of acute respiratory distress syndrome and death. Neutrophils secrete cytokines and chemokines that attract more immune cells such as T lymphocytes and monocytes. Cytokines such as G-CSF, CXCL10, MCP1, MIP1A, and TNF-α are responsible for attracting neutrophils to the inflammatory sites. These findings may highlight the role of neutrophils in the severity of illness in COVID-19 patients.[91] In COVID-19 patients, the release of NETs can protect the host. In addition, neutrophils produce TNF, IL-6, IL-8, and reactive oxygen species.[62] However, the overproduction of NETs causes lung tissue damage (killing lung epithelial cells) via enzymes such as neutrophil elastase (NE) and myeloperoxidase.[62,66] Additionally, the increase in platelet-neutrophil aggregates results in increased levels of NET and immunothrombosis formation. Immunothrombosis causes microvascular

Table 1: A summary of the role of inflammatory cells in SARS-CoV-2, SARS-Cov, and MERS-CoV infections

Inflammatory cell type	SARS-CoV-2 (Reference)	SARS-CoV (Reference)	MERS-CoV (Reference)
T helper cells: Th1, Th2, and Th17	IL2, IL7, IL10, GSCF, IP-10, MCP1, CCL3 (MIP1A), TNFα,[63] IL-10,[46] GM-CSF,[45,74] CCL5[96]	IL-2, IL-12, IFN-γ, TNF-α, IL-17,[95] IL-4, IL-5, IL-10,[80] CCL5[96]	IFN-γ , TNF-α, IL-15, IL-17,[42] IL-17[94]
CD8+T cells	Perforin, granzymes, IFN-γ,[81] GM-CSF[74]	IFN-γ, TNF-α, CD107a,[28] IL-2[73]	IFN-γ and granzyme[31]
Natural killer cells	IFN-γ[90]	IFN-γ, IL-6, IL-8, IL-10, IL-12[28]	IFN-γ, TNF-α[42]
Monocytes/ macrophages	IL-10,[46] IL-6, TNF-α, IL-10[74]	IFN-γ, IL-6, IL-8, IL-10, IL-12,[28] TNF, IL1-β, nitric oxide , CCL2, CXCL10[97]	IFN-γ, IL-15,[42] IL-8, IL-12[41]
Neutrophils	NETs,[62] ELANE[91]	IP-10, CCL-2, CXCL-1, CXCL-3,[75] ELANE[91]	NETs, granules[44]
Dendritic cells	IL-12, type I IFNs,[93] IL-15,[90] IL-10,[46] IL-15[90]	TNF-α , IL-6, CCL2, CCL3, CCL5, CXCL10[76]	IL-6 and TNF-α, IFN-γ, IL-12, IP-10[98]
B lymphocytes	IgG , IgM,[101] IL-10[46]	IgM, IgG, IgA[100]	IgM, IgG[99]

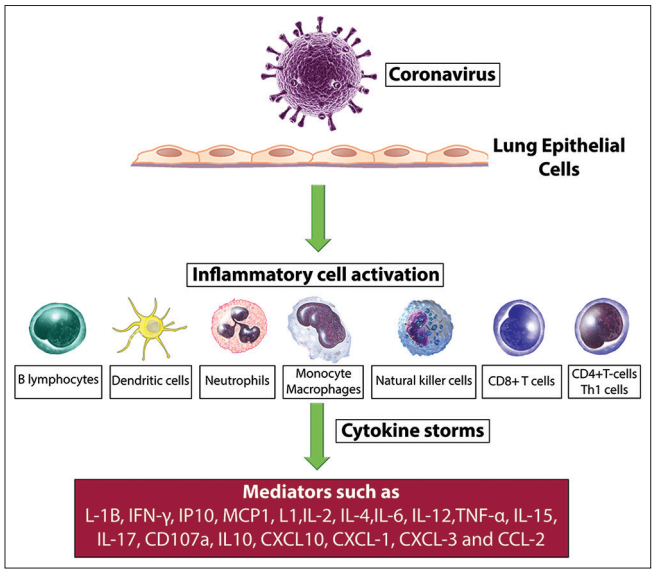

Figure 1: Corona virus binds to Lung Epithelial Cells which causes the activation of inflammatory cells and cytokine storms

occlusion in the lung.[92] A previous animal study also found that neutrophilia is associated with hemorrhagic lesions in the lungs of rats with coronavirus infection. Upregulation of ELANE (a NE) in patients with SARS and COVID-19 patients might be the reason for the hemorrhaging of lungs, one of the leading causes of death.[91]

DCs are a diverse group of antigen-presenting cells and play pivotal roles in the initiation and regulation of innate and adaptive immune responses. DCs produce IL-12 and type I IFNs.[93] In addition, DCs produce IL-15 which activate NKcells.[90] Table 1 summarizes the role of inflammatory cells in SARS-CoV-2, SARS-Cov and MERS-CoV infections.

Diagnosis and Detection Methods

Figure 1 summarizes the pathophysiological mechanism of coronavirus infection resulting in the excessive production of cytokines. Early detection of virus-infected patients has a great impact on the prevention of transmission. The diagnosis of COVID-19 is based on the combination of epidemiologic information such as a history of travel to or residence in affected areas 14 days prior to symptom onset, clinical symptoms, CT findings, and laboratory tests (RT-PCR) on respiratory tract samples.[102] Beside to oropharyngeal swabs specimens, SARS-CoV-2 can be detected in urine, blood, and anal swabs.[103] Analysis of saliva and the GCF is a noninvasive diagnostic method. SARS-CoV-2 can be detected in saliva samples in the early phase of infected patients. In a previous study, saliva was detected in 91.67% of SARS Cov-2 patients a few days after hospitalization.[104] In this study, to confirm that salivary glands were infected with SARS-Cov-2, saliva was directly collected from the opening of salivary glands.[105] Interestingly, very recent study has found that tear sample from a patient with conjunctivitis can also be used as a tool for detection of SARS-CoV-2.[106] Chest CT is a useful diagnostic tool in most patients with COVID-19. In some patients with negative qRT-PCR results but with clinical symptoms, typical CT features are visible. A meta-analysis found that the sensitivity and specificity of initial chest CT scan for highly suspicious individuals for COVID-19 are 87 and 43%, respectively. It means that a chest CT scan can be considered as an adjuvant diagnostic tool.[107] A recently published work has indicated that SARS-CoV-2 antigen and RNA can be detected in formalin fixed paraffin-embedded specimens using immunohistochemistry.[108] Detection with genomic sequencing is also a rapid and complete diagnostic method. This technique is able to investigate SARS-CoV-2 evolution during transmission. Also, this technique can identify several pathogens in a single patient.[109] SARS-CoV antibodies include spike and nucleocapsid proteins. Detection of IgM and IgG against SARS-CoV-2 is a fast and simple screening method.[110] A very recent study has indicated that the combination of IgM and IgG antibodies is an accurate diagnostic test with 84.5% sensitivity and 91.6% specificity.[111] Polymerase chain reaction (PCR) test was rapidly developed for

diagnosis of the virus.[112] RT-PCR is the most reliable method for COVID-19 diagnosis. A previously published study on the accuracy of RT-PCR results has indicated that specimens from bronchoalveolar lavage fluid are positive in 93 and 72% of cases, respectively.[113] It seems that pharyngeal swabs have lowest sensitivity and nasal swabs is more sensitive than pharyngeal swabs.[114] Besides, RT-PCR is the gold standard for sputum samples.[111] However, the exact sensitivity and specificity of RT-PCR tests for COVID-19 are not clear, but it is suggested that a positive test is highly suggestive of true infection, but a negative test does not rule out the disease.[114]

In the first 5 days of SARS-CoV infection, throat swabs, nasopharyngeal aspirates, and sputum samples are the most useful clinical specimens, but later viral RNA could be found more readily in stool specimens.[115] Respiratory tissue samples are used to identify patients with MERS-CoV.[116] RT-PCR is also a suitable test for detection of MERS-Cov.[117]

Therapeutic Strategies For

There is still no effective treatment for MERS-CoV or any other coronavirus infections. Supportive care strategies and preservation of renal, hepatic, and neurological function may help clinicians to deal with the infection.[118] To avoid the innate immune responses, SARS-CoV and MERS-CoV use several strategies. Therefore, suppression of IL-1 family members and IL-6 can be considered as a therapeutic strategy in many inflammatory diseases, including viral infections. Besides, IL-37 and IL-38 are able to suppress IL-1β and are considered as potential therapeutic cytokines.[63] Remdesivir was first developed for the treatment of Ebola virus infection. A recent published study has reported that a combination of remdisevir and IFN-β has an antiviral function in MERS-CoV patients.[119]

Due to high level of IL-6, blockade of interleukin-6 signaling may become a new method for the treatment of severe patients. Tocilizumab is a blocker of IL-6R which blocks IL-6 signal transduction pathway.[50] In addition, blocking of IL-6 in severe cases of COVID-19 infections results in increasing the absolute lymphocyte blood count and cytotxic functions of NK cells.[78,120] Due to the elevated expression of IL-1β in most COVID-19 patients with severe symptoms, inhibition of IL-1 has been suggested as a therapeutic strategy.[121] Also, leukotriene B4 that activates neutrophils is able to kill human coronavirus, respiratory syncytial virus, and influenza B virus. Besides, adaptor-associated protein kinase 1 inhibits SARS-CoV-2 viral infection through clathrin-mediated endocytosis. Endocytosis is a way that viruses enter human host cells.[122] Currently, remdisevir is suggested as an antiviral drug for SARS-CoV-2. Nevertheless, further clinical trials are needed to assess the efficacy and safety of remdesivir for SARS-CoV-2 pneumonia patients.[123] Antiinflammatory drugs including corticosteroids can reduce the effect of cytokine storms and lung damage. A short course of systemic corticosteroids can be tolerated among patients with SARS-CoV-2.[3] The use of glucorticosteroids has been considered as a therapeutic strategy, even so, the timing of administration and the dosage play key roles in the outcome in severe cases. Too early administration of glucocorticoids increases the viral load because it can inhibit the initiation of immune responses. It is suggested that glucocorticoids to be used in severe cases to prevent acute respiratory syndrome.[20] Vaccine seems to elicit both the humoral and cellular immune system. An ideal vaccine is characterized by safety and protection efficacy in large-scale clinical trials. To date, several vaccines have reached to the final stages. Currently, it is not possible to compare different vaccines.[124] Vaccines against SARS-CoV-2 can be divided into three main groups: RNA-, DNA-, and peptide-based vaccines.[125] The phase III clinical studies of mRNA vaccines show efficacy by 95%.[126]

Conclusions

In the past two decades, the world has faced with three coronavirus outbreaks which have caused global health consternation. The new coronavirus, SARS-CoV-2, has infected more people than SARS-Cov and MERS-CoV as it can easily be transmitted from person to person. Epidemiological studies indicate that majority of individuals are asymptomatic. Therefore, an effective and an efficient tool is required for rapid testing. Dysregulation of cytokine levels have been reported in infected patients and immune responses have a great impact on the determination of course of infection. However, cytokine levels differ in severely affected patients and those with moderate or mild symptoms. So, identification of cytokine level can help in recognition of patients with severe disease. Still, further studies are needed to assess the immune differences between different types of coronavirus infections and different patients. Besides, due to high mortality rate, it is crucial to develop antiviral therapeutics and vaccine. To achieve this goal, it is needed to understand the molecular mechanisms of the virus life cycle. A comparative study of SARS-CoV-2 outbreak with the previous coronavirus outbreaks may provide additional light into the most effective therapeutic strategies. As COVID-19 is a serious threat to public health, identifying biomarkers for disease is an urgent need. To identify biomarkers, it is necessary to recognize the involved cytokines and factors.

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Conflicts of interest

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