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Immune Profiling of SARS-CoV-2; What We Know and What We Don't Know

Mahshid Zohouri[1], Atri Ghods[1], Mina Roshan Zamir[1], Fazel Shokri[2], and Abbas Ghaderi[1]

[1] Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[2] Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Coronavirus disease 2019 (COVID-19), described as World War 3, is the current worldwide health challenge and nearly all countries have so far faced this disaster. There is still no cure because of the complicated pathogenesis, however, there are several studies on track investigating different aspects of the immune response to the virus. In this review, we will provide an overview of recent investigations that have analyzed immune cells in patients with COVID-19. We will then discuss the differences in immune profiles between healthy controls and various clinical presentations, including asymptomatic, mild, moderate, and severe cases.

Keywords: Adaptive immunity; Coronavirus disease 2019; Immunology; Innate immunity; Physiopathology; Severe acute respiratory syndrome coronavirus 2

INTRODUCTION

After severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) epidemics, which caused lethal coronavirus-related diseases in 2002 and 2012, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late December 2019. Genome sequencing from five patients in Wuhan, China, with an unusual presentation of acute respiratory distress syndrome (ARDS), revealed a new member of beta-coronaviruses.[1] Later, in early 2020, WHO designated the new coronavirus (nCoV) as SARS-CoV-2, and the coronavirus disease as coronavirus disease 2019 (COVID-19).[2]

In human coronaviruses, SARS-CoV is the most similar one to SARS-CoV-2 with 79.6% genome identity. Among the coronaviruses family, the most similar virus to SARS-CoV-2 is the bat coronavirus RaTG13, with 98% similarity.[3] SARS-CoV-2 also has a high amino acid similarity segment in the receptor binding domain (RBD) of the spike protein with pangolin strain GD410721.[4] Therefore, the bat was nominated as the natural reservoir, and the pangolin as an intermediate host.[5]

The virus causes a different spectrum of disease severity from asymptomatic to ARDS and multiple organ failure. What makes different individuals go towards asymptomatic, mild, moderate, severe, or critical diseases is still unknown; however, some parameters such as primary viral load, hosts' immune health status, types of human leukocyte antigen (HLA), and underlying diseases have been reported to play a crucial role in disease severity.[6-8]

After entering the cells, the virus replication machine starts working and contributes to several pathologies both by the direct action of the virus and also

Corresponding Author: Abbas Ghaderi, PhD;
Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, P.O.Box:71345-3119, Iran. Tel: (+98 713) 2334 589, Fax: (+98 713) 2304 952, E-mail: ghaderia@sums.ac.ir

inflammation caused by the immune defense mechanism. In fighting against the virus, the immune system defense works as a double-edged sword. What makes this system go towards eradicating the virus or causing a severe inflammatory response is still unknown. In this review after discussing the viral structure and pathogenesis, we will discuss different aspects of the immune system in patients with COVID-19 to understand the mechanisms underlying disease progression. Finally, by comparing the immune profile in patients based on disease severity, we will look into the parameters that may disrupt a balanced response.

Viral Structure

This enveloped, single-stranded, positive-sense RNA virus with a genome length of about 30kbps belongs to the coronaviridae family, orthocoronavirinae subfamily, and subgenus of sarbecovirus.[1,9] The terminal two-thirds of the viral genome consists of ORF1a/1b, which encodes for viral replicase complex. The remaining one-third of the genome consists of other ORFs encoding four main viral structural proteins:[10] nucleocapsid (N) protein that forms a helical ribonucleoprotein and is surrounded by a lipid envelope derived from the host cell; spike protein (S), a glycoprotein that helps virus' fusion and entry to host cells and is composed of S1 and S2 parts; membrane (M) protein for viral budding and release; and lastly envelope protein (E).[11]

Pathophysiology

The nose is probably the main route for viral entry to the human body, with subsequent aspiration and seeding to the lung.[12] However, SARS-Cov-2 has a broad organotropism as shown by autopsy findings (*i.e.*, kidney, heart, liver, and brain), but it preferentially infects the respiratory system.[13] Further, it could enter the blood and cause multi-organ damage.[14] The primary determinant for host tropism of viruses is spike protein-receptor interaction. To date, angiotensin-converting enzyme 2 (ACE2), CD147, transmembrane protease serine 2 (TMPRSS2), cathepsin B/L, and/or furin protease activity have been introduced as essential structures for SARS-CoV-2 entry.[15-17] Co-expression of ACE2 and TMPRSS2 has been shown on the cells of the respiratory tree, cornea, and gastrointestinal system (esophagus, ileum, colon, gallbladder, and common bile duct).[18] Recently, another viral receptor known as receptor tyrosine kinase AXL has been introduced, which binds to the N-terminal domain of the SARS-CoV-2 S protein.[19]

In the respiratory system, ACE2 is especially expressed by nasal epithelial cells, type II pneumocytes, endothelial cells, and macrophages.[18,20] After the attachment of the virus to the receptor via the RBD on the S1 protein, proteases in the recipients' cells, such as furin, cleave the spike on the S1/S2 multibasic compartment. Finally, the progenies are released after fusion with the plasma membrane and infect other cells.[10] RBD of the S protein in SARS-CoV-2 possesses a 10-fold higher affinity with ACE2 in comparison with SARS-CoV, leading to more potent cell entry.[21] Furthermore, several studies have reported that SARS-CoV-2 RBD has two different conformations, "lying down" and "standing up" states, with a bias towards the lying down state, which restricts the RBD's accessibility to immune cell factors such as neutralizing antibodies and leads to SARS-CoV-2 immune evasion.[22-24]

What exactly happens to the human body after viral entry and replication is still a crux. Based on the symptoms, patients with COVID-19 are classified into four categories: mild, moderate, severe, and critical.[25] One possible impact of viruses on the human body is cellular damage and apoptosis that happens directly by the virus or as a defensive mechanism by the immune system,[26,27] a process that may contribute to symptoms ranging from mild to severe. In the critical group, the immune system can contribute to the syndrome of acute respiratory distress by dysregulated cytokine release.[28] Moreover, one unique and fearsome symptom that has been reported in COVID-19 patients is vascular thrombosis, which has been reported as pulmonary thromboembolism,[29] coronary artery disease,[30] and even a cerebrovascular attack.[31] After viral entry, ACE2 expression on target cells decreased.[32, 33] Consequently, loss of balance between ACE2 and angiotensin II type 1 (AT1) receptors results in vasoconstriction, enhanced inflammation, and eventually thrombosis.[33] It has been hypothesized that endothelial dysfunction is a result of direct virus damage or crosstalk between the complement and the coagulation system. Terminal complement components (*e.g.* C5a and C5b-9) could cause endothelial damage by several mechanisms.[34] Pathologic studies suggest features of immune-mediated vasculitis with infiltration of monocytes and lymphocytes around blood vessels and also vascular wall thickening in these patients.[35] Altogether, the Virchow triad of endothelial damage, stasis, and a

hypercoagulable state all come together in patients with COVID-19, contributing to the development of thrombosis.

The Immune System

Innate Immunity

Innate immunity, as the leader of the immune system, could limit viral spread by causing an early response. On the other hand, it could cause tissue damage as a consequence of over-response by initiating cytokine release syndrome.[36,37] Macrophages (MQ) and dendritic cells (DC) are among the major contributors.

Patients with COVID-19 have a higher number of neutrophils,[38,39] a higher neutrophil to lymphocyte ratio (NLR),[38,40] a lower number and percentage of monocytes,[40,41] basophils, and eosinophils.[39,40]

Although the total number of natural killer (NK) and natural killer T (NKT) cells was lower in the peripheral blood of patients,[39,40] in an analysis of bronchoalveolar lavage fluid (BALF), NK cells were reported to be low at the early stages of the disease, while the proportion increased at later stages (Table 1).[38,42]

Table 1. Amount of different cell types in innate immunity of patients with COVID-19 compared to normal individuals.

Cell Type (Innate)	Amount Compared to Normal
Neutrophil	Increased
Neutrophil/Lymphocyte ratio	Increased
Monocytes	Decreased
Basophils	Decreased
Eosinophils	Decreased
$CD1c^+$ B cluster and plasmacytoid dendritic cells	Increased
NK cells	Decreased
NKT cells	Decreased
C5a, C5b-9	Increased

NK: natural killer; NKT: natural killer T

The analysis of PBMC showed that non-structural protein (NSP) 9 and NSP10 in the virus promote the production of IL-6 and IL-8 via targeting the NF-κB repressor.[33] In addition to non-structural proteins, the M protein, as a structural component, inhibits the production of interferon (IFN)-I and -III via disrupting the retinoic acid-inducible gene I (RIG-I)/melanoma differentiation-associated protein 5 (MDA-5) mitochondrial antiviral signaling proteins (MAVS) signaling pathway.[43] Besides, several other cyto/chemo -kines are also induced by the virus; high levels of chemokine ligand 2 (CCL2), 8, 3L1, C-X-C motif chemokine ligand 1(CXCL1), 2, 9, 10, and IL-33 are detected in BALF; and high titers of CXCL8, 9, 10, 16, 17, TNF superfamily member 10 (TNFSF10), tissue inhibitor of metalloproteinases 1 (TIMP1), monocyte chemoattractant protein-1 (MCP-1), C5, amphiregulin (AREG), neuregulin 1 (NRG1), IL-2R, IL-1β, 4, 5, 6, 8, 10, 17A, 18, tumour necrosis factor α (TNF-α), and interferon gamma (IFN-γ) are also detected in peripheral blood,[39,40,44-47]. This pro-inflammatory milieu enhances the migration of additional innate cells, decreases the cytolytic functions of NK and $CD8^+$ T cells, and triggers the activation of auto-reactive T and B cells in autoimmune susceptible individuals in an antigen-independent manner.[39,48] Interferon signaling is the

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major defense mechanism of innate immunity against viruses, but data about IFNs in patients with COVID-19 are controversial. Although it has been shown that open reading frame 3 (ORF3), ORF6, and NSP1 as components of SARS-CoV-2, inhibit IFN signaling pathways, some studies reported activation of IFN signals in the blood of patients with COVID-19.[14,49,50] Another study revealed that this upregulation was not significant in either blood and BALF; however, interferon-stimulated genes (ISGs) were robustly elevated, and some of them were specific to nCoV.[38,51,52] Interestingly, it has been reported that ACE2 is an ISG in human airway epithelial cells, and IFN upregulates ACE2 expression and leads to higher infectivity of SARS-Cov-2.[53]

Moreover, *in vitro* analysis showed that increasing viral load induces secretion of IFN-I and III but at a low level.[54] It seems that patients with severe disease have a robust response of type 1 interferon in contrast with the suppressed response reported in early infection.[55] However, decreased secretion of IFN-I and III has been demonstrated in COVID-19 patients.[54] Since IFN-γ prevents SARS-CoV-2 replication in human intestinal epithelial cells, interventions by recombinant interferons may control the viral infection.[56] Additionally, NSP1 and OFR6 induce dysfunction of the signal transducer and activator of transcription 1 (STAT1), which causes the hyperactivation of STAT3. As the consequence, plasminogen activator inhibitor-1 (PAI-1) is upregulated, which further induces an environment that is both proinflammatory and prothrombotic (Figure 1).[49] Interestingly, highly neutralizing IgG auto-Abs against type I IFNs have been reported in 15% of critical COVID-19 patients. These observations may imply that low levels of IFNs in serum could be due to these autoantibodies.[53]

SARS-CoV modulates transforming growth factor beta (TGF-β) signaling through the binding of N protein to mothers against decapentaplegic homolog 3 (Smad3) and elevating Smad3/p300 transcription levels, which leads to lung fibrosis. Since the N protein of SARS-CoV and SARS-CoV-2 show 90% identity in amino acid sequence, it implies that a similar mechanism may be involved in SARS-CoV-2 infection triggering lung fibrosis.[57]

Special monocytes in the peripheral blood of patients have been detected with markers and cytokine profiles of both M1 and M2. They express $CD11b^+$, $CD14^+$, $CD16^+$, $CD68^+$, $CD80^+$, $CD163^+$, $CD206^+$ and secret TNF-α, IL-6 and IL-10 (Figure 2).[32] The patients have larger monocytes in their blood and a higher percentage of $CD14^+CD16^+$ inflammatory monocytes.[32,41] The $CD169^+$ macrophages in the spleen and lymph nodes could be infected by SARS-CoV-2. These cells produce a large amount of IL-6 and highly express Fas, therefore causing lymphoid tissue damage by promoting necrosis and lymphocyte apoptosis (Figure 1).[58] NK cells express and transcript significantly more natural killer group 2A (NKG2A), lymphocyte-activation gene 3 (LAG3), programmed cell death protein 1 (PD1), and T cell immunoglobulin and mucin domain 3 (TIM3) as markers of exhaustion (Figure 2).[20,59] These cells are also less activated; patients have lower percentages of $IL\text{-}2^+$, $IFN\text{-}\gamma^+$, $TNF\text{-}\alpha^+$, and $CD107a^+$ NK cells, and NK cells have lower percentages of perforin and granzyme A and lower intensity of granzyme B.[20] Congruently, CD56bright NK cells, which produce IFN-γ and TNF-α, are depleted in the PBMC of COVID-19 patients.[59]

It is worth mentioning that the pathogenesis driven by neutrophils is not only exerted by tissue infiltration but also is enhanced by neutrophil extracellular trap (NETosis) contributing to organ failure and critical condition of COVID-19 severity.[60] Moreover, the proportion of $CD1c^+$ B cluster and plasmacytoid dendritic cells are increased in the blood.[14]

Complement System

The complement system is the key mediator in innate immunity. High serum levels of C5a and C5b-9, as the membrane attack complex (MAC), have been reported in patients with severe COVID-19 (Table 1). The level of C5b-9 was also associated with the severity of the disease.[61, 62] Furthermore, lung biopsy showed diffuse complement activation with C3 deposition. Tissue immunohistochemistry (IHC) staining also demonstrated strong staining for mannan-binding lectin (MBL), MBL-associated serine protease-2 (MASP-2), and C5b-9.[61] Moreover, strong tubular C5b-9 deposition had been revealed in the autoptic kidney of the patients.[34] It seems that the N protein of SARS-CoV-2 triggers the MASP-2-dependent complement pathway.[63] Alongside this, a trial of recombinant anti-C5a antibody administration in patients with severe and critical ARDS was associated with improvement of both clinical and paraclinical parameters at least in some patients.[61]

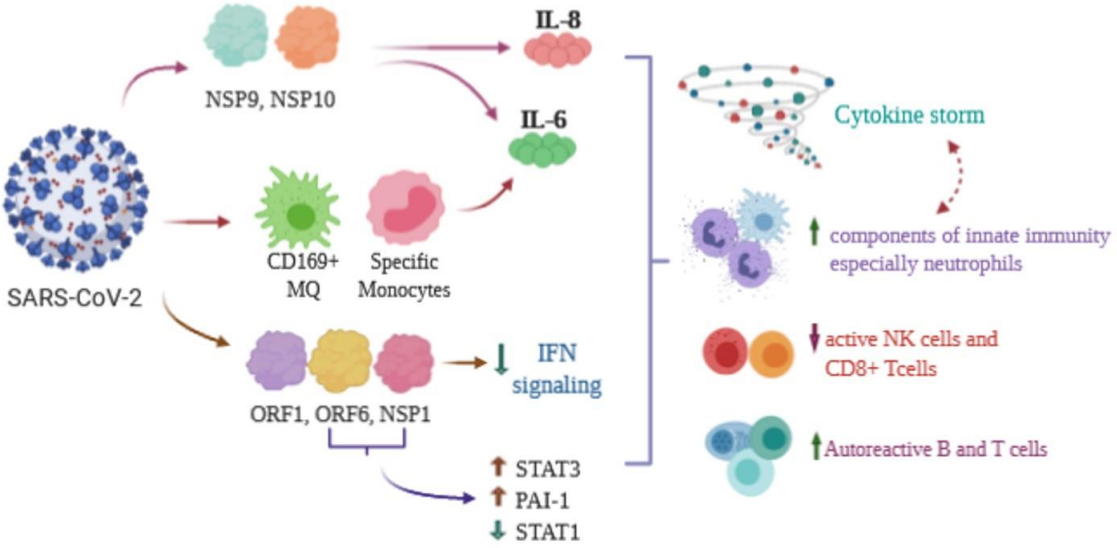

Figure 1. NSP9 and NSP10 by induction of IL-8 and IL-6, specific MQs and monocytes in the patients infected with SARS-CoV-2 via increasing production of IL-6 cause a proinflammatory milieu. Besides, ORF1, ORF6, and NSP1 decrease IFN signaling, which decreases the primary response and further causes a robust activation of innate immune cells. ORF6 and NSP1 also decrease SATAT1 signaling with compensatory activation of STAT3 and therefore cause upregulation of PAI-1. All these results in dysregulated activation of immune cells, secretion of cytokines, decrease in the activity of NK cells and $CD8^+$ T cells, and finally activate autoreactive lymphocytes in genetically susceptible individuals.
NSP: non-structural protein; IL: interleukin; MQ: macrophages; ORF: open reading frame; IFN: interferon; STAT: signal transducer and activator of transcription; PAI-1: plasminogen activator inhibitor-1; NK: natural killer.

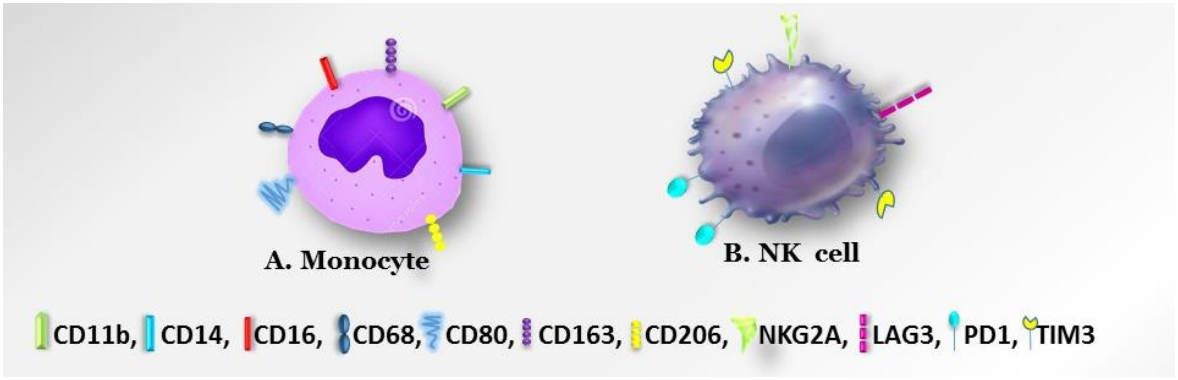

Figure 2. Special monocytes in the peripheral blood of patients expressing markers of both M1 (CD80) and M2 (CD163, CD206). Increased expression of immune regulatory molecules on NK cells which indicates their exhaustion due to an inflammatory environment.
NK: natural killer; NKG2A: natural killer cell protein group 2-A; LAG3: lymphocyte-activation gene 3; PD1: programmed cell death protein 1; TIM3; T cell immunoglobulin and mucin domain-containing protein 3

Adaptive Immunity

As a consequence of effective innate immunity, adaptive immune responses are activated to eradicate the virus. Patients with COVID-19, regardless of disease severity, have lymphopenia which remains persistent in severe groups.[41,64,65] Accordingly, splenic white pulp atrophy and declined generation of lymphoid follicles have been observed in COVID-19 patient autopsies.[35,66]

This low lymphocyte count might be the result of the direct cytotoxic effect of the virus, leukocyte adhesion, and extravasation as a consequence of endothelial dysfunction, recruitment to tissues such as the lung, or apoptosis by activation of the P53 signaling pathway.[44,65,67]

B Cell

Although humoral immunity may not be considered as the main immune response against viral infections, it is complementary and essential.[68] On the other hand, sub-neutralizing antibodies could help virus entrance via enhancing its uptake by Fc receptors in a process called antibody-dependent enhancement (ADE).[69] Although this phenomenon was not shown to increase viral replication, shedding, and spread, it is suggested that ADE can lead to inflammation and tissue injury due to the activation of Toll-like receptors (TLRs) and the production of pro-inflammatory cytokines.[70] In line with these data, some studies have shown higher antibody levels in patients with severe COVID-19 diseases.[71-73]

The total number of B cells has been reported to be decreased in a study in patients with COVID-19,[39] although immunoglobulins (IgM, IgG, and IgA) were within the normal range (Table 2).[40] The frequency of naïve B cells has been reported to be similar to healthy controls, while plasmablasts and T-bet$^+$ B cells were significantly increased, and memory B cells decreased in the peripheral blood. Although CXCR5 expression is reduced on B cells, Ki67 expression is increased on all subsets of B cells, but PD1 is expressed at higher levels only on plasmablasts (Figure 3).[47]

Antibody-secreting cells appear in the blood of patients with COVID-19 on day 8 and before the resolution of symptoms.[74] IgM level is increased gradually in the first week of symptoms' onset, peaked in the second week and then decreased to the background level. IgM switches to IgG at week two (especially IgG1 and IgG3) and maintains a high level even after 48 days.[3,75,76] IgA antibodies are also reported in serum samples of patients with COVID-19.[72,77,78] They can be detected 3-6 days after symptoms onset and have an increasing trend until day 14.[77] In addition to blood, IgA is also produced in lung mucosa as a secretory molecule (sIgA).[79] Besides, the RBD-specific antibody reaches a detectable level after 8 days.[75] Altogether, IgA, IgG, and IgM are detectable after about 2- 3 weeks in the sera of nearly all patients with a slight decrease in IgM level after week three.[80]

To investigate the effective immunity of the humoral system, rhesus monkeys were re-challenged with SARS-CoV-2 in the early recovery phase; no viral dissemination or any clinical manifestation of the disease was observed. Moreover, an undetectable level of viral RNA in most tissues of autopsied animals was reported. Besides, enhanced levels of antibody, cellular immunity, and lower monocytes percentage in re-challenged animals as compared to the primary infection were reported.[81-83]

Several studies are now on track to evaluate the effectiveness of convalescent plasma therapy on patients with severe COVID-19. They reported that plasma infusion before day 14 of the starting symptoms, could improve patients' clinical symptoms as well as oxyhemoglobin saturation within 3 days. Moreover, an increase in lymphocyte count, resolution of lung lesions, and viral load were also observed in most of the infused patients.[84] Alongside these advantages, convalescent plasma therapy may lead to ADE and its inflammatory consequences due to the presence of non-neutralizing polyclonal antibodies in the infused plasma. Therefore, the administration of the specific neutralizing monoclonal antibodies might be a more effective strategy.[85]

Recent research studies have revealed that mRNA vaccines are capable of inducing a potent and persistent germinal center response, leading to the recruitment of cross-reactive memory B cells, as well as the activation of new clones that target specific epitopes within the SARS-CoV-2 spike protein. As a result, these vaccines elicit a strong and durable antibody response with high affinity, which is protective against the virus.

Data from SARS-CoV showed a detectable level of antibody for about 3 years, however, IgG levels in patients with SARS-CoV-2 significantly decreased only after 2-3 months.[86,87] Similarly, a rapid decrease in anti-SARS-CoV-2 antibody titers has been indicated in asymptomatic and mild patients, implying that these persons may have low protection levels against reinfection with SARS-CoV-2.[87,88] Rapid generation of

durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins, as critical components of long-lasting immunity following viral clearance, has been recently demonstrated in convalescent patients.[89]

Although the N and S proteins of the virus are both immunogenic, the B cell epitopes localized within the RBD subunit are the main targets for virus-neutralizing antibodies and vaccine design.[72,90,91]. In contrast to the antibody reaction to N protein, which was mostly directed against linear epitopes as shown by reactivity to synthetic peptides and decreased native protein, the antibody response to RBD appears to be mainly through the identification of disulfide-bond dependent conformational epitopes.[92]

T Cell

Although innate and humoral immunity limit viral infections, cell-mediated immunity is crucial for eradicating pathogens and making immunological memory. After activation via antigen-major histocompatibility complex (MHC) complex, $CD4^+$ T cells spur other arms of the immune system into action, and $CD8^+$ T cells destroy infected cells directly. In addition, the innate and adaptive arms activate Th1 cells against nCoV by secreting high amounts of cytokines such as IFN-γ, interferon gamma-induced protein 10 (IP10), MCP1, and IL-1β. Besides, in contrast to SARS-CoV, Th2 cells are also activated in these patients and secrete many cytokines such as IL-4 and IL-10.[64]

Patients with COVID-19 have a lower total number of T cells,[20,39,40] $CD4^+$,[39-41,65] $CD8^+$,[39,47] $CD8^+$ regulatory T cells,[40] and higher $CD4^+$/ $CD8^+$ T cell ratio,[39,47] in peripheral blood and also a higher proportion of T cells in BALF (Table 2).[42] Therefore, the reduction in the total number of T cells in the periphery can be at least partially attributed to their recruitment to the lung, which in turn causes an increased number of T cells in that area.[78]

Among helper T cells, the number of $CCR6^+$ Th17 and percentages of Th1 cells increased while the percentages of Th2 and Th17 cells is reported to be decreased in the peripheral blood of these patients (Table 2).[20,46,65] The frequencies of Treg and T follicular helper (Tfh) cells were within a normal range, however, another study showed central Tfh cells to be increased.[46, 47] In a recent study, Tfh cells and germinal centers were significantly lower in the draining lymph nodes in deceased patients as compared to convalescent patients.[93] As such, it can be speculated that a defect in the germinal center formation may lead to impaired antibody class-switching from IgM to IgG in deceased patients. The source of SARS-CoV-2 RBD and N antigens-specific IgG in these patients may be originated from short-lived extrafollicular plasmablasts producing IgG during primary B cell response with no extensive affinity maturation. Indeed, such weakly reactive IgG and IgM antibodies may be responsible for ADE, which has already been reported in SARS-CoV2 and other coronaviruses as well as human immunodeficiency virus (HIV) and Dengue virus infections.[94-96]

Despite a lower number, T cells have activated status as evidenced by a higher proportion of $HLA\text{-}DR^+$ and $CD38^+$ T cells with higher expression of CD69, CD38, and CD44.[41,65] Moreover, the frequency of $CD4^+$ T cells expressing Ki67, CD38, and HLA-DR is increased while $CD4^+$ IFN-γ producing cells are decreased. [39, 41, 47] Besides, $CD4^+$ T cells have a higher expression of OX40, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and lower expression of CXCR5 (Figure 3).[41,46,47] These mixed phenotype T cells may be representative of activated cells which gradually go towards exhaustion.[78] Otherwise, because SARS-CoV-2 infects DCs, these dysfunctional DCs may contribute to the exhausted phenotype.[28]

$CD8^+$ T cells have a higher frequency and proportion of $Tim3^+PD\text{-}1^+$, $CD39^+$, $Ki67^+$, $CD38^+$, $HLA\text{-}DR^+$ and $HLA\text{-}DR^+CD38^+$ subsets, and higher expression of NKG2A.[20,41,46,47] Additionally, a lower percentage of $CD107a^+$, $IFN\text{-}\gamma^+$, and $IL\text{-}2^+$ subset of $CD8^+$ T cells with a lower concentration of granzyme B has been reported in different studies.[20,39,41] Controversial results have also been reported showing $CD8^+$ T cells with a high concentration of cytotoxic granules such as perforin and granulysin, which could at least partly account for tissue injury in COVID-19.[65] Polyfunctional $CD8^+$ T cells as an important fighter against viruses are also reduced in these patients.[39]

Specific $CD4^+$ and $CD8^+$ T cells against M, S, and ORF proteins have been identified in more than 70% of patients with SARS-CoV-2.[97,98] S protein-specific T cells and to a lesser extent, T cells specific for NSP3, NSP4, ORF3a, and ORF8 have been detected in COVID-19 patients.[97,99] However, these T cells have also been detected in unexposed individuals, though S-reactive $CD4^+$ T cells which target N-terminal sequences or RBD that are almost exclusively specific for COVID-19.[97,98] In addition, S-specific $CD4^+$ T cells response is correlated with higher IgG and IgA titer which further

underlines the role of $CD4^+$ T cells as the coordinator of the immune response against the virus.[97]

Studies that analyzed the SARS-CoV-2-specific immune profiles are limited. A recent study revealed that $CD4^+$ T cells that are specific for SARS-CoV-2 are mostly IFN-γ producing Th1, inducible T-cell co-stimulator $(ICOS)^+$ circulating T follicular helper (cTfh), and T central memory (Tcm) with high expression of CD127. Although it was revealed that CXCR5 is expressed at low levels on T cells from the peripheral blood of patients, this marker is highly expressed on SARS-CoV-2-specific $CD4^+$ T cells. Interestingly, SARS-CoV-2-specific $CD8^+CD27^+CD28^+$ Temra cells which are long-lived cytotoxic cells persist in patients recovered from the mild disease.[100]

Memory T cells are the main body's defense against viral pathogen re-exposure. It has been shown that in the convalescence phase, SARS-CoV-2 specific T cells with memory phenotype are increased even in the absence of detectable circulating antibodies.[101] The frequency of $CD4^+$ T cells with the phenotype of central memory T cells is increased in COVID-19 patients especially the mild groups, whereas naïve T cells are increased in severe patients and central transitional memory T cells as well as naïve $CD4^+$ cells are decreased in mild groups.[39,46,47] The proportion of transitional memory $CD8^+$ T cells is low, while the effector memory subset of $CD8^+$ T cells is high in severe individuals.[46] It may take several years of investigation to reveal sufficient data on the durability of memory T cells in SARS-CoV-2 patients. However, studies on SARS-CoV have shown that memory T cells could be identified even six years post-exposure.[102] A recent study has demonstrated that cellular immunity can persist for at least six months after infection with SARS-CoV-2, even in patients with mild disease.[103]

Table 2. Amount of different cell types in adaptive immunity of patients with COVID-19 compared to normal individuals

Cell Type (Adaptive)	Amount Compared to Normal
Lymphocyte	Decreased
B cell	Decreased
Plasmablast	Increased
IgM, IgG, IgA	NL
T cell	Decreased
$CD4^+$	Decreased
$CD8^+$	Decreased
$CD4^+/CD8^+$	Increased
$CD8^+$ Regulatory T cell	Decreased
Th1, Th1Th17	Increased
Th2, Th17	Deacreased

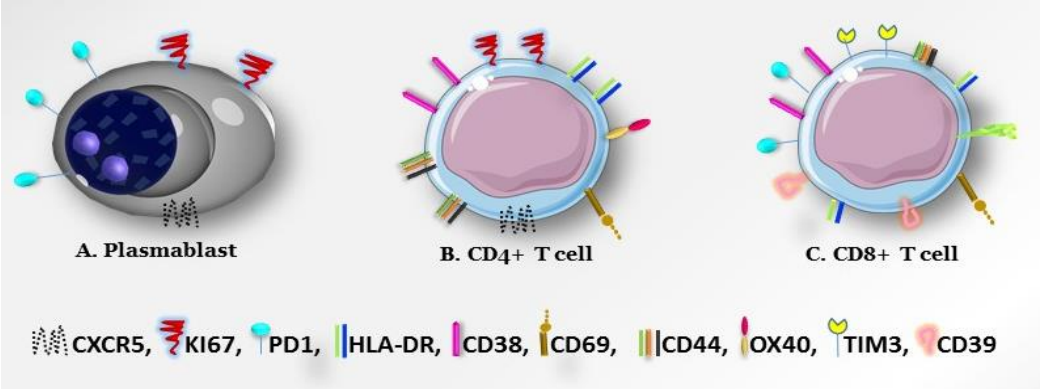

Figure 3. A. Plasmablasts with the expression of PD1 as an exhaustion marker and decreased expression of CXCR5 as a T follicular helper marker are increased in COVID patients. B, C. Mixed phenotype of both activated and exhausted cells on $CD4^+$ and $CD8^+$ T cells from PB of patients.
CXCR5: C-X-C chemokine receptor type 5; PD1: programmed cell death protein 1; HLA-DR: human leukocyte antigen – DR isotype; TIM3: T cell immunoglobulin and mucin domain-containing protein 3

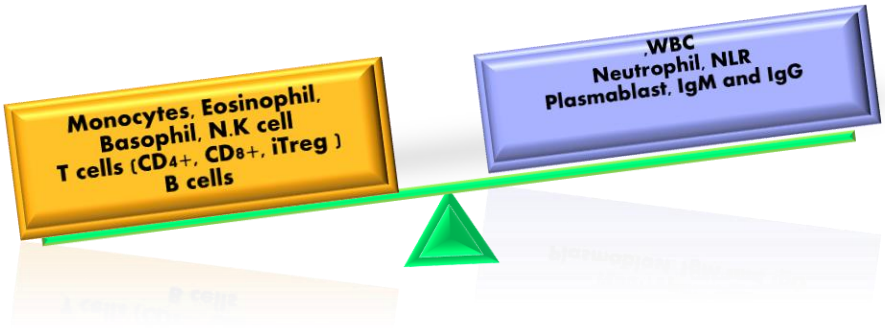

Figure 4. The difference in components of the immune profile while a patient transits from mild disease with SARS-CoV2 to a more severe type. WBC, neutrophils, NLR, plasmablast, IgM, and IgG increased, while monocyte, eosinophil, basophil, NK cell, CD4+ and CD8+ T cells, iTregs, and B cells decreased.
NLR: neutrophil to lymphocyte ratio; iTreg: induced Treg; NK: natural killer; WBC: white blood cell

Immune Profiling of Patients Based on Disease Severity

What makes the immune response against the virus cause severe or mild symptoms in different subjects is still not fully understood. Analyzing the immune profile in patients with a different spectrum of disease might help to delineate the underlying mechanisms.

Cell Count

Patients with severe disease compared with milder cases have higher leukocytes count,[40] particularly neutrophils,[20,40] and a lower percentage of monocytes, although with a higher percentage of inflammatory types,[40, 41] lower percentage of eosinophils,[40] basophils,[40] and lower number of NK cells.[20,39,40,46] They also have differences in the profile of adaptive immunity; including a lower lymphocyte count,[20,40] a higher neutrophil-to-lymphocyte ratio,[40]and a lower number of T and B cells,[20,40] $CD4^+$ T cells,[39,46] and also lower $CD8^+$ T cells (Figure 4).[20,41,46] However, one study has shown that while $CD4^+$ and $CD8^+$ T cells are decreased in terms of absolute count, their function is normal.[40] In contrast to severe types, patients with mild disease have a larger population of total T and $CD8^+$ T cells with antigen-specific expanded clones.[14,42] Profiling of immune cells in BALF has revealed more MQs and a lower proportion of NK cells and T cells in patients with severe disease.[42]

The total number of T helper cells is underrepresented in severe groups with a lower ratio of memory helper T cells/ naïve helper T cells. Besides, they have a lower percentage of CD28 positive cytotoxic

suppressor T cells and iTregs.[40] Nevertheless, the percentages of Th1, Th2, Th17, Tfh, and Treg cells were similarly represented between mild and severe groups.[46] In contrast to this study, some studies have recently reported a significantly decreased percentage of Tregs ($Foxp3^+$, $CD3^+$, $CD4^+$, $CD25^{high}$, $CD127^{low}$) in severe SARS-CoV-2 patients.[104,105]

Patients with ARDS have a higher proportion of plasmablasts.[59] Serum levels of virus-specific antibodies are increased in different groups of patients;[76] however, within two weeks post-onset of symptoms, patients with severe disease display higher levels of IgG and/or IgM antibodies.[72,80] Moreover, in cases who died of COVID-19, N and RBD-specific IgM was higher compared to recovered cases, while the level of N and RBD-specific IgG was not significantly different between these two groups.[106] When compared to asymptomatic patients, specific IgG for the virus is higher in symptomatic subjects. The reduction in the virus-specific IgG level happened in the early convalescent phase of both symptomatic and asymptomatic groups and this decrement is more prominent in the latter.[87] Moreover, germinal centers were lost in the lymph node and spleen of patients with severe disease as shown by autopsy findings. Reduced numbers of Bcl-6^+ germinal center B cells and Tfh cells that contribute to affinity maturation were also reported.[96]

Moreover, there are also some differences between critical and severe groups. While patients with severe disease have some features of autoimmunity, critical cases seem to be immunodeficient. In critical cases, some subtypes of PBMCs are decreased or even disappeared, including some clusters of $CD8^+$ and $CD4^+$ T cells as well as plasmacytoid dendritic cells. In contrast, in severe cases, some subsets of immune cells such as naïve $CD8^+$ T cells, $CD8^+$ cytotoxic T cells, $CD4^+$ cytotoxic T cells, and plasmacytoid dendritic cells are increased.[14] Both severe and critical patients displayed a higher IgM level, while IgG titer was not elevated in the critical patients.[76]

Immunophenotyping and Functional Analysis

Highly inflammatory monocyte-derived ficolin-1 $(FCN1)^+$ MQs predominate the lung of patients with severe disease and replace the fatty acid-binding protein 4 $(FABP4)^+$ alveolar MQs.[42] Additionally, monocytes that are highly capable of secreting GM-CSF and IL-6 are high in severe cases.[41] CD56dim and granzyme A expressing NK cells as an important part of antiviral host defense are depleted in severe groups and CD56bright NK cells as an important producer of IFN-γ and TNF-α are depleted in both mild and severe cases.[39,59] Finally, the lower cytotoxic potential and also TNF-α production by NK cells are correlated to the higher serum level of IL-6.[39] Expression of all genes related to HLA class II has also been reported to be down-regulated, especially in severe cases, hence, MHC-antigen interaction as part of the activation of adaptive immunity is more disrupted in severe groups.[59]

IFN production is deranged in patients with COVID-19. In severe cases, several signaling pathways related to IFNs such as interferon α, β, γ pathways have been reported to be activated, however, in cured cases, interferon-related signaling is just mildly elevated and the mitogen-activated protein kinase (MAPK) pathway is the most enriched one.[14] One study showed that the elevation of ISGs in $CD14^+$ monocytes was correlated with age and time from fever onset, but not ARDS and ventilation support.[59] On the other hand, some studies revealed that low interferon and ISG levels concurrent with persistent blood virus load are characteristics of disease severity. At the same time, these patients have an exacerbated inflammatory response that is at least partially caused by nuclear factor kappa B (NF-κB) signaling.[107] Moreover, in a study on BALF, both types I and III IFNs as well as other inflammatory cytokines were more abundant in patients with severe disease.[52]

In the early stages of infection in patients with mild disease, CCL5 is elevated, while early production of IL-10, IL-1RA, and later elevation of IL-6 and IFN-γ were reported in the severe groups. No difference was reported between mild and severe cases for TNF-α and GM-CSF production,[108] though contradicting results concerning elevated serum levels of TNF-α have also been reported in severe cases.[107]

Overall, the severe group has the cardinal manifestation of secondary heamophagocytic lymphohistiocytosis with cytokine profile characterized by increased IL-2, IL-2R, IL-6, IL-7, IL-8, L-10, granulocyte-colony stimulating factor (GCSF), IFN-γ, TNF-α, monocyte chemo-attractant protein 1 (MCP-1), inducible protein 10 (IP-10), and macrophage inflammatory protein 1-α (MIP-1). Therefore, the increased level of these cytokines is associated with disease severity.[40,64,109]

In patients with severe disease, $CD4^+$ T cells have higher expression of CD38, CD44, CD69, OX40, PD-1, TIM3, and also they have more percentages of IL-6^+ and

GM-CSF$^+$ CD4$^+$ T cells.[42] In addition, IFN-γ$^+$GM-CSF$^+$ Th1 cells are increased in severe cases.[41] Moreover, CD8$^+$ T cells with expression of CD38, HLA-DR, TIM3, and PD1 are higher in these patients and they also have more intensity of CD69, CD38, CD44, and GM-CSF.[41,46] In these CD8$^+$ T cells, metabolites and energy generation are upregulated, while T cell migration and calcium ion signaling are increased in cytotoxic T cells from mild cases.[42] Contradicting results have also been reported, showing no functional differences between T cells in terms of disease severity.[40] Pathological studies in the autopsied lung of severe patients revealed massive infiltration of the lung interstitium by CD4$^+$ and CD8$^+$ T cells, MQs, and granzyme B (GZMB)$^+$ cells.[46] As a result, most of the lung damage in these patients seems to be associated with inflammatory cells rather than the virus (Table 3).[110]

Table 3. Immune profiling of patients based on disease severity

	Increase	Decrease
Severe Cases	Leukocytes count (Neutrophils)	Percentage of monocytes, eosinophils, basophils
	Neutrophil to lymphocyte ratio (NLR)	Number of NK cells
	MQs (in BALF)	Lymphocyte count
	Plasmablasts	Number of T and B cells
	IgG and/or IgM antibodies (within two weeks post-onset of symptoms)	Number of CD4$^+$and CD8$^+$ T cells
	Highly inflammatory monocyte-derived FCN1$^+$ MQs (in the lung)	NK cells and T cells (in BALF)
	GM-CSF and IL-6 secreting monocytes	Total number of Th cells
	Types I and III IFNs (in BALF)	Ratio of memory helper T cells/ naïve helper T cells
	Early production of IL-10, IL-1RA, and later elevation of IL-6 and IFN-γ	Percentage of CD28$^+$ cytotoxic suppressor T cells and iTregs
	Serum levels of TNF-α	Percentage of Tregs (Foxp3$^+$, CD3$^+$, CD4$^+$, CD25^{high}, CD127^{low})
	IL-2, IL-2R, IL-6, IL-7, IL-8, L-10, GCSF, IFN-γ, TNF-α, MCP-1, IP-10, MIP-1	Number of Bcl-6$^+$ germinal center B cells and Tfh cells
	CD4$^+$ T cells with a higher expression of CD38, CD44, CD69, OX40, PD-1, TIM3	CD56^{dim} and granzyme A expressing NK cells
	Percentages of IL-6$^+$ and GM-CSF$^+$ CD4$^+$ T cells	CD56^{bright} NK cells
	IFN-γ$^+$GM-CSF$^+$ Th1 cells	Expression of HLA class II-related genes
	CD8$^+$ T cells with expression of CD38, HLA-DR, TIM3, and PD1	
	Massive infiltration of the lung interstitium by CD4$^+$ and CD8$^+$ T cells, MQs, and GZMB$^+$ cells	
Mild Cases	Total T and CD8$^+$ T cells	CD56^{bright} NK cells
	T cell migration and calcium ion signaling in cytotoxic T cells	
	CCL5 (in the early stages of infection)	

BALF: bronchoalveolar lavage fluid; NK: natural killer; FCN1: ficolin-1$^+$; MQs: macrophages; IFN-γ: interferon gamma; GM-CSF: granulocyte-macrophage colony-stimulating factor; TNF-α: tumour necrosis factor α; GCSF: granulocyte-colony stimulating factor; Tfh: T follicular helper; TIM3: T cell immunoglobulin and mucin domain 3; CCL5: chemokine ligand 5; PD: 1programmed cell death protein 1; MCP-1: monocyte chemo-attractant protein 1; IP-10: inducible protein 10; MIP-1: macrophage inflammatory protein 1-α

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CONCLUSION

Upon detection, the human body starts fighting against the virus. On the other hand, the virus deviates the immune system from its programmed route through different unknown mechanisms and in several pathways, causing a high neutrophil-to-lymphocyte ratio, low number of NK cells, and also B and T lymphocytes. Therefore, cross-talk between innate and adaptive immunity does not take place properly. As a consequence, it becomes uncertain whether an adequate number of memory T cells is produced, which is an important factor that affects vaccine development strategies. What makes the immune system eradicate the virus and cause mild disease or lose the competition and results in ARDS is a hot topic in current investigations. In-depth analysis of the immune profile in patients with a spectrum of disease severity would provide clues. Furthermore, answering such questions would help in designing more effective medications to guide the immune system toward the eradication of the virus and also in making new vaccines that activate individuals' immunity more efficiently.

STATEMENT OF ETHICS

This study is a narrative review and does not need the statement of ethics.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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