



## Behaviors of Human T cells in SARS-CoV-2 Infection: Lessons and Tips

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### ABSTRACT

Cell-mediated immunity (CMI) is crucial in controlling the highly aggressive and progressive SARS-CoV-2 infection. Despite extensive researches on severe COVID-19 infection, the etiology and/or mechanisms of lymphopenia, decreased T cell-mediated responses in patients, cytokine release storms (CRS), and enhanced pro-inflammatory mediators are not fully understood. Several T cell subpopulations, including innate-like lymphocytes (ILLs) and conventional T cells, are involved in COVID-19 infection; however, their contribution to immunity and complications remains to be more elucidated. CD16<sup>+</sup> T cells are among the effective players in the development of T helper1 (Th1) responses in COVID-19 infection, while their robust cytolytic properties contribute to lung tissue damage. While CD56<sup>+</sup>CD16<sup>bright</sup> NK cells play a protective role, natural killer T (NKT) cells, mucosal-associated invariant T (MAIT) cells, and  $\gamma\delta$  T cells and their roles in COVID-19 require further investigation. The involvement of the other T cell subsets, such as Th17, along with neutrophils, adds to the complexity of the situation. In this review, we presented and discussed the findings of recent studies on T cell responses and the contribution of each type of immune cells to COVID-19.

**Keywords:** Cell-mediated Immunity, COVID-19,  $\gamma\delta$  T cells, Mucosal-Associated Invariant T cells, NKT cells, T helper cells

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Cite this article as:

Shams A, Khosravi S, Zareiye A,  
Lalehzari Y, Nematollahi R, Basti  
S. Behaviors of Human T cells in  
SARS-CoV-2 Infection: Lessons  
and Tips. *Iran J Immunol.* 2023;  
20(4):382-399,  
doi: 10.22034/iji.2023.98326.2567.

Received: 2023-03-12

Revised: 2023-06-28

Accepted: 2023-07-16

### INTRODUCTION

Cell-mediated immunity (CMI) involves multiple cells working together in a coordinated manner to induce optimal immune responses. Conventional T cells expressing  $\alpha\beta$  T cell receptor (TCR) and unconventional T cells or innate-like lymphocytes (ILLs) with  $\gamma\delta$  TCR are both involved in COVID-19 infection. Unconventional T cells are

typically found in epithelial environments such as the epidermis, gastrointestinal and genitourinary tracts. Their function mainly is to identify infectious pathogens and modulate inflammatory responses in these tissues. Mucosal-associated invariant T (MAIT) cells and invariant natural killer T (iNKT) cells are two well-known unconventional T cells that respectively recognize small-molecule derivatives of riboflavin presented on major

histocompatibility complex, class I-related (MR1) molecules and glycolipids in the context of CD1d (1, 2). Despite substantial research on conventional T cells and ILLs, the precise involvement of these cells in COVID-19 is unknown.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of the coronavirus family. SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) are more harmful to humans than other coronaviruses, according to extensive studies (1). To the best of our knowledge, the SARS-CoV-2 spike protein changed significantly in receptor binding sites, resulting in SARS-CoV-2 and the recent COVID-19 pandemic (3). SARS-CoV-2 pathogenicity has been linked to single-stranded RNA, nucleocapsid protein, envelope protein, membrane protein, and spike glycoprotein (S). Human angiotensin-converting enzyme 2 (hACE2) is identified as the virus's primary receptor via interaction with S-glycoprotein. Other receptors, however, recognize the virus, including lymphocyte function-associated antigen 1 (LFA-1) and transmembrane serine protease 2 (TMPRSS2), which play essential roles in the virus's rapid and fulminant progression (4).

A review of prior studies on SARS-CoV-1 and MERS shows that T cell-mediated immune responses play an indisputable role in the fate of infection. Despite the fact that COVID-19 has only been around for a short time, many studies have been conducted to determine the quality of the immune responses against the virus, but many questions remain unanswered, including the role of a well-coordinated T cell response, the rate of contribution of each immune cells, the characteristics of the primary early immune response, and the memory immune response to COVID-19.

#### *CD8<sup>+</sup> T Cells in COVID-19*

CD8<sup>+</sup> T cells or cytotoxic T cells (CTLs) are regarded as a distinct acquired immunity component in the eradication of

viral infections in a major histocompatibility complex (MHC-I)-dependent manner. For well-organized immune responses by the cells, CD4<sup>+</sup> T cells or helper T cells (Th) leadership is essential. According to many studies, close cooperation between CTLs and Th1 (a specialized Th subset) in controlling viral infection is indispensable (5). Researchers have found several antigenic proteins in the SARS-COV-2 virus that affect CTL activation by downregulating MHC-I. The specialized structures related to the virus including open reading frames (ORFs) such as ORF8, ORF3a, and ORF7a proteins, suppress MHC-I expression in antigen-presenting cells (APCs) (6, 7). The ORF3a, as a viroporin, suppresses innate immunological responses by a variety of methods, including antigen presentation inhibition, autophagy, and apoptosis induction. While ORF3a appears to downregulate MHC-I through wide protein interaction inhibition, ORF7 in the endoplasmic reticulum hinders the transport of peptide-MHC-I complexes (4). Many studies are being conducted to determine the role of SARS-COV-2 accessory proteins in viral pathogenicity. Recent findings showed mutations in MHC-I-related immunogenic proteins of SARS-COV-2 including S:R273S, nsp3:T1456I, endornase:P205L, N:P6T and M:L129R suppress antiviral functions of CTLs. Indeed SARS-CoV-2 specific CD8<sup>+</sup> T cells producing IFN- $\gamma$  in the patients hardly reach to 1% (8).

Primary adaptive immune responses to COVID-19, on the other hand, result in the generation of protective tissue-resident memory T cells (Trm) (9). The formation of CD8<sup>+</sup> Trm is known to inhibit the transmission of flu viruses (10). Large numbers of these Trm-like CD8<sup>+</sup> T cells were seen in the epithelium of lung airways during the SARS-COV-2 invasion (9). SARS-Cov-2 appears to restrict the T cell response by limiting antigen presentation, resulting in exhaustion of cytotoxic CD8<sup>+</sup> T cells, a reduction in memory CD4<sup>+</sup> T cells, and a limitation of regulatory T cells (Tregs) (11). Low Th cells

(less than 1500/ul) and decreased CD8<sup>+</sup> T cells are thought to be an independent risk factor for poor prognosis in ICU patients (12) (Table 1).

#### *CD4<sup>+</sup>T Cell Responses in COVID-19*

Following activation, CD4<sup>+</sup> T cells or T helpers, as the backbone of adaptive immunity, are differentiated into several subpopulations with specific transcription factors and cytokine signature. Th1, Th2, Th9, Th17 follicular helper T cells (Tfh) and Tregs are major subtypes of CD4<sup>+</sup> T cells (5).

#### *The Roles of Th1 and Th2 Cells in COVID-19*

Th1 and Th2 proportions in many pathologic situations determine the disease's fate. The Th1 cells augment inflammatory responses by producing mainly IL-1, IL-6, IL-12, IFN- $\gamma$ , and TNF- $\alpha$ , whereas Th2 compromise the responses by producing antagonist cytokines. Appropriate Th1 and Th2 cooperation seems critical in SARS-CoV-2 infection. However, their excessive production of cytokines as part of cytokine releases storm (CRS) often leads to pulmonary immunopathology and lung tissue damage. To our knowledge, the onset of SARS-CoV-2 invasion is an effective Th1 reaction exploiting macrophages and CTLs for eradicating the virus. However, Th2 responses, according to new references, lead to poor COVID-19

prognosis. Table 1 summarizes the data on the contribution of each T cells subsets in the COVID19 infection (5). Masahiro et al. explained the association between resistance to SARS-COV-2 in children and higher frequency of highly activated IFN- $\gamma$ -producing Th1 cells. Furthermore, in the blood of youngsters, naive and fresh T cells with a large diversity of TCRs are more common than in adults and the elderly. The aged, on the other hand, have greater memory and exhausted T lymphocytes due to decreased thymus synthesis (13, 14). The functional experiment revealed that IFN- $\gamma$  and TNF- $\alpha$  are anti-infectious, whereas IL-2 plays a dual role. Exhausted T cells not only emit the virus and allow it to propagate readily, but the infected cell itself works as a virus spreader. Macrophages release large levels of pro-inflammatory cytokines and neutrophil chemoattractant in the presence of low-level acquired immunity, resulting in tissue damage and ultimately loss of lung function. Some specialists believe that using Bacillus Calmette-Guérin (BCG) to boost Th1 responses may have protective effects on SARS-COV-2 (9). Th1 responses, on the other hand, must be generated in a safe environment, such as when the BNTb163b2 vaccination produces immune-mediated hepatitis with aberrations in CD8<sup>+</sup> T cell activity (15) (Table 1).

**Table 1. T helper cells and cytotoxic T cells involvement in COVID-19 infection**

| Reference                | Number and type of patients | Results   | Other important findings   |
|--------------------------|-----------------------------|---|--|
| Francisco, 2021 (5)      | 55 recovered                | Declined Th1 and Th17, highly activated Th2 cells   | Elevated IL-15 and exhausted Th cells  |
| Stine, 2021 (94)         | 203 recovered               | CD8 <sup>+</sup> /HLA-A2 <sup>+</sup> specific T cells development in patients                            | Spike protein induced CMI  |
| Rupp et al., 2022 (16)   | 20 severe                   | CD38 <sup>+</sup> Ki67 <sup>+</sup> CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells development in patients | CD38 <sup>+</sup> Ki67 <sup>+</sup> Th1 negatively correlated with plasma IL-6 |
| Wu et al., 2021 (17)     | 342 ICU patients            | Declined CTLs   | High mortality   |
| Xiaoqi et al., 2022 (19) | 64 hospitalized patients    | T CD4 <sup>+</sup> $\leq$ 200 cells/ $\mu$ L: poor prognosis  | Reverse correlation between IL-6 and T cell counts                             |
| Imeneo, 2022 (95)        | 420 severe                  | Decreased peripheral blood T lymphocyte and IFN- $\gamma$ production capacity                             | Associated with most severe cases  |

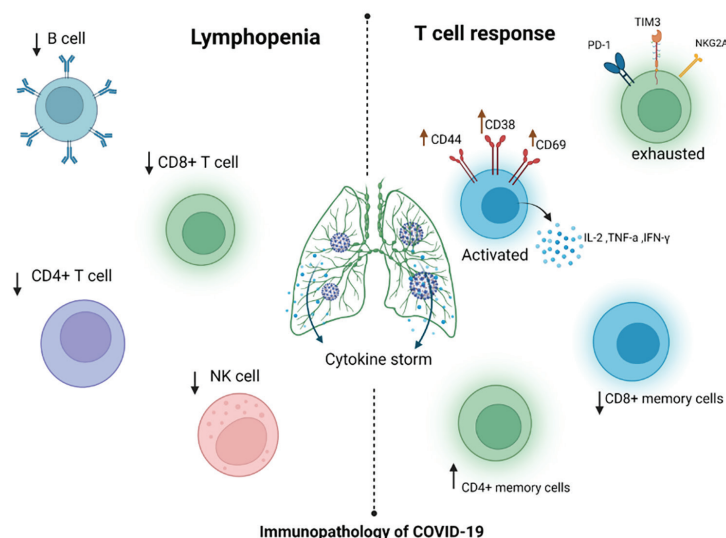
Th: T helper, CMI: Cell-mediated immunity, HLA: Human leukocyte antigen, CTL: Cytotoxic T cell, IFN- $\gamma$ : Interferon- $\gamma$

According to Rupp and Dongling reports, human Th cells express several receptors for SARS-CoV-2 that make them potential targets for the virus invasion, resulting in lymphopenia in many infected patients (16, 17). Some mechanisms are explained for T cells invaded by SARS-CoV-2 including ACE2R, TMPRSS2, lymphocyte LFA-1, and dipeptidyl peptidase (DP), which lead to lymphopenia in the patients. Accordingly, COVID-19 also has more trophies for Th2 cells because of increased expression of the DP receptor on the cell surface, resulting in a Th1/Th2 imbalance that may be connected to disease severity (5). In the infected individuals, the inhibitory receptor-programmed cell death protein 1 (PD-1) is overexpressed on CD4<sup>+</sup> and CD8<sup>+</sup> peripheral blood lymphocytes (18). Furthermore, in contrast to lymphopenia, a primarily neutrophil-related leukocytosis was seen in the COVID-19 infection, associated with a poor prognosis. As a result, in severe COVID-19 individuals, a portion of the lymphopenia is due to the suppressive effects of granulocytic myeloid-derived suppressor cells (G-MDSCs) (19). The IL-6 cytokine also plays a role in lymphopenia by pyroptosis of T cells in COVID-19 patients in addition to

its effects on impairing Th1 cell activity and induction of Th2 (19, 20). Another significant mechanism that leads to lymphopenia is CRS, caused mainly by macrophages infected with COVID-19 (21). M1 macrophages and epithelial cells via the toll-like receptor 3, 7 (TLR3/7), or retinoic acid inducible gene 1 protein (RIG1)/ melanoma differentiation-associated protein 5 (MDA5) and respiratory tissue's dendritic cells (DC) under Th1 cells commanding are supposed to be the main cells in the CRS phenomenon in the infection. The inflammasome and interferon regulatory factor (IRF) 3/ nuclear factor  $\kappa$  B (NF $\kappa$ B) signaling are induced by M1 macrophages leading to the production of pro-inflammatory mediators such as IFN- $\gamma$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IL-18 (20).

#### CD16<sup>+</sup> T Cells in COVID-19

CD16<sup>+</sup> T cells are identified a highly activated T cell population that may express  $\alpha\beta$  or  $\gamma\delta$  TCR. The cells generally are generated during inflammation progresses such as COVID-19 infection, providing a favorable environment for the growth of Th1 cells with high pathogenic and damaging potential. CD16<sup>+</sup> T cells are rich



**Fig. 1.** Various lymphocytes involve in immune responses against SARS-CoV-2 in lung. COVID-19 causes lymphopenia, lymphocyte activation and malfunction and increased pro-inflammatory cytokine production. Patients' CD4<sup>+</sup> and CD8<sup>+</sup> T cells display CD69, CD38, and CD44 and virus-specific T cells from severe cases have a central memory phenotype with high IFN- $\gamma$ , TNF- $\alpha$ , and IL-2 levels. Lymphocytes reveal an expressed phenotype with upregulated PD-1, TIM3, and NKG2A. COVID-19 is characterized by increased cytokine production, specifically IL-1, IL-6, and IL-10. PD-1: Programmed cell death ligand 1; TIM3: T cell immunoglobulin mucin-3; NKG2D: Natural killer group 2 member D

in cytolytic proteins (perforin, granzyme B (GrB)), lysosomal membrane glycoprotein1 (LAMP1), and syntaxin (STX11), and they are directed to inflamed lungs by CXCR3 and CCR6 chemokine receptors (22, 23) (Fig. 1). According to new studies, the elevation of activated CD16<sup>+</sup> T cells in severe COVID-19 leads to activation of the complement system causing epithelial and endothelial cell damage in the COVID-19 infection (9).

#### *Th17 Cells and COVID-19*

The immune system combats viral infections, like other intracellular infections, by polarizing T cell responses to Th1 and Th17 phenotypes, which promote inflammation. Th17 cells express retinoic acid-related orphan receptor  $\gamma$ t (ROR $\gamma$ t), and are induced by IL-23 and IL-6. Th17 cells perform antiviral activity by secreting pro-inflammatory cytokines such as IL-6, IL-17, and TNF- $\alpha$ . Several studies have found a link between disease pathogenesis and increased Th17 levels in COVID-19 patients. Previously, the same results were obtained with SARS-CoV-1 and MERS-COV (24). Despite the protective responses of Th1 in mobilizing various immune system components, Th17, by recruiting neutrophils to the lungs, caused tissue damage in COVID-19 patients (25). SARS-CoV-2 specific CCR6<sup>+</sup> Th17 cells found in patients with acute COVID-19 play an important role in COVID-19 pathogenesis by IL-22, IL-26, IL-21, and IL-17 (A to F). Furthermore, during COVID-19 infection, IL-17 attracts eosinophils to the lungs, causing inflammation and pulmonary eosinophilia (26). Despite Th1 induction of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  dependent inflammation, Th17 cells are specialized in chemotaxis and neutrophil behavior regulation (27-29). IL-17A contributes to acute respiratory disease syndrome by increasing lung tissue destruction as a result of altered neutrophil activity. In COVID-19 patients, potent Th17 is consistently equipped by matrix metalloproteinase and IL-22, causing tissue damage and devastating edema (28). As

a result, it was suggested that inhibiting cytokines such as IL-17 may reduce inflammation and improve the patient's outcome (24, 25, 27-32).

On the other hand, some studies have shown a decrease in blood Th17 count in COVID-19 patients (32-34). For the first time, the cooperation between Th17 and CD8<sup>+</sup> T cells in severe and critical COVID-19 patients was explained. As a result, CD8<sup>+</sup> T cells activate Th17 cells which lead to immune thrombosis via neutrophil activation and NETosis (27). Because both Th17 and neutrophils can cause immune-mediated injuries, targeting them both may be beneficial in COVID-19 patients. Thiamine deficiency has been shown to increase pro-inflammatory responses in both Th1 and Th17 cells (31). JAK2 inhibitors such as Fedratinib may suppress Th17 production and impede the cytokine storm in COVID-19 patients. Curcumin, a natural healing factor derived from turmeric, reduces Th17 responses and inflammation and may improve COVID-19 patients' outcomes (30).

#### *Th22 Cells and COVID-19*

Th22 is a novel subset of T cells that can be differentiated by IL-6, TNF- $\alpha$  and IL-21 as well as ROR $\gamma$ t as a transcription factor, however, T-bet and TGF- $\beta$  repress Th22 cells formation. Th22 cells have the ability to produce IL-22 and IL-13 and TNF- $\alpha$ . Since Th22 cells do not produce IFN- $\gamma$  or IL-17 they are different from Th1 and Th17. Although Th1 and Th17 can secrete IL-22, the primary producers of IL-22 are Th22 cells. Studies show that the cellular abundance of Th22 cells (CD3<sup>+</sup>, CD4<sup>+</sup>, IL-22<sup>+</sup>, IL-17A<sup>-</sup>) is higher in all adult patients compared with the age-matched healthy controls, whereas there are no significant differences between adolescent patients and age-matched healthy controls (35, 36). In the critical phase of COVID-19, the immune response dramatically increases several cytokines, including IL-22. This process is called CRS and leads to fatal complications. As the disease progresses, IL-22-induced signaling pathways shift from

protective to pathogenic. Accordingly, IL-22/Th22 cells may play an important role in the pathological process of novel coronavirus infection, but the detailed mechanism needs more research (36-38).

#### *Th9 Cells in COVID-19*

T helper 9 (Th9) cells are a minor subset of Th cells which considered as a source of IL-9 cytokine in human. For decades IL-9 production had been believed to be related to Th2 function but nowadays studies demonstrate that Th17, iTreg, and Th9 cells are other sources of IL-9 production. PU.1 and interferon regulatory factor (IRF) 4 are essential transcription factors for Th9 maintenance and development as well as other Th subsets such as Th17 and Th2. Respiratory syncytial virus (RSV) elevates IL-9 levels in the bronchial secretions so the decrement in IL-9 levels can cause viral clearance (39). Th9 cells frequency had been reduced at 12, 16, 24 weeks after COVID-19 infection. Although in severe/critical COVID-19 phases, Th9 cells demonstrated more reduction compared with the mild/moderate phases. On the other hand, Th22 and Th17 migratory central memory cells demonstrated increment while their proportion among COVID-19 patients' groups did not differ (36).

#### *NKT Cells in COVID-19*

Human NKT cells split into CD4<sup>+</sup> and CD4<sup>-</sup>CD8<sup>-</sup>NKT subsets, and their activation is mostly dependent on CD1d antigen presentation. Their strong capability for IL-4 and IFN- $\gamma$  production distinguishes them as regulatory immune system cells. The cells also express CD3, semi-invariant V $\beta$ 11 V $\alpha$ 24JQ TCR, and CD56, which are inherited by T and NK cells, respectively (40). NKT cell infiltration into the lungs during the acute phase of COVID-19 infection likely mediates pulmonary inflammation regardless of the illness severity (41). NKT cells have been linked to COVID-19 pneumonia, according to Kim and colleagues (42). Zingaropoli's research demonstrated that the cells fell

below 2.3% of blood T cells within 1-2 days of hospitalization, resulting in severe COVID-19 disease (43). Zhang and Mazzoni et al. did not confirm the findings (44, 45). According to other studies, the severity of COVID-19 has a direct association with decreased blood NKT cells in patients (46). Based on the recent findings, determining circulating NKT cells using CD3 and CD56 could be a potent predictive biomarker for severe COVID-19 (47). Most studies have employed C3/CD56 to quantify NKT cell frequency and functional analysis, although other markers, such as CD1d and MR1 tetramers, will determine the NKT cell population much more precisely. Based on the research available, we can conclude that NKT cells in severe COVID-19 cause inflammation by producing IFN- $\gamma$ , leading to disease progression. Although not all studies approved of a decline in NKT cell counts in severe COVID-19 patients, the majority of the authors reported it, and some of them believed that the frequency may be regarded as a prognostic indicator in the patients.

#### *NK Cells and COVID-19*

The most potent innate response to viral infection is provided by natural killer (NK) cells (48). Killer cell immunoglobulin-like receptor (KIR) and immunoglobulin-like inhibitory receptor for transcription (ILT2) are well-known HLA-G-mediated NK cell inhibitory receptors, whereas NKG2D operates as an activating receptor (49). Recent research has found that NK cells in COVID-19 patients express high levels of inhibitory receptors NKG2A and KIR2DL1, as well as CD107 (a hallmark of CD8<sup>+</sup> T cell degranulation) and a clear downregulation of IFN- $\gamma$ , GrB, and TNF- $\alpha$ . Saresella et al. also have found higher levels of ILT2 and decreased cytotoxic activity of NK cells in infected people. They also demonstrated that, whereas CD56<sup>+</sup>CD16<sup>bright</sup> NK cells are prominent in recovered COVID-19 patients, CD56<sup>dim</sup>CD16<sup>bright</sup> NK cells suppress the virus in COVID-19 patients via antibody-dependent cellular cytotoxicity (ADCC) (48). To our

knowledge, the CD56<sup>dim</sup>CD16<sup>bright</sup>NK cell population has substantial antiviral activity by producing large amounts of IFN- $\gamma$  (50), and this NK cell population is drastically reduced in COVID-19 patients (51).

Decreased levels of CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cell population have been shown in peripheral blood of COVID-19 patients that is most likely due to their recruitment into inflamed lungs (50). Vietzen and colleagues discovered a large increase in NKG2A on the surface of NK cells in severe COVID-19 patients, associated with the cells' functional exhaustion. NKG2C/CD94 NK cells serve a protective effect in patients (52). Furthermore, the majority of the patients' NK cells had high levels of lymphocyte activation gene 3 (LAG3), and as a result, the NK cells appear exhausted due to the expression of LAG3 (18). According to the researchers, CTL and NK cell dysfunction in COVID-19 patients is associated with increased NKG2A expression and lower internal levels of IL-2,

IFN- $\gamma$ , TNF- $\alpha$ , and GrB in the cells (53, 54). Bergantini and colleagues demonstrated the importance of CD56<sup>dim</sup> CD16<sup>bright</sup>, hyper activated NK cells with the CD25, CD69, and NKP44 phenotypes in the severity of COVID-19 infection (51) (Table 2).

The existence of distinct genetic variations of the NKG2C receptor and HLA-E, as well as the rate of receptor expression on NK cells, all contribute to the severity of COVID-19 infection (52). Several NK cell subpopulations have been implicated in COVID-19 infection, with CD56-CD16<sup>bright</sup> NK cells playing a protective role. The formation of distinct NK cell populations with opposing activities during COVID-19 infection is a vast subject that requires further investigation.

#### *$\gamma\delta$ T cells and COVID-19*

Human  $\gamma\delta$ T cells account for roughly 1-5% of blood lymphocytes and 10–100% of T lymphocytes in the stomach, lung, and skin (55). Although the role of  $\gamma\delta$  T cells in

**Table 2. NK cells in COVID-19 infection**

| Reference                     | Number and type of patients                 | NK Cell changes   | Other important findings  |
|-------------------------------|---|---|---|
| Saresella et al., 2021 (48)   | 30 recovering from severe and mild COVID-19 | -Reduction of CD56 <sup>dim</sup> CD16 <sup>bright</sup> and CD56 <sup>dim</sup> CD16 <sup>-</sup><br>-Increase in CD56 <sup>-</sup> D16 <sup>bright</sup> cells<br>-Reduction of CD56 <sup>dim</sup> CD16 <sup>dim</sup><br>-Impaired cytolytic activity | Associated with the development of a severe COVID-19                          |
| Björkström et al., 2021 (50)  | Not mentioned<br>Severe/Mild                | Reduction of CD56 <sup>dim</sup> and CD56 <sup>bright</sup> NK cells  | Contributed to the pathogenesis in severe COVID-19                            |
| Kim et al., 2022 (42)         | 3 severe/mild                               |   | Elevated NKT cell response in the lungs of COVID-19 patients                  |
| Zingaropoli et al., 2021 (43) | 45 severe                                   | Reduction of CD56 <sup>bright</sup> and increase in CD56 <sup>dim</sup> NK cells  | Major reduction of NKT cells associated to severe COVID-19 pneumonia          |
| Vietzen et al., 2020 (52)     | 361 severe                                  | KLRC2 <sup>del</sup> and HLA-E*0101 overrepresented in hospitalized patients  | NKG2C/HLA-E axis associated with severe infection                             |
| Koay et al., 2022 (47)        | Not mentioned                               | Reduction of NKT cells blood frequency  | Circulating NKT cell as a powerful predictive biomarker for severe COVID-19   |
| Tomić et al., 2021 (54)       | 20 severe                                   | Reduced expression of autophagy markers and expansion of MDSCs  | Correlated with poor T cell responses in the COVID-19 patients                |
| Bergantini et al., 2021 (51)  | 35 severe                                   | Increase in CD56 <sup>dim</sup> D16 <sup>bright</sup> Decrease in NK cells<br>D56 <sup>bright</sup> CD16 <sup>neg/dim</sup> NK cells  | High adaptive/memory-like NK cell frequencies in patients with severe disease |

NK cell: Natural killer cell, NKT cell: Natural killer T cell, NKG2A: natural killer group 2A

the containment and eradication of influenza A virus, cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) has been established (56), our understanding of SARS-CoV-2 remains enigmatic. As mentioned, the category of unconventional T cells includes three basic lineages: MAIT, iNKT and  $\gamma\delta$ T cells. Unconventional T cells are involved in SARS-CoV-2 pathogenesis in mucosal barriers by producing IFN- $\gamma$  and IL-17A (57).  $\gamma\delta$  T cells account for fewer than 16% of CD3<sup>+</sup> T cells in various human tissues (58). So far, three major types of  $\gamma\delta$  T cells have been identified: V $\delta$ 1 T cells (found in the dermis, liver, intestinal epithelium, and spleen), V $\delta$ 2 T cells (found in the blood and may have their TCR linked with a V $\gamma$ 9 chain), and V $\delta$ 3 T cells (found in mucosal tissues and blood). Activation of V $\delta$ 1 T cells by Nkp30 ligation results in the generation of CCL5, CCL4, and CCL3, which limit HIV replication (59), and activation of V $\delta$ 2 T cells by granzymes-perforin induces cell death in influenza-infected cells (60). In vitro investigations reveal that when exposed to SARS-CoV-2-infected cells, V $\delta$ 2 T cells display cytotoxic activation and generate IFN- $\gamma$  (61). In contrast to the decline in MAIT and iNKT, the frequency of  $\gamma\delta$ T cells in COVID-19 patients did not alter significantly (62). COVID-19 patients' circulating MAIT and iNKT cells, as well as, to a lesser extent,  $\gamma\delta$ T cells, produced less IFN- $\gamma$  than in the healthy donors, indicating a concentration of activated cells in inflamed lung tissue (63). CD69 on unconventional T cells (except for V $\delta$ 1V $\delta$ 2- $\gamma\delta$  T) was higher in discharged patients than upon admission. On day 15, this was related with increased levels of programmed cell death protein1 (PD1) on iNKT cells rather than  $\gamma\delta$  or MAIT T cells in intensive care unit (ICU) patients (64). Del Bello et al. revealed that  $\gamma\delta$  T cells cannot respond to immunodominant components of SARS-CoV-2, such as nucleocapsid and spike proteins, instead attracting envelope or membrane proteins or viral RNA from robust virus T cell responses (65). V $\gamma$ 9V $\delta$ 2

T lymphocytes are the most common type of circulating  $\gamma\delta$  T cell capable of recognizing stress signals in infected transformed cells. Phosphoantigens (pAgs), which are non-peptide phosphorylated intracellular domains of the cell surface protein butyrophilin3A1 (BTN3A1), activate human V $\gamma$ 9V $\delta$ 2 T cells (66). According to research, V $\gamma$ 9V $\delta$ 2 lymphocytes aggregate in the lungs of vulnerable elderly COVID-19 patients and contribute to the spread of inflammation (67). Furthermore, the cells are fully differentiated (CD45RA<sup>+</sup> CD27<sup>-</sup>) and have highly active phenotypes (CD16<sup>+</sup> CD8<sup>+</sup> CD56<sup>+</sup>). SARS-CoV-2 replication is inhibited by V $\gamma$ 9V $\delta$ 2 T cells via cytotoxic and non-cytolytic IFN- $\gamma$  dependent mechanisms (66).

Overall, the ratio of immature neutrophils to V $\delta$ 2 T cells predicts the onset of hypoxia and pneumonia. Certain illnesses, such as inflammatory bowel disease, aging (since older individuals have higher baseline levels of CRP, TNF- $\alpha$ , and IL-6 and lower levels of V $\delta$ 2 T cells than younger people), and medicines such as azathioprine, which reduce V $\delta$ 2 T counts, might also be risk factors for COVID-19 infection. The relationship between neutrophils and V $\delta$ 2 T cells allows for more accurate and early COVID-19 prognosis (68). Exhausted TCRV $\gamma$ -9 and TCRV $\gamma$ -non9 T lymphocytes concentrate in inflamed lungs in COVID-19 patients, according to new studies, and anti-IL6R treatment promotes  $\gamma\delta$  T cell lymphocyte differentiation (69).

S100A8/A9 was discovered in three of the enlarged subgroups: classical monocytes 1 (CM1), low-density granulocytes (LDGs), and CD16<sup>+</sup>CD8<sup>+</sup> T. Low-density neutrophils (LDNs) inhibit CD4<sup>+</sup> T, CD8<sup>+</sup> T, T, and MAIT cells in severe COVID-19 infection by upregulating oxidative stress S100A8/A9 (70). The ratio of peripheral T cells to LDN cells might serve as an early indicator of COVID-19 severity (71). Odak's study demonstrated that in moderate COVID-19 patients, the number of NK, NKT, and  $\gamma\delta$  T cells did not decrease, however in severe patients, the number of effector-like (eff-1)



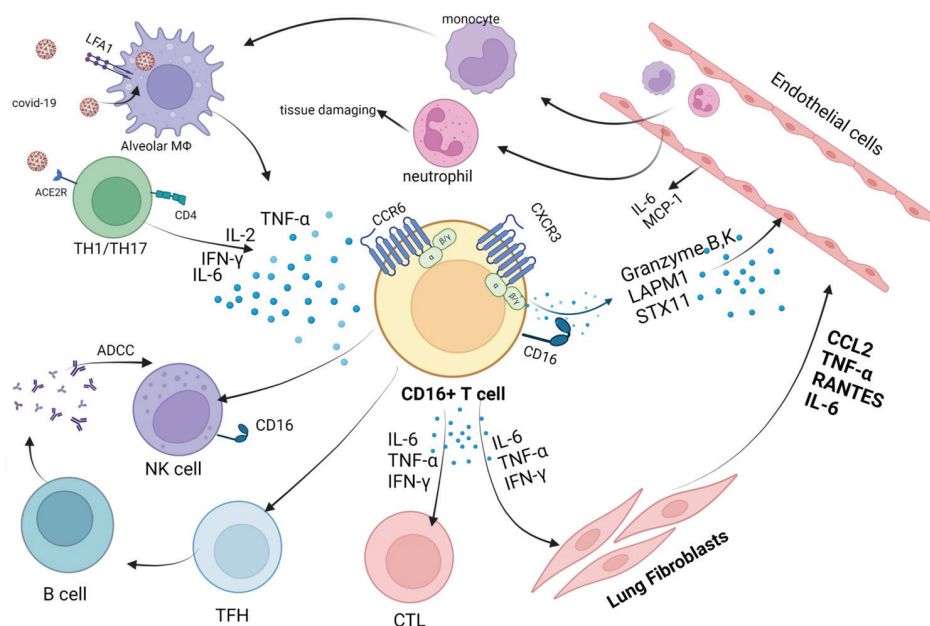
cells decreased while absolute cell counts and frequencies of naive-like (naive-1) cells increased (72). Adequate research has shown that in COVID-19 infection,  $\gamma\delta$  T cells have a strong proliferation capability and tend to exhibit CD4 markers, and their exhaustion is not PD-1 reliant (73). Lung dysfunction and fibrosis are evident in the late stages of acute respiratory distress syndrome (ARDS), with pro-inflammatory cytokines such as IL-6, IL-1 $\beta$ , and TGF- $\beta$ 1 (which is increased by neutrophils) involved in pulmonary fibrosis (74) (Fig. 1). SARS-COV-2 increases the expression of iUL16 binding protein (ULBP) and MHC class I chain-associated protein A and B (MICA/MICB) in host cells, causing  $\gamma\delta$  T lymphocytes to be activated by NKG2D receptors (75). In viral infections,  $\gamma\delta$  T cells provide co-stimulatory signals that activate

naïve  $\alpha\beta$ -T cells (Fig. 2). Furthermore,  $\gamma\delta$  T cells can limit viral replication by producing soluble molecules known as non-toxic antiviral mediators (Fig. 3) (62).

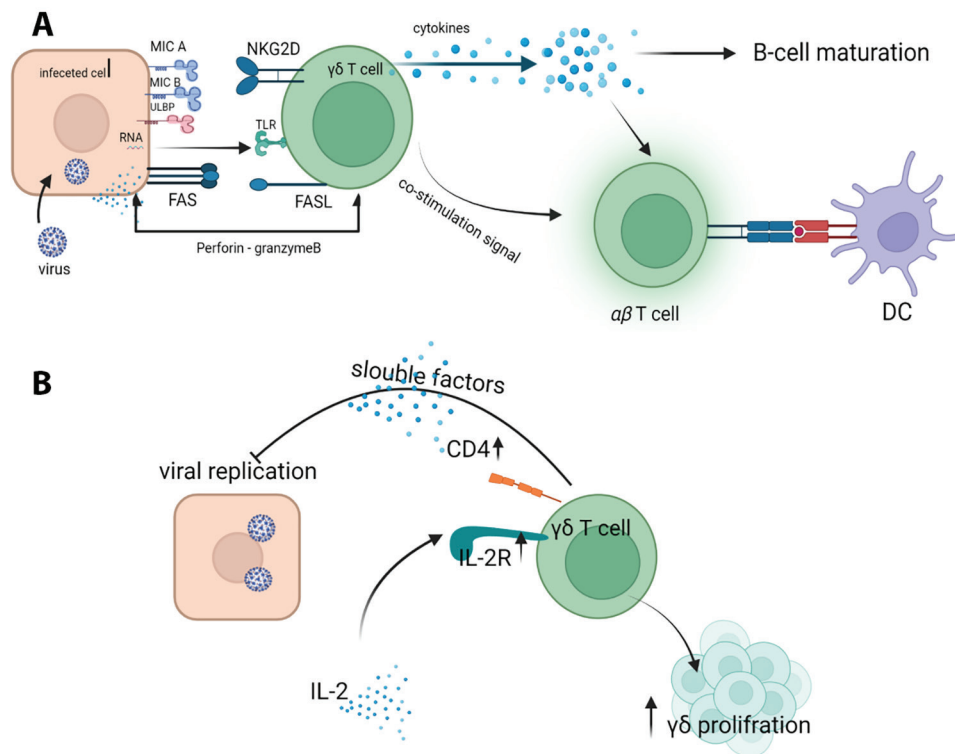
To summarize, effector  $\gamma\delta$  T cells guard against COVID-19 infection by generating antiviral mediators (IFN- $\gamma$ ) and direct cytotoxic mechanisms. The naive and exhausted cells are seen in the patients' lungs, causing the infection to develop. On the other hand, in severe infection, the tight interaction of depleted  $\gamma\delta$ T cells with neutrophils plays a key role in fibrosis and permanent COVID-19 symptoms.

### Regulatory T Cells and COVID-19

Tregs and induced CD4<sup>+</sup> regulatory T (iTregs) cells are found in peripheral lymphoid organs and play important roles in immune control and self-tolerance. Three



**Fig. 2.** CD16<sup>+</sup> T cell play prominent role in inducing immune responses in COVID-19 infection. High levels of CXCR3 and CCR6 chemokine receptors on CD16<sup>+</sup> T cells, induced under inflammatory responses, direct the cells to inflamed lungs. In severe COVID-19 patients, T cells express perforin, Grb, LAMP1, and STX11, which increases their degranulation potential. In turn, granzyme K and B stimulate endothelial cells to release more IL-6 and CCL2 (MCP1). Local CD16<sup>+</sup> T cells inducing immune complex and endothelial cells (mediating monocyte and neutrophil chemoattractant release) are associated with severe COVID-19. NK cells and CTLs are activated by CD16<sup>+</sup> T cell cytokines leading to upregulation of anti-viral responses of the cells, which in some conditions causes lung tissue damage in infected patients. Furthermore, CD16<sup>+</sup> T cells-derived cytokines help IgG antibody production, which is contributing to ADCC by binding to CD16 on NK cells. On the other hand, virus (COVID-19)-IgG complex transduce cytotoxic activity signals using CR1 on CD4<sup>+</sup> and CD8<sup>+</sup> T cells. TFH responses in lymph nodes are essential for GC responses, long-lived plasma cells, and memory B cells. LAMP1: Lysosomal associated membrane protein 1; STX11: Syntaxin-11; MCP-1: Monocyte Chemoattractant Protein-1; CR1: Complement Receptor 1; ADCC: Antibody-dependent cellular cytotoxicity; TFH: Follicular helper T; GC: Germinal center



**Fig. 3.** A: SARS-CoV-2 enters host cells and binds NKG2D receptors on T cells, increasing ULBP and MICA/MICB levels. On T cells, TLRs identify SARS-CoV-2 viral RNA. Fas-FasL or perforin-Grb can kill infected host cells. Other cellular alterations include cytokine increase, DC antigenic presentation function, and B cell maturation. T cells activate naive  $\alpha\beta$  T cells by processing and presenting viral antigens to start an adaptive immune response. B: Non-toxic antiviral T cell function can suppress virus replication. In COVID-19 patients, T cells express CD4, CD25 (IL-2Ra) levels increase, and IL-2 increases T cell proliferation. NKG2D: Natural killer group 2-member D; ULBP: UL16-binding protein; MICA/B: Major histocompatibility complex (MHC) class I chain-related protein A/B; TLRs: Toll-like receptors; DC: Dendritic cells

subgroups of Tregs are established based on the expression of CD45RA and Foxp3. The Foxp3<sup>high</sup>/CD45RA<sup>low</sup> population suppresses inflammatory responses by generating IL-10 and TGF- $\beta$  (76). While reduced Treg cell function in COVID-19 patients has been described, no association with infection severity has been verified (77). COVID-19 increases antibody production by decreasing follicular regulatory T cells (Tfr) in germinal centers, according to Zahran and colleagues (78). Increased Th17/Treg in children has been linked to resistance to COVID-19 in studies (79). Russo demonstrated that ATP stimulation of purinergic receptors drives Th17 (ROR $\gamma$ t<sup>+</sup>) growth while inhibiting Treg activation (80). Several contentious studies have been carried out to evaluate the relationship between Treg frequency and

phenotype and the severity of COVID-19 in adult patients. Neumann and colleagues discovered a direct link between higher frequency and the activated Treg phenotype and COVID-19 severity (81), while others discovered a negative correlation (24, 82, 83). More information about Treg characteristics has been provided. CD25<sup>high</sup>FoxP3<sup>+</sup> Treg subset amplification has been seen in pediatric COVID-19 patients (76), while CD25<sup>high</sup>FoxP3<sup>+</sup> Treg was enlarged more in adults with mild COVID-19 symptoms than in pediatric patients (84).

Studies on COVID-19-infected patients with common variable immune deficiency (CVID) confirmed significant changes in the frequency of CD8<sup>+</sup> Tregs and CD4<sup>+</sup> Tregs compared with the controls (infected peoples without CVID). However, circulating

T follicular helper (cTfh) cells significantly reduced in the patients (85). In contrast to the control group, solid organ transplant (SOT) recipients with severe COVID-19 had a high prevalence of Treg and fatigued T cells (PD-1<sup>+</sup>, CD39<sup>+</sup> in CD8<sup>+</sup> and CD39, T cell immunoreceptor with Ig and ITIM domains (TIGIT) on CD4<sup>+</sup> T cells) (65). COVID-19 behavior was also examined during pregnancy, when tolerogenic dendritic cells (tmDC) and Treg cells are prevalent. Infection with SARS-CoV-2 generates both T regulatory cells and pro-inflammatory T cells in pregnant women. Specific viral epitopes on iTreg cells have been discovered to be plentiful regardless of pre-existing conditions or the timing of pregnancy and infection, whereas Treg cells in convalescent COVID-19 women have not been found (86) (Table 3).

According to a review of the literature, Treg in COVID-19 infection hinders efficient immune responses against COVID-19 by producing anti-inflammatory cytokines, particularly IL-10. As a result, iTreg in adults and the elderly is more common than COVID-19 in children, indicating that the infection is more severe in these populations. Based on the most recent studies that we analyzed, some researchers stated that in 478 severe COVID-19 patients, a drop in Treg was detected. However, a similar tendency was not observed in 84 severely affected individuals. Although many markers are examined for Tregs, the majority of research used the CD45RA<sup>-</sup>/PFOXP3/CD4/CD25 marker to characterize active iTregs. Analysis of 368 mild COVID-19 patients, on the other hand, revealed no change in cell

**Table 3. Summary of studies on the roles of regulatory T cells in COVID-19 infection**

| Reference                  | Number and type of patients     | Tregs' changes  | Other important findings  |
|----------------------------|---------------------------------|---|---|
| Chen et al., 2020 (96)     | Mild:10<br>Severe:11            | Reduction of CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 and CD45RA <sup>+</sup> Tregs<br>Decrease in CD4 <sup>+</sup> T cells producing IFN- $\gamma$ | Associated with disease severity  |
| Wang et al., 2020 (82)     | Mild:30<br>Severe:35            | Reduction of CD45RA <sup>+</sup> Tregs  | Associated with the pathogenesis of extremely severe SARS-CoV-2 infection |
| Qin et al., 2020 (83)      | Mild/moderate:166<br>Severe:286 | Reduction of CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>low</sup> Tregs   | Associated with disease severity  |
| Neumann et al., 2020 (81)  | Mild/moderate:23<br>Severe:20   | Increment of IL-10-producing Tregs  | Associated with disease severity  |
| Yang et al., 2020 (14)     | Asymptomatic                    | Increment of CD45RA <sup>-</sup> FoxP3 <sup>high</sup> Tregs  | Associated with disease severity  |
| De Biasi et al., 2020 (11) | Not mention                     | Increment in the percentage of CD45RA <sup>+</sup> CCR7 <sup>+</sup> Tregs and increment of activated CD45RACCR7 <sup>+</sup> Tregs                 | Associated with disease severity  |
| Meckiff et al., 2020 (97)  | Severe:22                       | Reduction of Tregs in hospitalized patients/ Increase in cytotoxic Th and follicular Th cells   | Associated with disease recovery  |
| Salehi et al., 2021 (44)   | Severe:30                       | Decreased Tregs<br>Increase in Th17/ Th1/ IFN- $\gamma$ / IL-17<br>Reduction of Th2/IL-4/ IL-10   | Associated with disease severity  |
| Sadeghi et al., 2020 (24)  | Severe:40                       | Decreased Tregs<br>Decreased FoxP3/IL10/TGF $\beta$<br>Decreased Th17/Treg cell ratio   | Associated with inflammatory responses and the disease pathogenesis       |
| Simsek et al., 2021 (98)   | Severe:190                      | Increase in CD45RA/FoxP3  | Associated with disease severity  |

Treg: Regulatory T cells, IFN- $\gamma$ : Interferon- $\gamma$ , TGF $\beta$ : Transforming growth factor- $\beta$

frequency. However, other studies have found an increase in circulating cells. Prolonged inflammation, accompanied by inflammatory cytokines such as IL-6, induces Treg death, inhibiting Foxp3 expression and turning them into effector Th1 and Th17 cells, resulting in infection progression.

#### MAIT Cells and COVID-19

MAIT cells, an innate-like lymphocyte that provide effective mucosal immunity, account for 1–4% of peripheral blood T cells (87). There are two main modes of activation for MAIT cells: MR1-TCR dependent and cytokine (IL-12, IL-15, IL-18, and type I interferon) signaling (88). MAIT cells produce IFN- $\gamma$ , TNF- $\alpha$ , and IL-17A after activation while also killing their target directly via the granzyme-perforin system (89). Although MAIT cells cannot recognize viral antigens, cytokines such as IL-18, IFN- $\alpha$ , and IL-12 produced by nearby cells efficiently activate the cells (89, 90). According to recent research, MAIT cells play a role in the response to SARS-CoV-2 by producing pro-inflammatory cytokines (90). Invasion of highly activated MAIT

cells (CD69<sup>high</sup>CXCR3<sup>low</sup>) enriched by IL-17A in the airway epithelium is linked to a poor clinical outcome in COVID-19. Shi and colleagues discovered that the number of peripheral MAIT cells was constantly decreasing in severe COVID-19 patients, with the number of blood MAIT cells decreasing by a tenth when compared with the control group (87, 91). Similarly, in influenza and HCV infections, activated MAIT cells were reduced (Table 4) (92).

MAIT cells, on the other hand, have been shown to reduce inflammation in the airways of COVID-19 patients, according to Jouan et al. (64). Increased levels of CXCL10 and CX3CL1 in serum have been linked to MAIT cell activation, according to Parrot and colleagues. Both MAIT cell phenotypes, low CXCR3 and high CD69, were associated with poor clinical outcomes in COVID-19 patients (90). Increased numbers of CD69<sup>+</sup> peripheral MAIT cells may be a predictor of disease severity (87). More research is needed to determine the exact role of the cells in COVID-19 pathology.

MAIT cells' function and phenotype are highly variable in COVID-19 patients. MAIT

**Table 4. Summary of studies on MAIT cells in patients with COVID-19**

| Reference                  | Population study     | MAIT cell exchanges  | Other important information   |
|----------------------------|----------------------|--|---|
| Parrot et al., 2020 (90)   | 69 acute             | Reduction in circulating MAIT cells<br>Enrichment of MAIT cells in the airways   | MAIT cells' intense activation is related to poor outcome   |
| Deschler et al., 2021 (89) | 22 mild<br>21 severe | Reduced blood MAIT cells   | MAIT cells' intense activation is related to poor outcome   |
| Flament et al., 2021 (91)  | 208 severe           | Reduced blood MAIT cells   | -Changed MAIT cells' functions due to IFN- $\alpha$ -IL-18 imbalance augment the disease severity<br>-MAIT cells' intense activation is related to poor outcome |
| Jouan et al., 2020 (64)    | 30 severe            | Highly activated unconventional T cells such as MAIT cells<br>Intense and persistence phenotype conversion in circulating unconventional T cells | Highly activation of MAIT cells could be beneficial.  |
| Shi et al., 2021 (87)      | 134 severe           | Increased the number of pyroptotic MAIT cells, but not the number of apoptotic MAIT cells  | Greater clonal expansions of MAIT cells in severe cases than in moderate cases  |

MAIT cell: Mucosal-associated invariant T cell

cell mutations may have a negative impact on the immune response to SARS-CoV-2 by inducing a persistent immune response that causes tissue damage as well as effects on the functional antimicrobial immune response (89). More research showed MAIT cells producing GrB in the pulmonary fluids and blood of patients increased. The production of GrB from MAIT cells was a factor that was related to a fatal outcome (91). On the other hand, decreasing and losing MAIT cells constantly in the blood of COVID-19 patients have a detrimental effect on defending against microbial and fungi infections in the barrier areas. Microbial and fungi infection in the COVID-19 patients beside high dose of corticosteroid may be related to MAIT cells depletion. New finding shows the falling of MAIT cells frequency was more intense than in other T cells subpopulations in COVID-19 patients. The reduction in MAIT cell frequency may have several reasons. Firstly, the tissue homing receptor profile of MAIT cells shows various chemokine receptors such as CCR5, CC6, CXCR3, and CXCR6, supporting the concept that MAIT cells infiltrate inflamed airways (90). Secondly, increased apoptosis may be involved in the reduction of peripheral MAIT cells in patients with severe COVID-19 (87). Consistently, CD127 as IL-7 receptor decreased on blood MAIT cells. Thirdly, pyroptosis is one of the eminent causes of the death of MAIT cells in COVID-19 infection. The number of pyroptotic MAIT cells strikingly raised in the blood of COVID-19 patients (87). Furthermore, Deschler and et al reported an impaired anti-viral functions of stimulated MAIT cells by IL-12 and IL-18 in COVID-19 patients (89). According to new insights, secondary non-viral infections in COVID-19 patients may activate MAIT cells by MR1 and increase the severity of the disease (93). Of note, study on 208 patients with various stages of COVID-19 has confirmed a decrease in MAIT blood cells as well as an accumulation of the cells in the patients' infected lungs. According to this study severity of the disease

was related to IFN- $\alpha$ -IL-18 imbalance (91). Because MAIT cells play a dominant role in COVID-19 infection pathology, a treatment strategy based on the cell inhibition would benefit patients.

## CONCLUSION

Human T cells are heterogeneous populations with different functions depending on the time and location of deployment. Based on latest findings most CTLs and Th1 cells in severe COVID-19 patients show exhausted and memory phenotypes. Th17 cells not only exacerbate pathological outcomes of the infection by production of IL-17 but also cause serious lung tissue damage by recruiting neutrophils. So it suggests that inhibiting the cells in COVID-19 infection can effectively diminish mortality and long-term complication in infected patients. MAIT cells as specialized cells in mucosal defense also play substantial role in progressing COVID-19 infection. Therefore, control of the cells using IL-12 and IL-18 antagonists or MR1 inhibitors seems having useful effects in ameliorating COVID-19 pathology. Studies have proved that exhausted and naïve  $\gamma\delta$  T cells in severe hospitalized COVID-19 patients are dominant and actively help the virus expansion and are associated with fibrosis and lung tissue damages. We still do not have a clear knowledge of the cooperation of the MAIT and  $\gamma\delta$  T cells in COVID-19 infection and more studies are much needed. To our knowledge, iTreg are associated with the expansion of COVID-19 infection, a phenomenon observed in adult and elderly patients unlike children (Table 5). Finding the ways to prevent inducing the cells in the vulnerable patients could be considered as an effective treatment strategy in the future. Studies also revealed that CD56<sup>+</sup>CD16<sup>bright</sup>NK cells play a protective role in the COVID19 infection, whereas iNKT cells contribute to the progression of the infection by producing inflammatory cytokine. Taken together

**Table 5. Summarizes the main roles of human blood lymphocytes in COVID-19 patients**

| Type of cells               | Function in COVID-19   |
|-----------------------------|--|
| CTLs (8, 15, 17)            | CTL frequencies are lower in severe COVID-19, which is associated with poor prognosis of the infection. Memory tissue CTLs provided protection against COVID-19 infection.   |
| Th1 cells (5, 44, 72)       | Cell-mediated responses involving macrophage, CTL, and NK activation via IL-2 and IFN- $\gamma$ are essential for protective immune responses in COVID-19 patients   |
| Th2 cells (5, 27)           | Th2 involvement in severe COVID-19 is unclear, although patients with mild disease may have a normal Th2 cell response. Given the prominent role of Th2 cell responses in other lung diseases; further studies in the era are needed |
| Th9 cells (36, 99, 100)     | Reduced according to limited studies.  |
| Th17 cells (25)             | Increased Th17 levels in COVID-19 patients. Associated with recruiting neutrophils to the lung and tissue damage in COVID-19 patients.   |
| Th22 cells (36, 38)         | As the disease progresses, IL-22-induced signaling pathways shift from protective to pathogenic.   |
| TFH cells (85)              | Show increase in the COVID-19 infection.   |
| Regulatory T cells (81)     | Show increase in the COVID-19 infection.   |
| $\gamma\delta$ T cells (57) | The exact role is not clear currently.   |
| MAIT cells (87)             | The exact role is not clear currently.   |
| NK cells (52)               | CD56 <sup>+</sup> CD16 <sup>bright</sup> NK cells playing a protective role  |
| NKT cells (44)              | The exact role is not clear currently.   |

CTL: Cytotoxic T lymphocytes; MAIT: Mucosal-associated invariant T (MAIT) cells; TFH: T follicular helper cells

several strategies covering different T cells population manipulations are required for the successful treatment of the disease.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENT

The authors acknowledge Dr. Nasrin Esfandiari for proof-reading the article.

## AUTHORS' CONTRIBUTION

AS presented the idea, investigated and supervised the findings of this work. He discussed the results and contributed to the final manuscript. AZ was responsible for reviewing and writing the Th and CTL sections. YL investigated the roles of Treg and  $\gamma\sigma$  T cells in COVID-19. RN reviewed Th17/Th22 cells in COVID-19. SB surveyed MAIT and NK cell's functions in COVID-19. SK helped supervise the project. She organized the references, planned, and designed the figures.

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