Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Lymphopenia during the COVID-19 infection: What it shows and what can be learned

To the Editor

Since the current outbreak of the COVID-19, several studies have determined a correlation with the disease severity and lymphopenia, a condition defined by abnormally low counts of lymphocytes. In infected children, where the mortality rate is close to zero, lymphopenia is rarely observed. However, in the elderly, where there is a higher mortality rate, lymphopenia occurs more frequently, especially in severe cases. An increased neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, and increased levels of cytokines, such as IL-2R and its ratio to lymphocyte count, were found to be correlated with disease severity and poor prognosis. A better understanding of the underlying mechanisms that lead to the observed lymphopenia can help to better understand the disease pathogenesis and will provide insight into better management of such patients, especially for patients with comorbidities. Here we briefly discuss the possible mechanisms that may lead to lymphopenia in patients.

A closer look at COVID-19 patients suffering from lymphopenia almost always exhibit significant decreases in T cell counts. Patients admitted to the ICU showed a drastic decrease in $CD8^+$ T cells. HIV patients who were already on antiretroviral therapy with normal T cell counts did not show excess morbidity to the virus [1]. CD4 count also appears to be crucial, as demonstrated that HIV patients with lower than normal $CD4^+$ T cell counts had a more severe disease outcome with higher ICU admittance and death [1]. Whether undiagnosed HIV patients infected with the virus behave similarly remains unknown. A higher total T cell count, including both $CD4^+$ and $CD8^+$, has been shown to be a predictor of less severe disease and a more favorable clinical outcome. Upon recovery, lymphocyte counts return to normal in almost all cases [2,3]. In contrast, the decrease in B cell counts among severe COVID-19 patients is not as consistently observed as the decrease in T cell counts [2].

While the role of B cells and anti-SARS-CoV-2 antibodies in the recovery process remains to be fully understood, strikingly, B cell activity may not be key for the recovery. A multiple sclerosis COVID-19 patient, who was treated with ocrelizumab, an anti-CD20 B cell depleting antibody, made a full recovery after a few days of hospitalization [4]. Another study reported two COVID-19 patients with X-linked agammaglobulinemia (XLA), a rare genetic disorder resulting in a lack of mature B cells, showed full recovery as well [5]. The patients had developed pneumonia but did not require intensive care or mechanical ventilation. These studies question the necessity of B cells involvement in mounting a successful response to the SARS-CoV-2 infection. A different study showed that in severe cases of COVID-19 patients, the numbers of antibody-secreting cells are higher than mild cases [6]. Similarly, a number of studies showed that higher titers of virus-specific antibodies correlate with the severity of the disease. It is unclear whether these antibodies are protective or nonprotective, as studies on other similar viruses show two types of antibody response in patients; one is neutralizing and protective, while the other can exacerbate inflammatory responses and augment lung injury [7]. Thus, it is plausible that patients with severe COVID-19 with high antibody count may not mount an appropriate neutralizing antibody response, whereas patients who recover from COVID-19 may have a predominance of protective-neutralizing antibodies, as early human result of passive transfer of plasma show possible beneficial results [8]. Several early animal studies of COVID-19 vaccines also show the generation of protective neutralizing antibody response. However, additional studies are needed to better understand the contribution of high titers of antibodies in the disease pathogenesis in severe patients.

Based on the available literature, we hypothesize the followings as possible underlying causes for the observed lymphopenia in severe COVID-19 patients, especially the decrease in T cell counts:

1 The inflammatory cytokine storm is likely a key factor behind the observed lymphopenia. The serum level of pro-inflammatory cytokines, such as TNF-α and IL-6, have been closely correlated with lymphopenia, while recovered patients show close to normal levels of such cytokines. Autopsy studies on lymphoid organs collected from several patients who succumbed to the disease revealed massive lymphocyte death, which was attributed to high levels of IL-6 as well as Fas-FasL interactions. Treatment with tocilizumab, an IL-6 receptor antagonist, increased the number of circulatory lymphocytes, further suggesting IL-6 increase is a key player in the lymphopenia development. Another study suggests a significant association between IL-6 serum levels in COVID-19 patients and the impairment of the cytotoxic activity of not only T cells but also NK cells [9]. More studies are needed to better understand how the cytokine storm may affect the T and NK cells' behavior during the infection.
2 COVID-19 infection can result in exhaustion of T cells. A study found both $CD4^+$ and $CD8^+$ T cells from COVID-19 patients had increased cell surface expression of programmed cell death protein 1 (PD-1) and T cell immunoglobulin and mucin domain 3 (Tim-3), two markers of T cell exhaustion [10]. Expressions of PD-1 and Tim-3 were correlated with disease severity and intensive care requirement. Another study found increased expression of NKG2A on T cells, another marker of $CD8^+$ T cells exhaustion, as well as decreased expression of some T cell activation markers, such as CD107a and IFN-γ. Of note, the number of regulatory T cells (Tregs) did not change in relation to the severity of the disease, suggesting T cell exhaustion occurs in a process independent of Tregs. More studies are necessary to better understand how the SARS-CoV-2 infection results in T cells exhaustion.
3 The SARS-CoV-2 virus may infect T cells. A study reported that two human T cell lines (MT-2 and A3.01) with a very low level of human ACE2 mRNA, the receptor that the virus uses to enter the host, can be infected with the virus *in vitro*. However, the virus could not

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replicate within the infected cells, as measured by qPCR expression of the viral N gene [11]. Conversely, another study reported the lack of viral gene expression in COVID-19 patients' PBMCs, suggesting lymphocytes had not been infected [12]. More studies are needed to better understand whether any immune cell subsets can be infected, either directly or indirectly, for example through an antibody-dependent enhancement (ADE) mechanism.

4 The SARS-CoV-2 infection can interfere with T cell expansion. A report suggests that some genes involved in T cell activation and function, such as *MAP2K7* and *SOS1* are downregulated in the T cells of severe COVID-19 patients. The expression of most of these genes returned to normal levels upon recovery [13]. This may be a result of the dramatic change in the cytokine milieu during the infection. More studies are needed to better understand how proliferation and activity of T cells are affected during the disease progression.

Overall, lymphopenia and increased levels of certain cytokines, such as IL-6, have been closely associated with the disease severity. T cells likely play a pivotal role in shaping the initial immune response. A remarkable decrease in T cell counts is almost always observed in severe cases. Patients admitted to ICU show a dramatic decrease in T cells, especially $CD8^+$ T cell counts. Studies from other similar coronaviruses suggest that Th1 cell response and cellular immunity is the primary mechanism of control of the infection [14,15]. Taken together, this would suggest of immunosuppressive agents that suppress T cell response, especially Th1 cells, may be particularly detrimental in fighting COVID-19, and thus should be avoided in patients with premorbid autoimmune diseases.

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