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Altered cytokine levels and immune responses in patients with SARS-CoV-2 infection and related conditions

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\begin{abstract}
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic in early 2020. The infection has been associated with a wide range of clinical symptoms. In the severely affected patients, it has caused dysregulation of immune responses including over-secretion of inflammatory cytokines and imbalances in the proportion of naïve helper T cells, memory helper T cells and regulatory T cells. Identification of the underlying mechanism of such aberrant function of immune system would help in the prediction of disease course and selection of susceptible patients for more intensive cares. In the current review, we summarize the results of studies which reported alterations in cytokine levels and immune cell functions in patients infected with SARS-CoV-2 and related viruses.
\end{abstract}

1. Introduction

Firstly, identified in an outbreak in Wuhan city of China, the novel coronavirus disease 2019 (COVID-19) has caused a global pandemic in early 2020. Alternatively named as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus has been shown to induce various clinical manifestation in hosts ranging from asymptomatic conditions to severe symptoms including respiratory failure, shock, or multiorgan system dysfunction [1]. Identification of IgM and IgG antibodies in the infected persons implies the development of immunity against SARS-CoV-2 [2–4]. However, the virus might also induce dysregulation of immune responses in susceptible individuals as demonstrated by the decreased lymphocytes counts especially T cells, increased leukocytes counts and neutrophil–lymphocyte-ratio and other imbalances in the population of immune cells. Moreover, severely affected patients have shown raised concentrations of infection-related markers and over-secretion of inflammatory cytokines. Notably, this condition has been accompanied by a significant increase in the proportion of naïve helper T cells while reduction in memory helper T cells and regulatory T cells [5]. Based on the importance of immune responses in the determination of course of infection and the related complications, we performed a literature search to find the reported dysregulations in the levels of cytokines and immune cells in patients infected with SARS-CoV-19 and related viruses.

2. SARS-CoV-2

A recent study in Chinese patients affected with SARS-CoV-2 has shown elevated plasma concentrations of IL-1β, IL-1RA, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GMCSF, IFN-γ, IFN-γ-induced protein (IP)-10, monocyte chemotactic protein 1 (MCP1), MIP1A, MIP1B and TNF-α in both patients needed ICU admission and non-ICU patients compared with healthy controls at initial assessment. Notably, author reported significant over-production of IL-2, IL-7, IL-10, GCSF, IP-10, MCP1, MIP1A, and TNF-α in ICU patients compared with other group of SARS-CoV-2 infected persons [6]. Another study has demonstrated associations between severity of SARS-CoV-2 infection and levels of IL-2R, IL-6, IL-8, IL-10 and TNF-α. Moreover, disease severity was associated with both WBC and lymphocyte counts as well as quantities of neutrophils and eosinophils. Authors have suggested the IL-2R level > 793.5U/mL, WBC > 9.5*10^9/L or neutrophil count > 7.305*10^9/L among parameters that indicate progression of SARS-CoV-19 infection to critical conditions. Thus, inflammatory responses were shown to be correlated with the severity of SARS-CoV-19. Besides, IL-6, TNF-α and IL-8 have been suggested as therapeutic targets [7]. A longitudinal study of cytokine levels and lymphocyte count in affected patients has...
revealed remarkable and continuous decreases in lymphocyte counts but elevations in neutrophil counts in severely infected cases compared with mild cases. Additionally, severely affected individuals had substantial reductions in T cells population, particularly CD8 + T cells, and upsurge in IL-6, IL-10, IL-2 and IFN-γ levels. Notably, T cell counts and cytokine concentrations in severe SARS-CoV-2 infected patients who stayed alive gradually returned to their levels in the mild cases. The most significant prognostic marker to show the course of infection has been the neutrophil-to-CD8 + T cell ratio [8]. IL-6 has also been among the up-regulated infection-related markers in the serum of patients with SARS-CoV-2 pneumonia [9]. Another study has demonstrated significant decrease in lymphocyte subsets both in severe and mild groups of patients with SARS-CoV-2 infection. Reduction in CD8 + T cells and increase in IL-6 levels were more prominent in the severely affected patients. Moreover, significant differences were detected between the severe and mild groups in CD4 + T, CD8 + T, IL-6 and IL-10 [7].

3. SARS-CoV

Cellular immune responses to SARS-CoV infection have been previously assessed in an animal study. Animals were exposed to the virus through an intranasal route. Such viral administration resulted in induction of pneumonia which was accompanied by over-production of TNF-α, IL-6, CXCL10, CCL2, CCL3, and CCL5. Notably, increased cytokine and chemokine levels were associated with relocation of NK cells, macrophages, and plasmacytoid dendritic cells (pDC) into the lungs. The viral clearance was accompanied by another round of cytokine and chemokine release and an inflow of T lymphocytes occurred. Depletion studies showed the essential role of CD4 + T cells in reduction of immunemediated interstitial pneumonitis and enhancement of elimination of SARS-CoV from the lungs [10]. An in vitro study has shown insignificant induction of IFN-β expression in SARS-CoV-infected macrophages, while up-regulation of CXCL10/IFN-γ-inducible protein 10 and CCL2/monocyte chemotactic protein 1 [11]. Wong et al. have assessed a number of cytokines and chemokines in patients affected with SARS. They detected significant increase in Th1 cytokine IFN-γ, inflammatory cytokines IL-1, IL-6 and IL-12, and some chemokines such as IL-8, MCP-1 and IP-10. Levels of chemokines were significantly decreased after corticosteroid therapy. They concluded induction of Th1 cell-mediated immune response in patients. This observation was associated with induction of monocyes/macrophages and neutrophils [12]. In another study in the pediatric setting, Ng et al. have demonstrated significant increase in circulating IL-1β concentrations. Yet, IL-6 and TNF-α cytokines were slightly increased at the primary phase of the disease [13]. Zhang et al. did not detect any significant difference IL-1 and TNF-α levels between normal controls, patients with SARS, severe SARS or those with SARS in convalescence. IL-6 levels were higher in SARS particularly those with severe SARS course. Yet, IL-6 levels were not different between convalescent patients and healthy control, indicating the presence of a positive correlation between the serum IL-6 levels and severity of the disorder. On the other hand, IL-8 and TGF-β levels were negatively correlated with SARS severity. IFN-γ and IL-4 concentrations were reduced, whereas IL-10 concentrations were elevated in convalescent SARS patients. Taken together, various immunoregulatory conditions are present during and subsequent to SARS infection [14].

4. Middle east respiratory syndrome coronavirus (MERS-CoV)

Lau et al. have assessed transcript levels of TNF-α, IL-1β, IL-6, IL-8, IFN-β, MCP1, TGF-β and IP-10 in cell lysates of polarized airway epithelial Calu-3 cells infected with MERS-CoV or SARS-CoV. MERS-CoV induced higher levels of IL-1β, IL-6 and IL-8, while lower levels of TNF-α, IFN-β and IP-10 compared with SARS-CoV. Their experiments confirmed the diminished induction of innate immunity and postponed proinflammatory cytokine production by MERS-CoV [15]. Kim et al. have reported higher neutrophil counts in severe MERS-CoV compared with mild cases. Moreover, IL-6 and CXCL-10 levels were higher in severe cases compared with mild cases. Besides, they could not detect IFN-α response in mild cases [16]. Zhou et al. have shown ability of MERS-CoV but not SARS-CoV in replication in monocyte-derived macrophages. MERS-CoV induced remarkably elevated concentrations of IL-12, IFN-γ, and chemokines compared with SARS-CoV [17]. Mahallawi et al. have demonstrated a noticeable pro-inflammatory Th1 and Th17 response in patients affected with MERS-CoV. These patients had elevated levels of IFN-γ, TNF-α, IL-15 and IL-17 compared to controls. Totally, cytokines profiles indicated an obvious pro-inflammatory immune reactions in the acute course of MERS-CoV infection [18].

5. Influenza

Pirhonen et al. have demonstrated induction of trivial amounts of IL-1β or IL-18 in influenza virus-infected monocytes, while GM-CSF-differentiated macrophages secreted high amounts of these cytokines. In vitro experiments indicated that the role of cellular differentiation in the aptitude of monocytes/macrophages to secrete IL-1β and IL-18 following exposure with virus infections [19]. In addition, Ramos et al. have reported lower monocyte counts and a marginally lower median level of IL-6 in patients infected with influenza compared with the control group [20]. Table 1 summarizes the results of studies which reported altered cytokine levels and immune functions in patients with SARS-CoVs and influenza infections and related conditions.

6. Discussion

Immune responses have indispensable functions in the determination of course of SARS-CoV infection. Dysregulation of cytokine levels have been demonstrated in almost all patients with this infection. Moreover, evident differences have been reported in the levels of several cytokines between severely affected patients and those with moderate or mild symptoms [6]. Identification of these aberrant reactions not only helps in recognition of patients who are predisposed to severe complications, but also would specify those would benefit from immune-modulating therapies. Due to the insufficiency of data on SARS-CoV-2, assessment of immune responses in other related disorders would provide a scheme permitting the anticipation of immune-related events which occur in the course of this novel infection. Yet, any of these viruses might exert some specific effects in the host. This speculation has been supported by an in vitro study on expression signatures of macrophages infected with SARS-CoV, human coronavirus 229E, and influenza A (H1N1) virus. Authors have reported slight or no induction of IFN-β in SARS-CoV-infected macrophages. Yet, expression of this cytokine was induced in the macrophages infected with human coronavirus 229E and influenza A virus [11]. Moreover, there were significant differences in the pattern of cytokine induction between SARS-CoV and MERS-CoV [15]. It is worth mentioning that although low level of SARS-CoV productive infection has been demonstrated in human monocytes/macrophages, these viruses have been identified in phagolysosomes but not on the cell surface implying absence of specific receptors for SARS entrance on macrophages [34]. Low efficiency of macrophage infections by this virus and production of IFN-α by these cells might be involved in the restriction of the infection in human subjects [34]. Similarly, SARS-CoV-2 cannot replicate in macrophages possibly due to lack of ACE2 expression (https://www.genecards.org/cgi-bin/carddisp.pl?gene=ACE2). Banerjee et al. have assessed an extensive spectrum of human immune cells for productive infection with this virus and verified lack of permissivity of human primary peripheral blood mononuclear cells to this virus [35].

Others have shown infection of human macrophages by SARS-CoV via an IgG-mediated antibody-dependent enhancement mechanism
| Disease | Case/Control | Sample | Finding Cytokines | Immune cells | Comment | Ref |
|---------|--------------|--------|------------------|--------------|---------|-----|
| SARS-CoV-2 | 40 cases: 13 Severe, 27 mild | Blood | Elevated IL-6, IL-10, IL-2, IFN-γ levels in severe cases | Increased neutrophil, decreased lymphocyte counts esp. CD8+T in severe cases | N/N ratio as a as a prognostic factor | [8] |
| | 99 cases | Blood | Increased IL-6 | | – | [9] |
| | 41 cases: 13 ICU/28 non-ICU patients | Plasma | Elevated IL-10, IL-2, IL-7, GSF, IP10, MCP1, MIP1a, and TNFα in ICU patients | | – | [3] |
| | 100 cases: 34 mild, 34 severe, 32 critical | Blood | A significant association between IL-6 and IL-10, IL-2, IL-8, TNFα, CRP, feroerin, procalcitonin, and disease severity | A significant association between WBC, lymphocyte, neutrophil and eosinophil counts and disease severity | IL-6, TNFα, IL-8 as promising therapeutic targets | [7] |
| | 53 cases: 34 severe, 19 mild | Plasma | Association of IP-10, MCP-3, IL-1ra with disease severity | | – | [6] |
| | 123 cases: 21 severe, 102 mild | Blood | Elevated IL-6 and IL-10 in severe NCP | Decreased CD4+T, CD8+T in severe NCP | T cell subsets and cytokines as predictive factors for severity. | [7] |
| SARS | 8 children before corticosteroid therapy/after 1-2 days/after 7-10 days | Plasma | Elevated IL-1β, IL-6, IL-8 induced by MERS, higher TNF-α, IL-6 and IL-8, reduced IL-10 | | – | [13] |
| | SARS infected cells / cells infected with RSV, FluAV, and hPIV2. | Caco2 cells | Induced high levels of IL-6, IFN-γ, IL-8, IL-10, IL-12, IL-15, IFN-γ, IL-17, IL-23, IL-28, and IL-33 | | – | [23] |
| | 88 cases 51 Ab positive/37 Ab negative | Serum | Elevated IFN-γ, IL-10, IL-12, IL-15, IL-17, IL-23, IL-28, TNF-α, and IL-18 | Increased lymphocytes, neutrophils, eosinophils, and monocytes | | [12] |
| | 228 cases | Serum | Elevated IL-6, decreased IL-8 and TGF-β | Accumulation of monocytes/macrophages and neutrophils | | [14] |
| | 61 cases: initial stage, peak stage, remission, recovery stage / 44 Healthy control | Serum | Elevated IL-6, IL-8, TNF-α, IL-16, TGF-β1, decreased IL-18 | | The mean concentration of IL-13 gradually decreased from initial stage to recovery. | [25] |

(continued on next page)
| Disease                     | Case/Control | Sample                           | Finding                                                                 | Immune cells | Comment                                                                 | Ref |
|----------------------------|--------------|----------------------------------|-------------------------------------------------------------------------|--------------|-------------------------------------------------------------------------|-----|
| Influenza                  | –            | HMC                              | IL-1β mRNA expression was induced by influenza A and Sendai viruses.   | –            | Virus induced IL-1β and IL-18 expression and activation is related to cellular differentiation and caspase-1-dependent pathway. | [19]|
|                            | 19 cases     | Nasal lavage fluid, plasma, serum | Elevated IL-6, IL-8, IFN-α                                             | –            | –                                                                       | [26]|
|                            | –            | Human primary alveolar and bronchial epithelial cells | IP-10, IFN-β, RANTES, IL-6                                             | –            | –                                                                       | [27]|
|                            | 77/17        | Nasal lavage fluid               | Lower IL-6                                                             | Lower monocyte counts | Pro-inflammatory cytokines levels were not elevated in patients with pneumonia. IL-10 levels predict ICU mortality. | [20]|
| ARDS                       | 51 cases at the time of ECMO installation/ 6 h later 300/300 | Plasma                      | Elevated IL-10 and IL-8 levels                                         | Higher Treg, CD14 + CD16+, CD14 + TLR4 + cell counts in survivors | –                                                                       | [28]|
|                            | 77/17        | Plasma                           | Higher TNF-α, IL-6 levels in patients                                  | –            | –                                                                       | [29]|
| Pneumonia                  | 15 severe/15 non-severe CAP | Blood                            | Elevated IL-6, IL-10, IL-8, CRP levels                                 | –            | IL-6 sharp decrease was associated with response to empirical antibacterial treatment by day 3. | [30]|
| Septic shock               | Endotoxin-stimulated septic monocytes/normal monocytes | Serum                      | Elevated IL-10, attenuated TNF-α in septic serum                       | –            | The persistent release of IL-10 leads to impaired proinflammatory cytokine release and the immune dysfunction in septic shock. | [31]|
|                            | 16 septic shock/ 11 circulatory shock         | Plasma                          | More increased IL-10 in septic shock cases                              | –            | The production of the IL-10 positively correlates with the intensity of the inflammatory response in septic shock. | [32]|
| Febrile illness            | 464 cases/431 survived/ 33 dead                | Plasma                          | Higher IL-10 and lower TNFα in patients who died                      | –            | IL-10 to TNFα ratio was associated with mortality of CAI.               | [33]|

Severe acute respiratory syndrome coronavirus 2, Neutrophil-to-CD8 + T cell ratio (N8R), 2019 novel coronavirus pneumonia (NCP), Human Macrophage Cell (HMC), human monocyte-derived macrophages (MDMs), human monocyte-derived dendritic cells (DCs), SARS sera antibody (Ab positive), cerebrospinal fluid (CSF), Acute respiratory distress syndrome (ARDS), extracorporeal membrane oxygenation (ECMO), community-acquired pneumonia (CAP), community-acquired infection (CAI).
The significant increase in plasma IL-1β concentrations in SARS patients have also implied the presence of a selective caspase-1-dependent route in induction of macrophages that are infected with this virus [13]. Animal studies have previously highlighted the role of CD4+ T cells in the inhibition of immune-mediated interstitial pneumonitis, enhancement of SARS-CoV elimination from the lungs, production of neutralizing antibody and cytokines and recruitment of lymphocytes to the lung [10]. However, there is no comprehensive data about the role of these cells in the course of SARS-CoV-2 infection. Yet, as noted by most of studies, the level of lymphopenia and upsurge of proinflammatory cytokines are determinants of severe SARS-CoV-2 infection [8]. Lymphopenia in severe cases include both CD4+ T and CD8+ T subsets [7]. Meanwhile, elevated levels of IL-6 and IL-10 has been suggested as factors that predict severe course of the disorder [7].

Taken together, the data presented above show some levels of similarity in the levels of proinflammatory cytokines particularly IL-6 and IL-10 as well as T cell subsets among different coronaviruses which indicate the role of these cytokines in the pathogenesis of infection-related complications. Future studies are needed to find the practical modalities to these abated responses and improve the clinical outcomes.

Declaring of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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