



Early Differences in Cytokine Production by Severity of Coronavirus Disease 2019

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(課程博士関係)

学位論文の内容要旨

Early Differences in Cytokine Production by Severity of Coronavirus Disease 2019

新型コロナウイルス感染症 COVID-19 の感染急性期
に産生されるサイトカインの重症度による違い

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LIDYA HANDAYANI TJAN

リディア ハンドヤニ チャン

1. Introduction

COVID-19 (Coronavirus Disease 2019) pandemic has affected many aspects of our daily lives. Clinical spectrum of COVID-19 ranges from an asymptomatic status to fatal infections. It has been shown that several components of the immune response including monocytes, macrophages, dendritic cells and natural killer cells, are dysregulated in patients with COVID-19 who have severe symptoms. However, the immune response features of asymptomatic and mild COVID-19 are little known. It is important to determine how patients with asymptomatic or mild COVID-19 react to SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) infection and suppress the spread of the virus. Because innate immunity is important for the host's evasion from the first virus attack, it may play a role in the pathogenesis of asymptomatic or mild COVID-19. We therefore focused on the acute phase of SARS-CoV-2 infection to gain insights into differences among the cytokines induced by the innate immune response in patients with COVID-19 of differing severities, because different cytokines may affect the features of the disease.

2. Materials and Methods

We collected blood samples from 95 COVID-19 patients with different disease severities (16 asymptomatic, 49 mild, 11 moderate, and 19 severe) during the early phase of infection (ie, < 10 days after symptom onset or after an asymptomatic patient's notifiable contact with a positive case patient). Serum samples were subjected to the measurements of cytokines, chemokines, and growth factors with the Bio-Plex Pro Human Cytokine Screening 48-plex panel (Bio-Rad), following the manufacturer's instructions, and results were read using the Bio-Plex 200 system.

3. Results and Discussion

Interleukin 12 and 2 levels were induced significantly higher in patients with asymptomatic or mild disease than in those with moderate or severe disease

Levels of cytokines, chemokines, and growth factors were measured in the 115 blood samples from patients with COVID-19 and healthy controls. Interleukin 12 (IL-12) and IL-2 levels were significantly higher in both the asymptomatic and mild disease groups than in the moderate and severe groups, but these levels were comparable between the moderate and severe disease groups and healthy controls.

IL-12 is secreted by dendritic cells and macrophages in response to microbial stimuli—including virus infection—and it acts on the IL-12 receptor, expressed mainly by activated T and NK cells. IL-12, together with IL-15, IL-18, and type I IFN, enhances the cytotoxic activity of NK cells and induces secretion of IFN- γ . IFN- γ secreted by NK cells activates macrophages to destroy phagocytosed microbes. IL-12 is also known as a key inducer of T-helper 1 cell differentiation. Interestingly, several studies showed that the numbers of peripheral NK cells were significantly lower in patients with severe COVID-19 than in healthy individuals or patients with mild COVID-19. Taken together it is possible that induction of IL-12 is required to maintain NK cell numbers in the early

phase of SARS-CoV-2 infection, and that this induction may play a role in evasion from virus spreading, as seen in asymptomatic patients and those with mild symptoms.

IL-2 is produced by CD4+ and CD8+ T cells, some B cells, and dendritic cells; its major function is to promote the proliferation of both CD4+ and CD8+ T cells. Importantly, IL-2 is also known as a growth factor for NK cells; it promotes the production of NK-derived cytokines and has a synergistic effect with IL-18 to enhance the cytotoxicity and expansion of NK cells. Based on these findings together, it seems reasonable that a higher induction of IL-2 was observed in our asymptomatic and mild COVID-19 groups compared with the moderate and severe COVID-19 groups in the acute phase, and several studies have consistently observed higher NK cell counts in patients with mild COVID-19 than in those with severe disease. However, further study is required to understand the correlation between the induction of IL-2 and NK cell numbers in patients with COVID-19.

Younger persons infected with SARS-CoV-2 are more likely to have asymptomatic or mild infection than older persons. Patients with asymptomatic or mild COVID-19 in our study were also significantly younger than those with severe disease. However, we could not see any correlation between age of the subjects and either serum IL-12 or IL-2 levels in this study. This analysis would support a possible protective role of early induction of IL-12 and IL-2 in the severity of COVID-19.

Interleukin 18 and 6 were induced higher in patients with symptomatic COVID-19 than in asymptomatic, and its level increased in accordance with disease severity

Our evaluation of other cytokines in patients with COVID-19 of differing severity revealed that the following cytokines were significantly higher in patients with COVID-19 than in healthy controls but did not differ by severity group: interleukin 15, 2Ra, 1Ra, 7, 10, 13, 1a, and 16, interferon (IFN) γ , monocyte chemoattractant protein 1, platelet-derived growth factor BB, and tumor necrosis factor α .

A different pattern was observed in serum interleukin 18 (IL-18) and IL-6 levels, which were significantly higher in the patients with symptomatic COVID-19 than in asymptomatic patients and healthy controls. They also increased in accordance with disease severity, although they were comparable in the asymptomatic and mild disease groups.

IL-18, known as an IFN- γ -inducing factor, is involved in the activation of NK cells, T-helper 1 and 2 cells, and macrophages. Its precursor, which is cleaved by caspase 1 to become biologically active IL-18, is constitutively expressed in monocytes, macrophages, dendritic cells, and endothelial cells. IL-18 has also been shown to have a very important role in acute respiratory distress syndrome (ARDS), a feature of severe COVID-19. High expression of IL-18 in our patients with severe COVID-19 and the common occurrence of ARDS in patients with severe COVID-19 seem correlated, through an underlying mechanism that must be explored further.

It has been reported that serum IL-6 levels were significantly lower in severe or critical COVID-19 than in other critical diseases (sepsis, cytokine release syndrome, and ARDS unrelated to COVID-19), suggesting that factors other than cytokine storm—including endovascularitis, direct viral injury and lymphodepletion, and virus-induced

immunosuppression—might be responsible for organ dysfunction in COVID-19. The other report showed that the endothelial trans-signaling of IL-6 induces the production of plasminogen activator inhibitor 1 in vascular endothelial cells, further explaining the endotheliopathy and coagulopathy that commonly occur in patients with severe COVID-19. Recent studies and our present results indicate that the induction of IL-6 may play a role in the severity of COVID-19.

4. Conclusion

In the present study, we investigated the difference among the cytokines induced by the innate immune response in patients with COVID-19 of differing severities. We were the first to report that during the acute phase of infection, IL-12 and IL-2 were induced significantly higher in patients with asymptomatic or mild disease than in those with moderate or severe disease. IL-12 and IL-2 are among cytokines of innate immunity which have important function to enhance cytotoxicity of NK cells. In addition, several reports have shown that the number of NK cells were significantly higher in patients with mild COVID-19 than in those with severe disease. Based on these findings together, it is possible that induction of IL-12 and or IL-2 are required to maintain NK cell numbers in the early phase of SARS-CoV-2 infection, and that this induction may play a role in evasion from virus spreading, as seen in asymptomatic patients and those with mild symptoms.

論文審査の結果の要旨			
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論文題目 Title of Dissertation	Early Differences in Cytokine Production by Severity of Coronavirus Disease 2019 新型コロナウイルス感染症 COVID-19 の感染急性期に産生さ れるサイトカインの重症度による違い		
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	Vice-examiner	古屋敷 智之	
副 査			
	Vice-examiner		

(要旨は1, 000字~2, 000字程度)

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The candidate, having completed studies on SARS-CoV-2 and COVID-19, with a specialty in the early differences in cytokine production by severity of COVID-19, and having advanced the field of knowledge in the area of clinical virology, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).