Cytomegalovirus antigen-specific T cell immune responses in patients with autoimmune diseases under different cytomegalovirus infection status

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To the Editor: Patients with autoimmune diseases have increased risk of active cytomegalovirus (CMV) infection due to immune dysfunction or the application of glucocorticoid and immunosuppressants. Cui et al. found that compared with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (27%), post-transplant patients (14.8%), and hematological malignancies (5.1%), patients with autoimmune disease had the highest incidence of CMV antigenemia (35.1%). Monitoring CMV antigen-specific T cell immunity is helpful for understanding the protective immunity against CMV infection and identifying the risk of CMV-related complications. Currently, the CMV antigen-specific T cell immune responses in patients with autoimmune diseases remains unknown.

T-SPOT.CMV kit (Oxford Immunotec, Abingdon, UK), based on enzyme-linked immunospot assay, is a rapid and high throughput method for detecting the functional CMV antigen-specific T cells by measuring the secretion of interferon-gamma (IFN-γ) stimulated by CMV specific antigen—phosphoprotein 65 (pp65) and immediate early protein-1 (IE-1). In our study, CMV antigen-specific T cell immune responses in patients with autoimmune diseases under different CMV infection status were investigated by T-SPOT.CMV kit. This study was approved by the institutional Ethics Committee of Peking Union Medical College Hospital (No. ZS-1412). All subjects enrolled in this study had signed the informed consent forms.

Patients with autoimmune disease were enrolled from Peking Union Medical Hospital between May 1, 2017 and September 30, 2020. For latent CMV infection group, the inclusion criteria included: (1) 18 to 80 years old; (2) CMV-viremia; (3) CMV DNA, pp65, and CMV-immunoglobulin negative; and (4) no clinical symptoms related to CMV infection. The active CMV infection group included patients with CMV viremia and patients with CMV disease. The inclusion criteria of CMV viremia group included: (1) 18 to 80 years old; (2) peripheral blood CMV DNA or pp65 positive; and (3) with no CMV infection related clinical symptoms. The inclusion criteria of CMV disease group included: (1) 18 to 80 years old; (2) CMV DNA or pp65 positive; and (3) had clinical symptoms associated with CMV infection (fever, hemocytopenia, or tissue invasive disease). Exclusion criteria of all groups included: (1) pregnancy or lactation and (2) HIV antibody positive.

A total of 50 patients with latent CMV infection and 50 patients with active CMV infection were enrolled. In active CMV infection group, 26 cases were diagnosed with CMV viremia, and 24 cases were diagnosed with CMV...
disease. There were no significant differences in age and gender distribution among the latent CMV infection, CMV viremia, and CMV disease group. Further patients’ characteristics were shown in Supplementary Table 1, http://links.lww.com/CM9/B344.

As shown in Figures 1A and 1B, the median frequencies of IFN-γ secreting T cells stimulated either by IE-1 or pp65 in active CMV infection group were significantly lower than that in latent CMV infection group (P < 0.001 and P < 0.001). Further, after being stimulated by IE-1, the median frequency of IFN-γ-secreting T cells in CMV disease group was significantly lower than that in latent CMV infection group (P < 0.001) and CMV viremia group (P = 0.015); the median frequency of IFN-γ-secreting T cells in CMV viremia group was significantly lower than that in latent CMV infection group (P < 0.001) and CMV viremia group (P < 0.001).

Moreover, the effect of lymphocyte counts on CMV antigen-specific T cell immune responses were analyzed. Both the age, gender distribution, and CMV infection status between different groups of CD4+ T cells count (200 cells/μL as the threshold) and between different groups of CD8+ T cells count (250 cells/μL as the threshold) were comparable. After stimulated by pp65, the median frequency of IFN-γ secreting T cells in group with CD4+ T cells count ≤200 cells/μL was significantly lower than that in group with CD4+ T cells count >200 cells/μL (P = 0.043); and the median frequency of IFN-γ secreting T cells in group with CD8+ T cells count ≤250 cells/μL was significantly lower than that in group with CD8+ T cells count >250 cells/μL (P = 0.03).

Consistent with the previous studies in transplant recipients,[4] our study found the CMV antigen-specific T cell immune responses were weakened in autoimmune diseases with active CMV infection including CMV viremia and CMV disease. Dynamic monitoring of CMV antigen-specific T cell immune responses in

![Figure 1: (A) Comparison of CMV specific T cell immune responses between latent CMV infection and active CMV infection group stimulated by IE-1 or pp65. (B) Comparison of CMV specific T cell immune responses between latent CMV infection, CMV viremia, and CMV disease group stimulated by IE-1 or pp65. CMV: Cytomegalovirus; IE-1: Immediate early protein-1; PBMC: Peripheral blood monocyte cell; pp65: Phosphoprotein 65; SFC: Spot forming cell.](image-url)
transplant recipients revealed that the frequencies of IFN-γ-secreting T cells stimulated by pp65 or IE-1 were significantly reduced after active CMV infection, suggesting that the occurrence of active CMV infection might be related to the insufficient T cell immunity against CMV. CD8+ T cells and CD4+ T cells are essential for the control of CMV infection. Qin et al[6] found that the decrease of lymphocytes, CD4+ T cells and CD8+ T cells count might be related to CMV disease among patients with systemic lupus erythematosus. Our data further revealed that the CD4+ T cells count and CD8+ T cells count might affect CMV antigen-specific T cell immune responses stimulated by pp65.

Monitoring CMV antigen-specific T cell immunity is conductive to identify the risk of CMV-related complications. Our study used the standardized T-SPOT.CMV method to evaluate CMV antigen-specific T cell immune responses in patients with autoimmune diseases under different CMV infection status. However, our study also had some limitations. First, small sample size limited the analysis of factors affecting the CMV antigen-specific T cell immune response. Second, our study could not infer the causal relationship between CMV antigen-specific T cell immune responses and the occurrence of active CMV infection. Further prospective studies are warranted in the future.

In conclusion, in patients with autoimmune diseases, lower CMV antigen-specific T-cell immune response was related to active CMV infection including either CMV viremia or CMV disease. CD4+ T cells and CD8+ T cells count might affect the CMV antigen-specific T cell immune responses.

**Conflicts of interest**

None.

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