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

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Abstract

Background: T-cell immunity is important for the control of cytomegalovirus (CMV) infection. The frequency of IFN-γ secreting T cells after stimulation with CMV-specific protein-1 (IE-1) and phosphoprotein 65 (pp65) antigen can help predict the risk of active CMV infection. Patients with autoimmune diseases have a high incidence of active CMV infection, but the CMV antigen-specific T cell immune response of this population is still blank in the world. This study aimed to use T-SPOT.CMV to investigate CMV antigen-specific T cell immune response in patients with autoimmune diseases under different CMV infection conditions.

Methods: Patients with autoimmune diseases in the Peking Union Medical College Hospital from March, 2017 to October, 2020 were continuously selected. According to the definition, the subjects were divided into latent CMV infection group and active CMV infection group. T-SPOT.CMV was used to evaluate CMV antigen-specific T cell immune response under different CMV infection status, and the possible influential factors of CMV antigen-specific T cell immune response were further analyzed.

Results: Fifty patients with latent CMV infection and fifty patients with active CMV infection were enrolled. After stimulated by immediate early IE-1 and pp65 antigen, the median frequency of IFN-γ secreting T cells in active CMV infection group were all significantly lower than that in latent CMV infection group ($p<0.001$), and in CMV disease group was significantly lower than that in latent CMV infection group ($p<0.001$). After stimulated by pp65, the median frequency of IFN-γ secreting T cells with CD4+ T cell counts < 200/ul was significantly lower than that of CD4+ T cell counts ≥ 200/ul ($p=0.043$), and those with CD8+ T cell counts < 250/ul was significantly lower than that of CD8+ T cell counts ≥ 250/ul ($p=0.03$). The frequency of IFN-γ secreting T cells stimulated by pp65 was significantly higher than IE-1.

Conclusions: In patients with autoimmune diseases, the CMV antigen-specific T-cell immune response in patients with active CMV infection was significantly lower than that with latent CMV infection. IE-1 was considered as a more stable antigen with better effect than pp65. Lymphocyte, CD4+T cell and CD8+T cell count might affect CMV antigen-specific T cell immune response.

Background

Cytomegalovirus (CMV) is prevalent worldwide. It was estimated that the serum positive rate of CMV in adults worldwide is about 40%-100% [1], while in China, the seropositive rate in adults was as high as 90% [2]. CMV cannot be completely eliminated by the immune response after primary infection. It will remain latent lifelong, and can be activated under certain conditions to cause recurrent infection. CMV infection is usually asymptomatic, or only presents with mononucleosis syndrome such as fever, sore throat, fatigue. However, it can cause damage to the lung, liver, gastrointestinal tract, bone marrow and retina, and even lead to severe organ specific complications, increasing mortality in immunosuppressive patients [3-5].

T cell immunity is considered essential to control CMV replication and prevent the progression to CMV disease [6]. The replication and activation of CMV can be effectively controlled by T cell immunity in a host with normal immune function. However in individuals with impaired immune function, the decrease in the number and function of CMV antigen-specific T Cell is associated with severe clinical complications caused by uncontrolled virus replication [7, 8]. Monitoring CMV antigen-specific T cell immunity is helpful for understanding the protective immunity against CMV infection and identifing the risk of CMV-related clinical complications.

It was estimated that human Cytomegalovirus (HCMV) had more than 200 open reading frames encoding proteins [9]. In latent CMV infection, 231 HCMV Open Reading Frame (QRF) encoded polypeptides were used as antigens to analyze interferon-gamma (IFN-γ) production of specific CD8 + T cells and CD4 + T cells stimulated by different antigens. The results showed that both UL83-coded pp65 and UL123-coded IE-1 can stimulate the immune response of specific CD8 + T cells and specific CD4 + T cells, and release IFN-γ. Compared with other antigens, pp65 and IE-1 had better immunogenic advantages [10].

T- SPOT.CMV is a rapid and high throughput method for detecting CMV antigen-specific T cell immunoassay. The basic principle involved in it is to detect the presence of functional CMV antigen-specific T cells by measuring the production of IFN-γ stimulated by CMV specific antigen [11]. Currently, this method is widely used in the field of transplantation with great application prospects in predicting active CMV infection and guiding preventive antiviral therapy [3, 12]. Studies have reported that the low CMV antigen-specific T cell immune response before and after transplantation is related to the high risk of active CMV infection [13-16].

A previous research conducted by our group revealed the methodology for detecting CMV antigen-specific T cell immunity for the first time in China, and found that the proportion of autoimmune disease patients in CMV disease group was very high [17]. It has been reported that patients with autoimmune diseases are at increased risk of active CMV infection due to the immune dysfunction or the application of glucocorticoid and immunosuppressants [18]. Cui et al. [19] have explored the positive rate of pp65 antigen test in patients with autoimmune diseases and non-autoimmune diseases in the Peking Union Medical College Hospital from 2008 to 2018. They found that the incidence of CMV antigenemia from high to low was as follows: autoimmune disease (35.1%) > HIV/AIDS (27%) > post-transplant patients (14.8%) > hematological malignancies (5.1%). This suggests that the risk of CMV antigenemia in patients with autoimmune diseases might be higher than that in patients of non-autoimmune diseases with low immune function.

Currently, the immune response of CMV antigen-specific T cells in autoimmune patients still remains blank. Hence, in this study, CMV antigen-specific T-cell immune response performed by T-SPOT.CMV in patients with autoimmune diseases under different CMV infection states was compared , and the possible influential factors of CMV antigen-specific T-cell immune response werd analyzed.

Materials And Methods

Study Design

Patients with autoimmune diseases who were hospitalized in the Peking Union Medical College Hospital from May 1, 2017 to September 30, 2020 and volunteered to participate in this study were selected. The inclusion criteria of patients in the latent CMV infection group were as follows: 1) 18-80 years old; 2) CMV-IgG positive; 3) CMV DNA, pp65 and CMV-IgM negative; and 4) no clinical symptoms related to CMV infection. The active CMV infection group included patients with subclinical CMV infection and CMV disease. The inclusion criteria of patients in subclinical CMV infection group are as follows: 1) 18-80 years old; 2) CMV DNA or pp65 positive; and 3) with no CMV infection related clinical symptoms. The inclusion criteria of CMV disease group are: 1) 18-80 years old; 2) CMV DNA or pp65 positive; and 3) have clinical symptoms associated with CMV infection, including viral syndrome (fever, hemocytopenia) or tissue invasive disease, except for other causes. Exclusion criteria of patients include: 1) pregnancy or lactation; and 2) HIV antibody positive.

CMV DNA positive is defined as the detection of viral load ≥ 500 copies/ml by real-time fluorescent quantitative polymerase chain reaction. CMV pp65 positive is defined as WBC count of peripheral blood with positive CMV pp65 antigen ≥ 1 WBC/2*10^5 WBC by indirect immunofluorescence method.

This study was approved by the institutional ethics committee of Peking Union Medical College Hospital (ZS-1412). We confirmed that all methods were performed in accordance with the relevant guidelines and regulations. All subjects have voluntarily participated in the study and signed the informed consent form.

T-SPOT.CMV Assay

Four milliliter of peripheral blood, anticoagulated with heparin, was collected from each participant. Peripheral blood mononuclear cells (PBMC) were isolated by gradient centrifugation within four hours. The AIM-V medium (Gibco™AIM V Medium liquid, Invitrogen, USA) was used to prepare a cell suspension with a concentration of 2.5×10^6 PBMCs/ml. 96 wells plate rack to restore to room temperature; AIM-V as a negative control, 5 μg/ml PHA as a positive control, and IE-1 and PP65 (final concentration of each peptide was 10 μg/ml) as specific antigens. Plates were incubated for 18–20 h at 37 °C in 5% carbon dioxide and then washed with washing buffer. After incubation, the detection antibodies (anti-IFN-γ-FITC) were added to each well and incubated for 2 h at room temperature. Then streptavidine red-conjugate and anti-FITC green were added and another 1 h of incubation was needed. Finally, fluorescence enhancer was loaded to make spots visible under the automated ELISPOT reader (AID iSpot, Strassberg, Germany). The specific T cells of IFN-γ in each reaction well were counted by ELISPOT reader. The background spot forming cells (SFC) in negative control well and positive control well should be ≤ 10 spots and ≥ 20 spots respectively. Otherwise, it will be regarded as an uncertain result.

Statistical analysis

Statistical analyses were performed using SPSS 21.0. The Kolmogorov-Smirnov test was used to examine whether the measurement data conformed to the normal distribution. Normally distributed

variables are denoted as means ± standard deviation (SD), abnormally distributed variables are denoted as median and interquartile range (IQR), and categorical variables are expressed as proportion (%). The group t-test or analysis of variance was used to compare the continuous variables that conformed to the normal distribution, and non-parametric rank sum test was used to compare continuous variables that were non-normally distributed. Chi-square test or Kruskal-Wallis rank sum test was used to compare the count data between the groups. $p < 0.05$ was considered significant.

Results

Demographic and clinical features of participants

A total of fifty patients with latent CMV infection and fify patients with active CMV infection were included. In the active CMV infection group, 26 cases were diagnosed with subclinical CMV infection, and 24 cases were diagnosed with CMV disease. The clinical manifestations of CMV disease included thrombocytopenia in 9 cases, fever in 9 cases, hepatitis in 3 cases and CMV pneumonia in 7 cases. The demographic and clinical features of participants are shown in Table 1.

CMV antigen-specific T cell immune response under different CMV infection states

The spots of T-SPOT.CMV in patients with latent CMV infection, subclinical CMV infection and CMV disease were shown in Figure 1. The number of spots in every negative control wells of all patients was 0 SFCs/2.5x10^5 PBMCs, and the number of spots exceeded 20 SFCs/2.5x10^5 PBMCs in the positive control wells.

Firstly, the CMV antigen-specific T cell immune response was compared between the latent CMV infection group and the active CMV infection group. As shown in Figure 2, after stimulated by IE-1 and pp65, the frequency of IFN-γ secreting T cells in active CMV infection group was significantly lower than that in latent CMV infection group [36 (IQR 9-75) vs 177 (IQR 57-353) SFCs/2.5*10^5 PBMCs, P < 0.001 and 137 (IQR 56-403) vs 420 (IQR 191-625) SFCs/2.5*10^5 PBMCs, p< 0.001]. By adding the number of spots stimulated by IE-1 and pp65 antigens, the frequency of T cells secreting IFN-γ in the active CMV infection group was significantly lower than that in the latent CMV infection group [199 (IQR 98-456) vs 642 (IQR 318-795) SFCs/2.5*10^5 PBMCs, p<0.001].

Furthermore, the CMV antigen-specific T cell immune response of latent CMV infection group, subclinical CMV infection group and CMV disease group was compared. The frequencies of IFN-γ-secreting T cells in each group were shown in Table 2. As shown in Figure 3, the frequencies of IFN-γ-secreting T cells after stimulation with IE-1 in the CMV disease group were significantly lower than that in the latent CMV infection group (p<0.001) and subclinical CMV infection group (p=0.015). The frequencies of IFN-γ-secreting T cells after stimulation with IE-1 in the subclinical CMV infection group was significantly lower than that in the latent CMV infection group (p=0.001). After stimulated by pp65 antigen, the frequencies of IFN-γ-secreting T cells in the CMV disease group were significantly lower than that in the latent CMV infection group (p<0.001) and subclinical CMV infection group (p<0.001). By adding the number of spots

stimulated by IE-1 and pp65 antigens, the frequency of IFN-γ-secreting T cells in the CMV disease group was significantly lower than that in the latent CMV infection group ($p<0.001$) and subclinical CMV infection group ($p<0.001$); and the frequency of IFN-γ-secreting T cells in the subclinical CMV infection group was significantly lower than that in the latent CMV infection group ($p=0.013$).

As shown in Figure 4, comparison of the frequency of IFN-γ-secreting T cells in the three groups after stimulated by IE-1 or pp65 antigen showed that the frequency of T cells secreting IFN-γ in the three groups after stimulated by pp65 was significantly higher than that by IE- 1.

The influence of lymphocyte counts on CMV antigen-specific T cell immune response

Lymphopenia is considered as a risk factor for various infection including CMV infection [20]. Our study further analyzed the effects of peripheral blood lymphocyte count on CMV antigen-specific T cell immune response. Clinically, when a patient's CD4+ T cell count is less than 200/ul, he would be susceptible to CMV infection [21]. In all patients with CMV infection, the median count of CD8+ T cell was 277/ul. In our study, the cut-off values of CD4+T cell and CD8+T cell count used were 200/ul and 250/ul. As shown in Table 3, after stimulated by pp65, the median frequency of IFN-γ secreting T cells with CD4+T cell counts less than 200/ul was significantly lower than that with CD4+T cell counts greater than or equal to 200/ul ($p= 0.043$); and the median frequency of IFN-γ secreting T cells with CD8+T cell counts less than 250/ul was significantly lower than that with CD8+T cell counts greater than or equal to 250/ul ($p= 0.030$).

Discussion

CMV infection is one of the most common opportunistic infections in patients with autoimmune diseases. The clinical manifestations of CMV infection are diverse, ranging from viral syndromes such as fever and hemocytopenia to tissue and organ damage of lung, gastrointestinal tract, liver, eyes or other organs. CMV infection can mimic the clinical manifestations of autoimmune diseases, thereby affecting the clinicians' judgment of the disease, bringing greater difficulties to treatment, and severely affecting the prognosis of patients [4, 22, 23]. At present, more and more attention is paid on the screening of CMV infection in patients with autoimmune diseases.

Currently, research on the application of monitoring CMV antigen-specific T cell immunity is mainly implemented in patients with solid organ transplantation and hematopoietic stem cell transplantation. Studies have demonstrated that low CMV antigen-specific T cell immune response was associated with high risk of active CMV infection or CMV disease. Monitoring of CMV specific T cell immunity can assist in predicting the risk of CMV replication and progression to CMV disease, and guide the duration of preventive anti-CMV therapy. This in turn helps in reducing the duration and intensity of CMV monitoring as well as the high cost associated with anti CMV treatment and drug side effects (such as nephrotoxicity, bone marrow suppression, etc.) in patients with low risk of CMV infection [24]. The CMV antigen-specific T cell immune response in patients with autoimmune diseases which are considered as a common immunosuppressive population, still remained to be unclear. The main purpose of this study is useing T-SPOT.CMV method to evaluate the CMV antigen-specific T cell immune response in different

CMV infection states, and to analyze the possible factors that affect the CMV antigen-specific T cell immune response in patients with autoimmune diseases

This study found that the frequency of IFN-γ-secreting T-cells stimulated by IE-1, pp65 and IE-1 & pp65 in patients with autoimmune diseases complicated with active CMV infection was significantly lower than those with autoimmune diseases complicated with latent CMV infection, and in patients with autoimmune diseases complicated with CMV disease was significantly lower than those with autoimmune diseases complicated with latent CMV infection. The results of the previous studies with regard to solid organ transplantation and hematopoietic stem cell transplantation recipients have shown that under the occurrence of CMV viremia or active CMV infection including CMV viremia and CMV disease, CMV antigen-specific T cell immune response stimulated by IE-1 and/or pp65 antigen was significantly lower than that of latent CMV infected patients before and after transplantation. This suggested that low CMV antigen-specific T cell immune response is related to the occurrence of active CMV infection [12, 15, 25-27]. In the prospective study conducted on dynamic monitoring of CMV antigen-specific T cell immune response in solid organ transplantation and hematopoietic stem cell transplantation recipients, the frequency of IFN-γ-secreting T-cells stimulated by pp65 and IE-1 showed no change or increase in latent CMV infected patients before and after transplantation, while the frequency of IFN-γ-secreting T cells stimulated by pp65 and IE-1 antigens was significantly reduced in active CMV infected patients [13, 14], suggesting that the occurrence of active CMV infection might be related to insufficient T cell immune response to CMV. This study illuminated that in autoimmune patients, the CMV antigen-specific T cell immune response of active CMV infection and CMV disease was lower than that of latent CMV infection, and this was consistent with that of the researches conducted in transplant patients.

T lymphocytes play an important role in the initiation and continuation of immune mechanism, and are considered as the body's main defensive cells against pathogens. Previous studies have found that 70%-80% of circulatory lymphocytes come from T lymphocytes, and the reduction in the number of circulatory lymphocytes affects the body's normal immune defense function [28]. For viral infections, CD8+ T cells are shown to be as the main effector cells, and CD4+ T cells play an important role in establishing the body’s long-term immune control of CMV infection. CD4 + T cells and CD8 + T cells play a synergistic role in the process of CMV infection control. Qin et al[29]. have analyzed the relationship between lymphocyte subsets and CMV infection status in 125 SLE patients, and the results showed that the lymphocytes, CD4+ T cell and CD8+ T cell count in the CMV disease group were significantly lower in the CMV viremia group and the inactive CMV infection group, but there was no significant difference in the CMV viremia group and the inactive CMV infection group. This indicated that the decrease of lymphocytes, CD4+T cell and CD8+T cell count might be related to the occurrence of CMV disease [29]. Similar to the results of the study by Qin, this study also showed that the lymphocyte count of CMV disease patients was significantly lower than that of subclinical CMV infection group and latent CMV infection group, but there was no significant difference between subclinical CMV infection group and latent CMV infection group, confirming that the decrease in lymphocyte count might be related to the occurrence of CMV disease. However, there was no significant difference in the number of CD4+T cells

and CD8+T cells among the three groups, and this might be related to the fact that the number of lymphocyte subsets detected in the enrolled patients was less (n = 47). Due to immune dysfunction associated with autoimmune diseases and the application of immunosuppressive therapy, the changes in lymphocyte subsets in this population remained much complicated. With regard to the effect of lymphocyte subsets on CMV antigen-specific T cell immune response, there are currently no studies on this aspect both at home and abroad, and large sample studies are necessary for furtherexploration.

Consistent with the study results of Liu [17], the frequency of IFN-γ-secreting T-cells after stimulation with PP65 was significantly higher than that after stimulation with IE-1 antigen, and the stimulatory effect of PP65 was better than that of IE-1. In addition, this study also found that different lymphocyte levels affected the number of IFN-γ-secreting T-cells after stimulation with PP65, while IE-1 antigen was unaffected by the lymphocyte levels. IE-1 was shown to be more stable and had better effect than pp65.

One of the basic goals of CMV antigen-specific T cell immunosurveillance is to stratify the risk of CMV-related complications (CMV disease) in order to improve the clinical management of patients. This study is first to use the standardized T-SPOT.CMV method to evaluate CMV antigen-specific T cell immune response in patients with autoimmune diseases under different CMV infection conditions in the global. It has an important clinical significance in predicting the value of CMV infection and CMV disease in patients with autoimmune diseases. However, this study also has some limitations. Firstly, although the underlying diseases of all patients are autoimmune diseases that require hormone or immunosuppressive therapy, there are certain similarities in the underlying diseases, leading to differences in the underlying diseases of different groups. Secondly, the small sample size limits the analysis of factors affecting the CMV antigen-specific T cell immune response in patients with autoimmune diseases. Finally, this study cannot infer the causal relationship between CMV antigen-specific T cell immune response and the occurrence of active CMV infection, so the further prospective exploration is warranted in the future.

Conclusion

In patients with autoimmune diseases, CMV antigen-specific T cell immune responses were different under different CMV infection states. The CMV antigen-specific T cell immune response in patients with active CMV infection and CMV disease was significantly lower than that with latent CMV infection. IE-1 was considered as a more stable antigen with better effect than pp65. Lymphocyte, CD4+T cell and CD8+T cell counts might affect CMV antigen-specific T cell immune response.

Abbreviations

CMV: Cytomegalovirus; IFN-γ: Interferon-γ; TNF-α: Tumor necrosis factor-α; IL-2: Interleukin-2; ELISPOT: Enzyme-linked immunospot assay; PBMC: Peripheral blood mononuclear cells; pp65: Phosphoprotein 65; IE-1: Immediate early protein-1; RT-PCR: Real time polymerase chain reaction; PHA:Phytohemagglutinin; PBS: Phosphate buffered saline; MHC: Major histocompatibility complex; SFC: Spot forming cell; IQR:

Interquartile range;CD4: Cluster of Differentiation 4 receptors; CD8: Cluster of Differentiation 8 receptors; WBC: White blood cell;

Declarations

Ethics approval and consent to participate

This study was approved by the institutional ethics committee of Peking Union Medical College Hospital (ZS-1412). All subjects have voluntarily participated in the study and signed the informed consent form.

Consent for publication

Not Applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conception and design: L.X.Q, S.X.C, Z.L.F, Z.B.T. Acquisition of the data: Z.W.J, C.J.T, D.Y.L, M.H.M, T.Y.T. Analysis or interpretation of the data: M.H.M, T.Y.T. Drafting of the manuscript: M.H.M. All authors reviewed and approved the final version of the manuscript. All authors had read and approved the manuscript.

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Tables

Table 1. Demographic and characteristics of the study subjects.

Characteristics	latent CMV infection (n=50)	subclinical CMV infection (n=26)	CMV disease (n=24)	P
Age (Mean ± SD)	46 ± 16	45 ± 16	43 ± 16	0.673
Male, n (%	38 (76.0	24 (92.3)	17 (70.8)	0.137
autoimmune disease*, n (%)	50 (100)	26 (100)	24 (100)	/
Hormone used within 3 months, n (%)	47 (94.0)	26 (100.0)	24 (100.0)	0.216
Immunosuppressive used within 3 months, n (%)	39 (78.0)	22 (84.6)	19 (79.2)	0.788
biological agents used within 3 months, n (%)	4 (8.0)	2 (7.7)	2 (8.3)	0.997
Lymphocyte count (/ UL), [median (IQR]	1115 (710-1892)	940 (582-1745)	535 (315-1300)	0.003
CD4 + T cell count (/ UL), [median (IQR]	318 (168-745)	420 (197-545)	155 (86-465)	0.149
CD8 + T cell count (/ UL), [median (IQR]	335 (215-535)	296 (198-807)	254 (152-360)	0.379

Mean ± SD: mean ± standard deviation; median (IQR): median (interquartile interval)

*Autoimmune diseases in latent CMV infection group included: systemic lupus erythematosus (n=16), ANCA associated vasculitis (n=3), dermatomyositis (n=2), rheumatoid arthritis (n=4), IgG4 related diseases (n=2), Behcet syndrome (n=2), systemic sclerosis (n=3), anti synthetase syndrome (n=1), Takayasu arteritis (n=2), systemic vasculitis (n=7), Granulomatous polyangitis (n=2), Sjogren's syndrome (n=1), autoinflammatory disease (n=2), recurrent polychondritis (n=1), Evans syndrome (n=1), connective tissue disease (n=1);

Autoimmune diseases in subclinical CMV infection group included: systemic lupus erythematosus (n=15), dermatomyositis (n=2), polymyositis (n=1), Sjogren's syndrome (n=2), giant cell arteritis (n=1), systemic vasculitis (n=1), rheumatoid arthritis (n=1), antiphospholipid anti body syndrome (n=1), primary biliary cholangitis (n=1) and connective tissue disease (n=1);

Autoimmune diseases in CMV group included: systemic lupus erythematosus (n=14), dermatomyositis (n=3), polymyositis (n=2), inflammatory myopathy (n=1), allergic purpura (n=1), connective tissue disease (n=2), and idiopathic pulmonary fibrosis (n=1).

Table 2. Comparison of CMV specific T cell immune response between latent CMV infection group and active CMV infection group

Antigens	latent CMV infection n=50	active CMV infection n=50
IE-1 [SFCs/2.5*105 PBMCs，Median (IQR)]	177（57-353）	36（9-75）
pp65 [SFCs/2.5*105 PBMCs，Median (IQR)]	420（191-625）	137（56-403）
IE-1+pp65 [SFCs/2.5*105 PBMCs，Median (IQR)]	642（318-795）	199（98-456）

Table 3. Comparison of CMV specific T cell immune responses with different CD4+T cell and CD8+T cell count

Antigens	CD4+T cells<200/UL （n=16）	CD4+T cells≥200/UL （n=31）	P
IE-1 [SFCs/2.5*105 PBMCs，Median (IQR)]	65（28-120）	64（27-269）	0.719
pp65 [SFCs/2.5*105 PBMCs，Median (IQR)]	158（81-293）	428（100-598）	0.043
IE-1+pp65 [SFCs/2.5*105 PBMCs，Median (IQR)]	244（149-377）	512（152-800）	0.080
	CD8+T cells<250/UL （n=15）	CD8+T cells≥250/UL （n=32）	P
IE-1 [SFCs/2.5*105 PBMCs，Median (IQR)]	66（12-180）	62（31-230）	0.673
pp65 [SFCs/2.5*105 PBMCs，Median (IQR)]	129（62-304）	376（105-594）	0.030
IE-1+pp65 [SFCs/2.5*105 PBMCs，Median (IQR)]	232（126-512）	462（160-758）	0.132

Figures

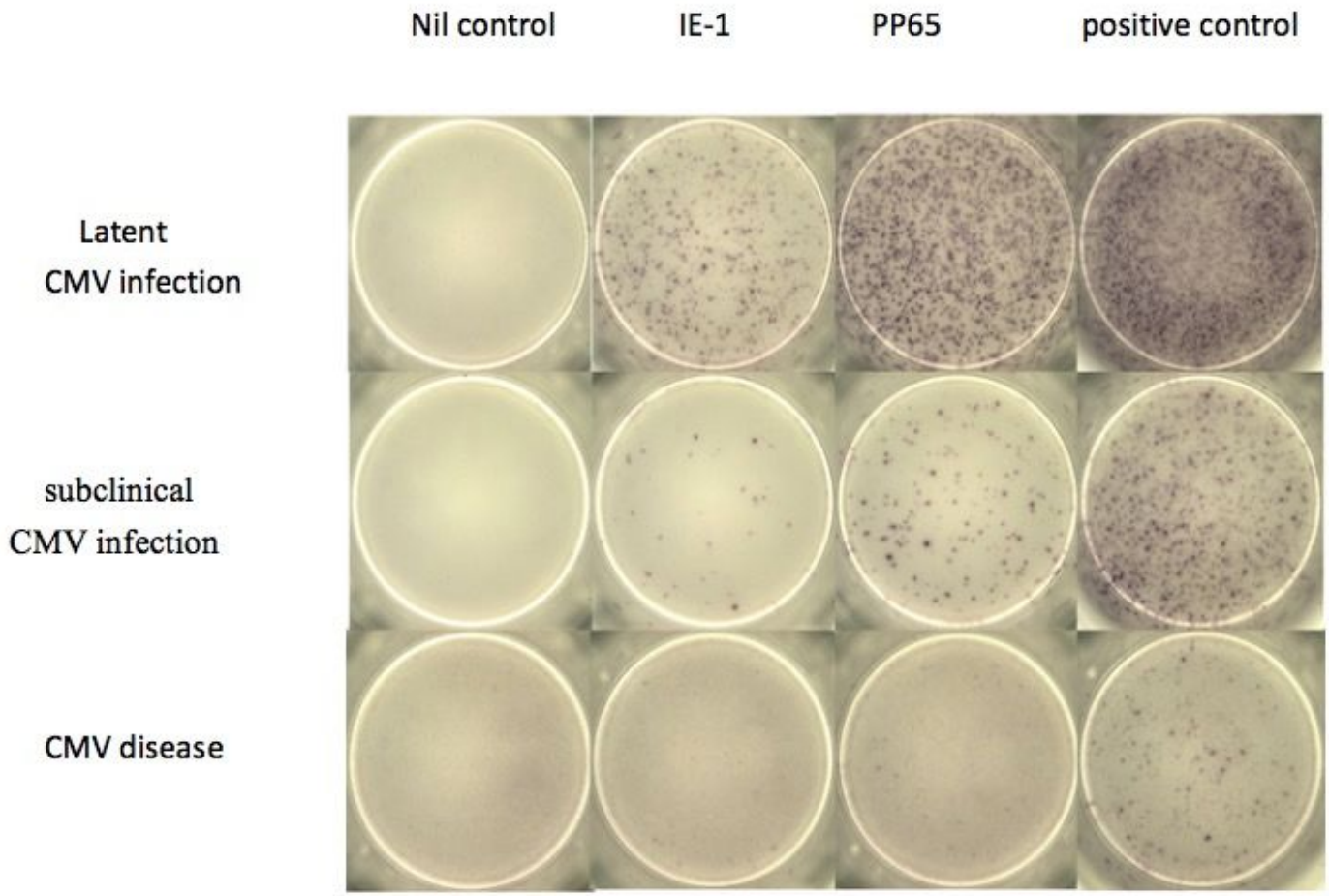

Figure 1

Three groups of patients with T- SPOT.CMV Schematic diagram of results.

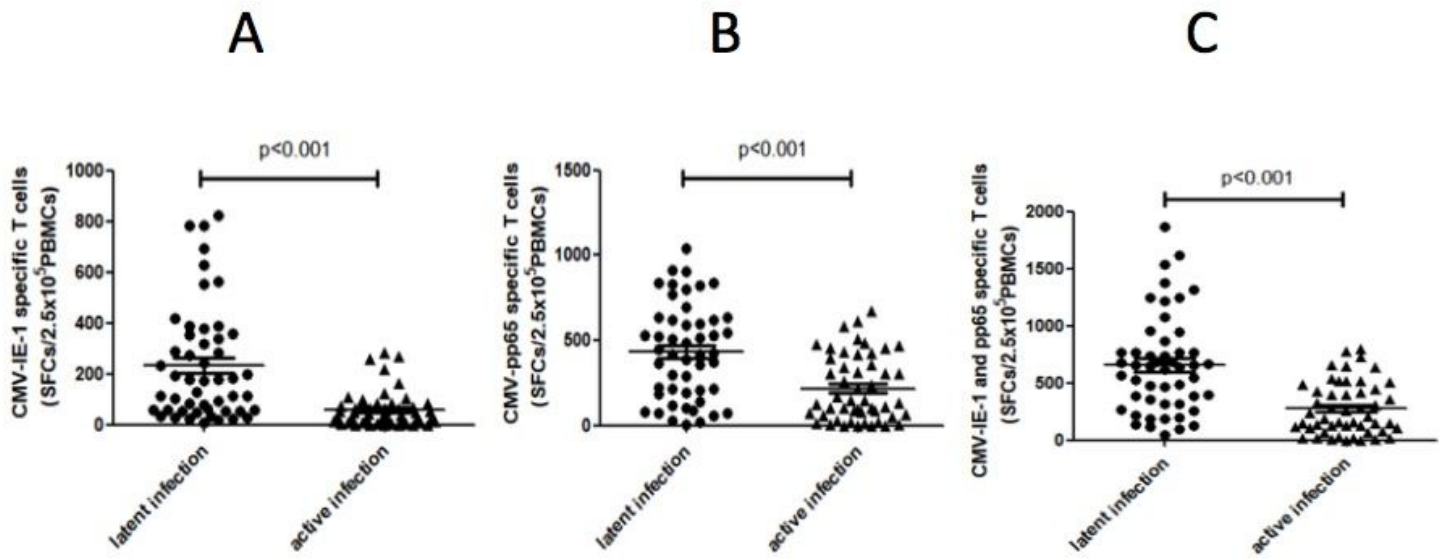

Figure 2

Comparison of CMV specific T cell immune responses between latent CMV infection group and active CMV infection group stimulated by different antigens. (A) Frequency distribution of IFN-γ-secreting T cells stimulated by IE-1. (B) The frequency distribution of IFN-γ-secreting T cells stimulated by pp65. (C) The frequency distribution of IFN-γ-secreting T cells stimulated by IE-1&pp65.

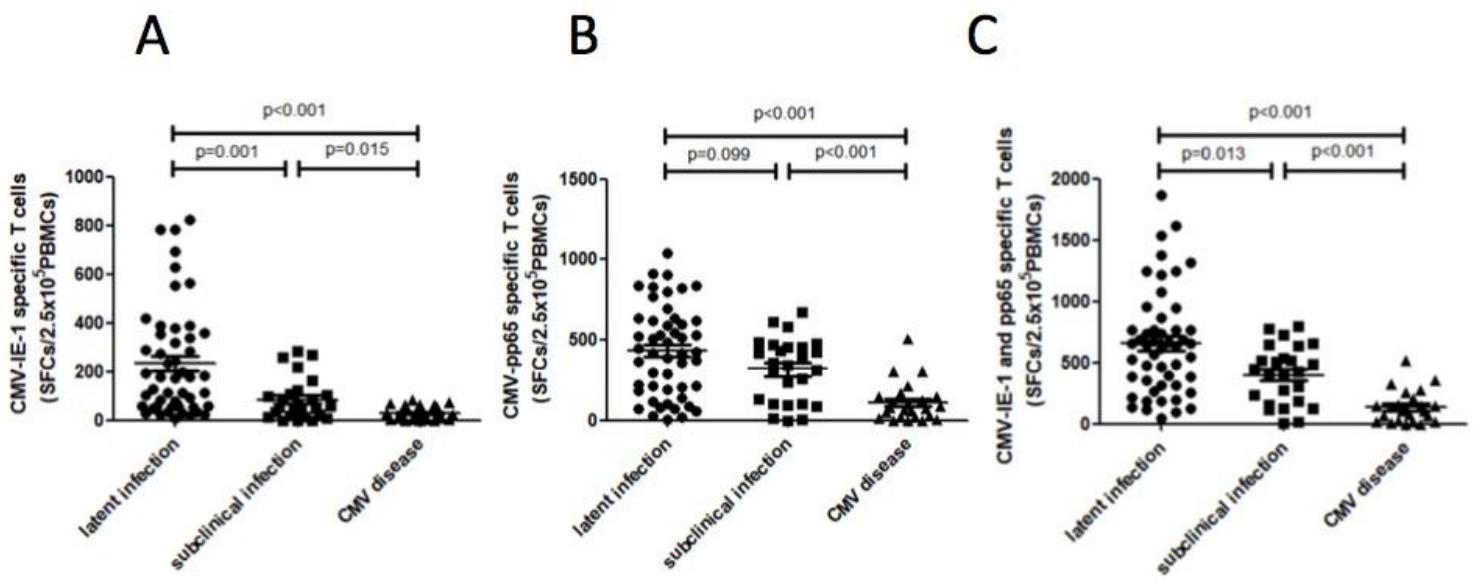

Figure 3

Comparison of CMV specific T cell immune responses in three groups (latent CMV infection group, subclinical CMV infection group and CMV disease group) stimulated by different antigens. (A) Frequency distribution of IFN-γ-secreting T cells stimulated by IE-1. (B) The frequency distribution of IFN-γ-secreting T cells stimulated by pp65. (C) The frequency distribution of IFN-γ-secreting T cells stimulated by IE-1&pp65.

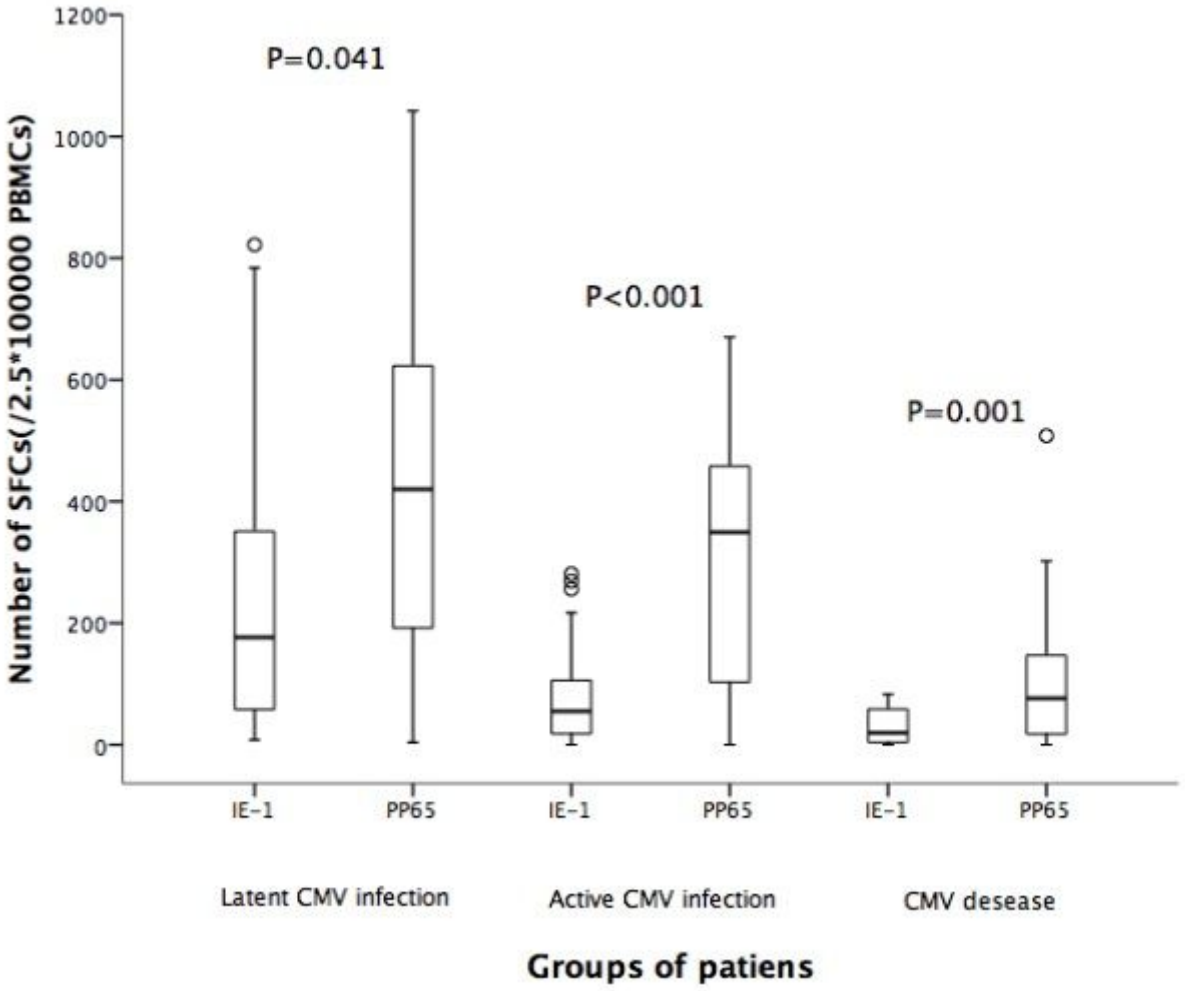

Figure 4

Comparison of CMV specific T cell immune responses in three groups (latent CMV infection group, subclinical CMV infection group and CMV disease group) stimulated by IE-1 / pp65 antigen.