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HIV-Specific IL-2$^+$ and/or IFN-γ$^+$ CD8$^+$ T Cell Reponses during Chronic HIV-1 Infection in Former Blood Donors

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Objective Conflicting data have been generated from previous studies to determine which kind of relationship exists between HIV-1 specific CD8 Tcell responses and HIV-1 viral load or CD4 count over the course of infection. In this study, 153 HIV-1 infected LTNPs were enrolled to investigate the role of HIV-1 specific CD8 T-cell responses in chronic HIV-1 infection among HIV-1 infected former blood donors. **Methods** The patients were stratified into three groups according to CD4 count: CD4≥500 cells/μL; 350 cells/μL≤CD4<500 cells/μL; CD4<350 cells/μL. PBMCs were isolated from the patients' anticoagulated blood samples. IL-2 and IFN-γ secretions of CD 8 T cells against 17 HIV-1 consensus B full peptide pools were analyzed by using ICS assay. **Results** An overall inverse correlation were observed between CD4 count and plasma viral load. Although no significant difference was observed during the comparisons of frequency/breadth of HIV-1 specific CD8 T cell responses, CD4 count stratification analysis showed that different correlation pattern existed in three strata: as for patients whose CD4 counts were less than 350 cells/μL, no significant correlations were identified between frequency/breadth of HIV-1 specific CD8 T cell responses and CD4 count/viral load; as for patients whose CD4 counts ranged from 350 cells/μL to 500 cells/μL, significant correlation was only observed between the response breadth of IL-2+IFN-γ+ CD8 T cells and CD4 count; however, as for patients whose CD4 counts were more than 500 cells/μL, direct correlations were identified between IL-2+IFN-γ+/IL-2+/IFN-γ+ CD8 T cells and viral load or CD4 count. **Conclusions** Universal consistent inverse correlation was only indentified between CD4 count and viral load. The relationship between HIV-1 specific CD8 T cell responses and CD4 count/viral load varied in different CD4 strata, which showed that better preserved CD4 T cells were correlated with better CD8 T cell functions.

Key words: HIV-1; subtype B'; CD8 T cell response; IFN-γ; IL-2; ICS

INTRODUCTION

The emergence of human immunodeficiency virus type 1 (HIV-1) specific CD8$^+$ T cells has been associated with the initial viral load drop during primary HIV-1 infection[1-2]. A similar association was also observed during SIV infection in nonhuman primates[3-7], an animal model of HIV-1 infection. However, it remains controversial for the role of HIV-1 specific CD8$^+$ T cell responses in containing HIV-1 replication and thereby restraining disease progression during chronic infection.

Several studies on chronic HIV-1 infection showed that the frequency of HIV-1-specific CD8$^+$ T cells inversely was correlated with plasma viral loads[8-9]. In primate models of HIV infection, depletion of CD8$^+$ T cells by infusion of the antibodies specific for the CD8 glycoprotein resulted in the failure of early control in early infection or a rise in viral load in the chronic phase[10-12]. The absence of HIV-1-specific CD8$^+$ T cells has been associated with very rapid HIV/AIDS progression[13]. However, several recent reports argued that the frequency of HIV-1 specific CD8$^+$ T cells might have no correlation[14-18] or even positively correlation[19-20] with plasma viral loads. The functionally impaired HIV-1-specific CD8$^+$ T cells were identified during chronic HIV-1 infection[21-23] and might partially

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account for the current controversial results. In order to precisely evaluate functional T-cell immunity, cytokine IL-2 was recently included as a functional marker for HIV-1 specific T cells. The frequencies of IL-2- and of IL-2/IFN-gamma-secreting HIV-1-specific $CD4^+$ T cells were negatively correlated with the levels of viremia[24-25]. Interestingly, IL-2-secreting HIV-specific $CD8^+$ T cells could support the proliferation of HIV-specific $CD8^+$ T cells in the absence of help from $CD4^+$ T cells[26], thereby functioning similarly to IL-2-secreting $CD4^+$ T cells. These data suggested that an inverse correlation between the IL-2-secreting HIV-1-specific $CD8^+$ T cells and viral loads might exist during chronic HIV-1 infection.

In present study, we examined the associations of HIV-1 specific $CD8^+$ T cell responses, quantified as $IL\text{-}2^+$ and/or IFN-gamma+$CD8^+$ T cells post HIV-derived peptide stimulation, with viral loads or CD4 counts in 153 HIV-1-infected former blood donors (FBDs) who remain naive to antiretroviral treatment. All study subjects were infected by Thailand B clade HIV-1 virus and were enrolled in Anhui province, one of the provinces in which the activities of unregulated blood/plasma donation during 1992-1995 in central China was prevalent. The unregulated blood/plasma donation caused the spreading of HIV-1 and currently accounted for 23% of HIV-1 prevalence in China by the end 2005[27]. After being infected for more than a decade, the HIV-1-infected FBDs are at the dichotomous stage either remaining as long-term nonprogressors or progressing into AIDS. Therefore, this cohort may provide us with an unique opportunity to examine the relationship between HIV-1 specific T cell responses and viremia or disease progression.

MATERIALS AND METHODS

Study Subjects

The study was initiated after its protocol sequentially approved by NIH USA, the Institutional Review Board (IRB) of China National Center for AIDS/STD Control and Prevention and the IRB of Anhui Provincial Center for Disease Control and Prevention, respectively. 153 HIV-1-infected FBDs naive to ART were enrolled in this study from Anhui province in central China. An HIV-1 seropositive FBD was eligible for this study only if all of the following criteria applied: age>18 years old; self-reported history of blood donation in 1995 or before; documentation of HIV-1 infection by ELISA and confirmed by Western Blot test; ART naive by self report; negative pregnancy testing for females; willing to provide adequate locator information for study retention purpose; willing and be able to provide informed consent; CD4 T cell counts>200 cells/μL at the last test 6 months ago. Written informed consents were obtained from all study participants at the enrollment.

Peptides and Antibodies

Since the FBDs were infected by B' clade HIV-1, a set of peptides spanning the full genome of consensus B clade (provided by NIH Research and Reference Reagent Program) were used as stimuli. Peptides (15 mer overlapping by 11 aminal acids with its next peptide) were used at a final concentration of 5 μg/mL for each peptide. All peptides were synthesized as free acids with more than 80% purity. Lyophilized peptides were reconstituted to 1mg/mL in 90% RPMI 1640 with 10% DMSO for the peptide mixture. 17 peptide pools were prepared, including Env1 (42 peptides), Env2 (42 peptides), Env3 (42 peptides), Env4 (42 peptides), Env5 (43 peptides), Gag1 (41 peptides), Gag2 (41 peptides), Gag3 (41 peptides), Pol1 (50 peptides), Pol2 (50 peptides), Pol3 (50 peptides), Pol4 (50 peptides), Pol5 (49 peptides), Nef (49 peptides), Tat+Rev (50 peptides), Vpr+Vpu (41 peptides), and Vif (46 peptides).

Quantification of Viral Loads, HIV-1 Genotyping and CD4 T-cell Counts

Plasma viral loads were detected by fluorescence real time PCR (LightCycler, Roche, U.S.A.; PCR Fluorogence Diagnostic, PG BioTech, China) according to the manufacturer's instruction, and the detection limit of PG assay was 400 HIV-1 RNA copies per mL plasma. HIV-1 subtype was determined with the sequences of env C2-C3 fragment and the entire gag gene. The CD4 cell counts from EDTA anticoagulated whole blood were determined with a monoclonal antibody cocktail composed of CD3 FITC, CD4 PE, and CD45 PerCP in a TruCount tube and with BD FASCalibur flow cytometer (San Jose, CA, USA) according to the manufacturer's instructions.

PBMC Stimulation and Intracellular Staining

Bulk PBMCs were isolated from anticoagulated patients' blood samples by Ficoll-Hypaque gradient centrifugation and were plated in round-bottom 96-microtiter plates (Costar, NY) at 0.5 million cells/well in 200 μL complete RPMI 1640 (RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin) containing 5 μg/mL for each peptide. Cells were cultured overnight and Brefeldin A (Sigma Aldirch) was added at 10 μg/mL

during the last three hour culture. A negative control with no peptide stimulation and positive control with 25 ng/mL PMA and 1.5 μg/mL Ionomycin (Sigma Aldrich, USA) were included for each subject PBMCs. In following incubation, the cells were collected and washed with PBS containing 1% bovine serum albumin and then stained with CD3 and CD8 antibodies. The cells were washed and fixed with 2% paraformaldehyde for 10 min at room temperature. In following fixation, the cells were washed with PBS containing 1% bovine serum albumin again, then were resuspended in 0.1% saponin solution and were stained with antibodies against human IL-2 and IFN-γ for 15 min at room temperature. In following staining, the cells were washed and resuspended in PBS containing 1% bovine serum albumin and were analyzed with FASCalinure (BD, CA). Direct fluorescence conjugated antibodies were obtained from BD Biosciences (San Jose, CA): IL-2-FITC, CD3-Pe-Cy5, IFN-γ-PE, CD8-APC, CD3-FITC, CD4-PE, and CD45-PerCP.

Cell Acquiring With Flow Cytometry

Cells were acquired with FASCalibur flow cytometer (BD Immunocytometry Systems, San Jose,CA). Data were analyzed by CellQuest Pro software. 20 000-50 000 lymphocytes were collected for each sample. Initial gating used a forward scatter Height (FSC-H) versus a side scatter Height (SSC-H) plot to identify lymphocytes. Subsequently, the events were gated through a side scatter Height (SSC-H) versus $CD3^+$ plot to define T cells. Based on this, a $CD3^+$ versus $CD8^+$ plot were used to define CD8 T cells. Finally, CD8 T cells were gated on IL-2 versus IFN-γ (representive diagram displayed in Fig. 2A).

Statistical Analysis

All data were background-subtracted by using values from negative control. Statistical significance of the association of log viral loads or CD4 counts with the frequencies of $CD8^+$ T cell responses were calculated through a Pearson Product Moment Correlation test. Statistical significance of the association of CD4 counts or viral loads with the breadth of $CD8^+$ T cell responses were calculated through Spearman Rank Order Correlation test.Comparisons between variables were based on the method of Kruskal-Wallis One Way Analysis of Variance on Ranks. P value < 0.05 was considered to be significant.

RESULTS

Characterization of Study Participants

153 HIV-1 infected FBDs, including 84 male and 69 female, were enrolled into this study with the average age of 44 years old. Their CD4, CD8 Tcell counts and viral loads in logs were 378±153 cells/μL, 856±465 cells/μL, and 4.34±0.76, respectively. All study participants were infected by Thailand B clade HIV-1 virus. They were further stratified into three groups according to their CD4 T-cell counts as CD4<350 (n=73), 350⩽CD4<500 (n=51), CD4⩾500 cells/μL (n=29). The ratio of male to female sex in two groups with CD4<500 cells/μL were close to 1:1 whereas it reached 2:1 in the group with CD4⩾500 cells/μL. No significant differences were observed in average age and CD8 T-cell counts. However, viral loads in group with CD4<350 cells/μL were significantly larger than that in 350<CD4<500 group (P=0.017) and in CD4⩾500 cells/μL group (P=0.025). Detailed information are provided in Table 1. A statistically significant inverse correlation was observed between CD4 counts and plasma viral loads (r=–0.275; P=0.0011) (Fig. 1).

TABLE 1

Characterization of 153 Studies Participants

	CD4<350 cells/μL	350 cells/μL⩽ CD4<500 cells/μL	CD4⩾500 cells/μL	Total
Number of Patients	73	51	29	153
Sex (Male:Female)	37:36	26:25	20:9	83:70
Mean Age (Years Old) (mean±SD)	44±8	43±7	44±9	44±8
CD4 Count (cells/μL) (mean±SD)	254±65	414±41	636±87	378±153
CD8 Count (cells/μL) (mean±SD)	757±314	870±523	851±592	856±465
Viral Load (Log)* (mean±SD)	4.61±0.72	4.08±0.72	4.04±0.71	4.34±0.76

Note. The patients enrolled in this study were former blood donors,infected during 1993-1995. All patients were infected by HIV-1B'(Thai B).
*Totally 15 patients' plasma viral loads were below detection limit which is 500 copies/mL.

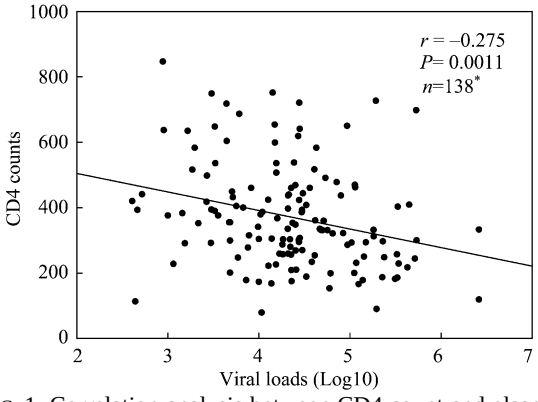


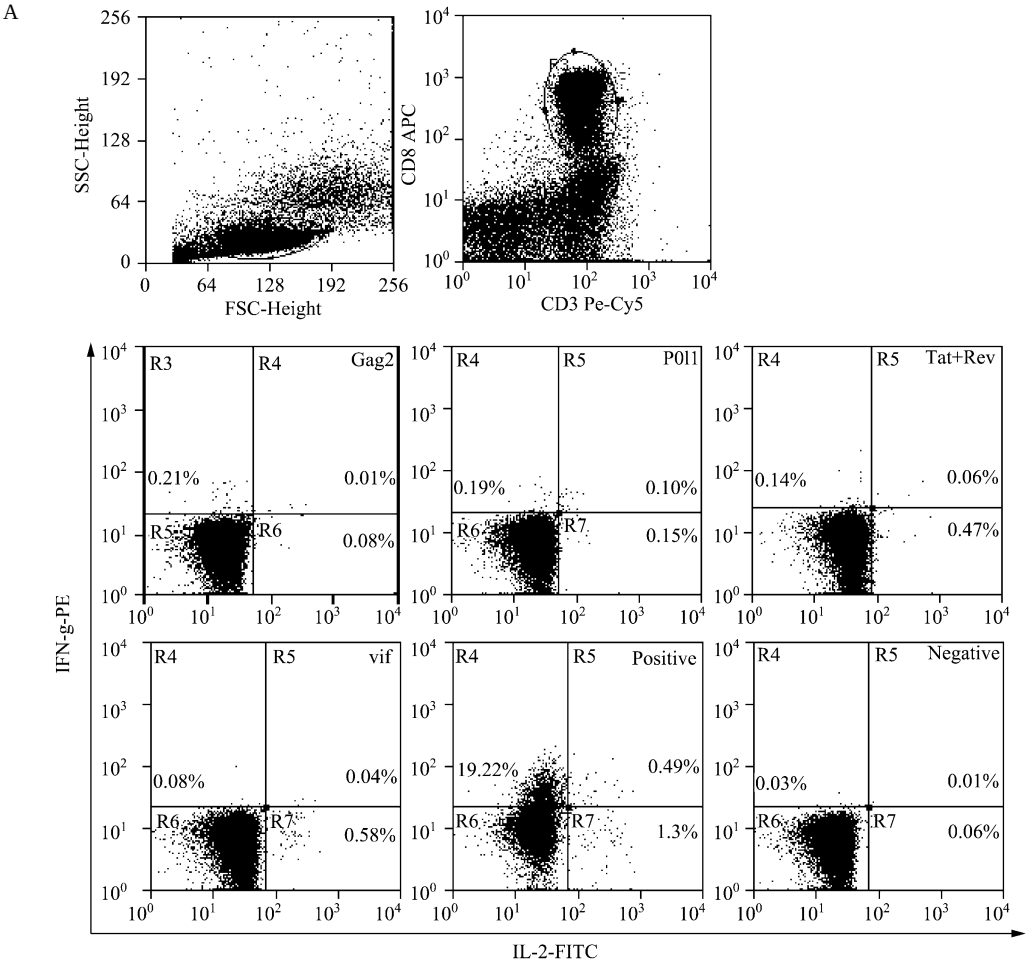
FIG. 1. Correlation analysis between CD4 count and plasma viral load statistically significant inverse correlation was observed between CD4 count and plasma viral load. *The plasma viral loads of 15 patients were undectable.

HIV-specific CD8$^+$ T Cell Responses against Different HIV-1 Antigens

As described above in methods section, 17 peptide pools were used to stimulate CD8$^+$ T cell responses with roughly equal number of peptides in each peptide pool. Three responding populations were identified, including single IFN-γ$^+$ cells, single IL-2$^+$ cells and dual IFN-γ$^+$/IL-2$^+$ cells, and were exemplified in Fig. 2A. In order to determine the dominant HIV-1 antigen, CD8$^+$ T cell responses against 17 peptide pools were pooled into 7 antigens, including Env, Gag, Pol, Nef, Tat+Rev, Vpu+Vpr, andVif. An unbalanced CD8$^+$ T cell response pattern was observed among different HIV-1 antigens. Pol is the most dominant antigen for CD8$^+$ T cell responses, Env and Gag were the second and third dominant antigens, respectively, and other HIV-1 antigens are less important, regardless of single IFN-γ$^+$ cells, single IL-2$^+$ cells or dual IFN-γ$^+$/IL-2$^+$ CD8$^+$ T cell responses (Fig. 2B).

Association of HIV-1 Specific CD8$^+$ T-cell Responses with Plasma Viral Loads or CD4 Counts

We next examined the association of the magnitude or the breadth of HIV-1 specific CD8$^+$ T cell responses



B

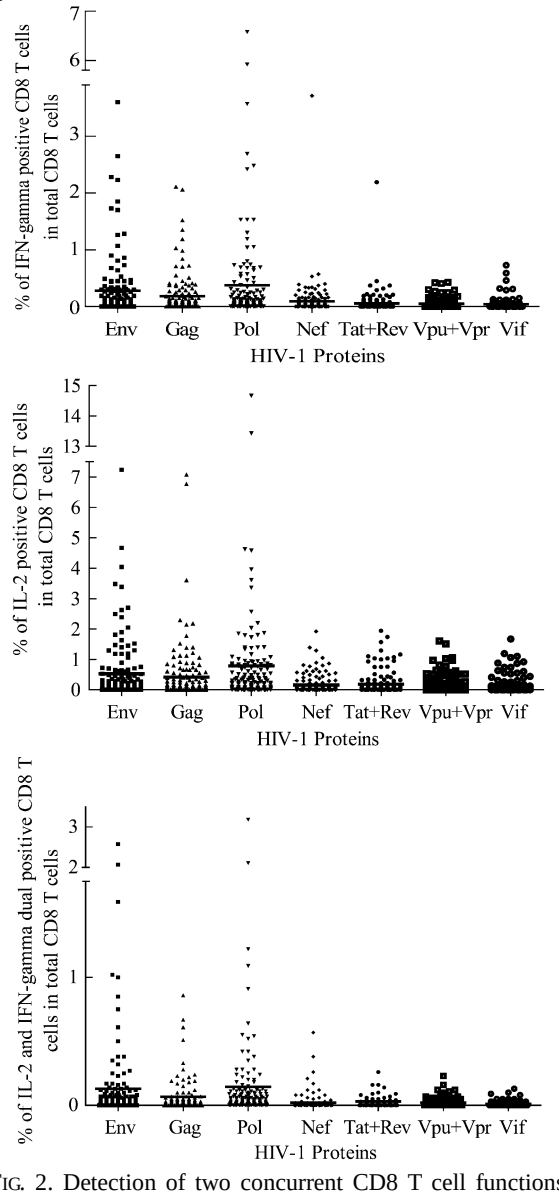
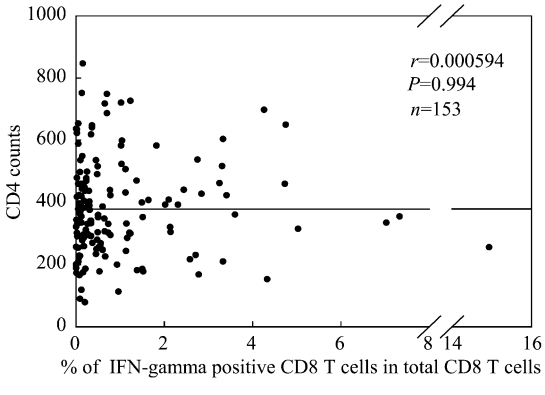


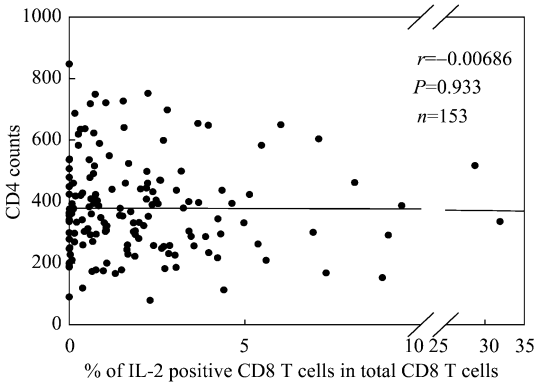
FIG. 2. Detection of two concurrent CD8 T cell functions and frequency comparisons of HIV-1 specific CD8 T cell responses against different HIV-1 proteinsGating scheme for identification of HIV-1 specific IL-2/IFN-γ secretion in CD8 T cells. Representive data of specific responses against different HIV-1 proteins generated from subject 020201328 are shown, after 8 h *in vivo* stimulation. See "Patients, materials and methods" for detailed explanation of the procedure. Frequency comparisons of HIV-1 specific CD8 T cell responses against different HIV-1 proteins. HIV-1 proteins were divided into 7 groups: Env, Gag, Pol, Nef, Tat+Rev, Vpu+Vpr, Vif. Specific responses against each peptide in the same group were summed up, and comparisons were performed according to the method of Kruskal-Wallis One Way Analysis of Variance on Ranks. Responses against Pol were the most high, Env was the second, then was Gag (detailed statistical data are not shown).

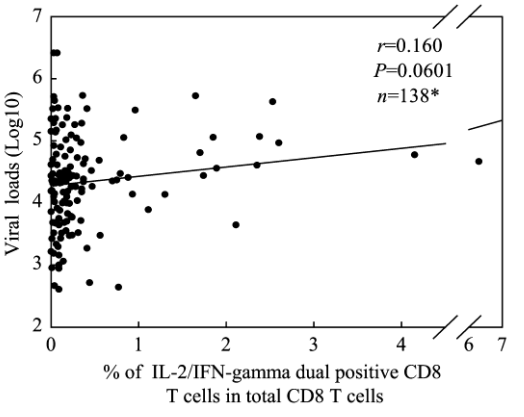
with plasma viremia or disease progression surrogate CD4 counts. $CD8^+$ T cell responses against 17 peptide pools were pooled together for each study subject as the magnitude of immune responses and the number of responding peptide pools out of 17 for each subject were considered as the breadth of $CD8^+$ T cell immune responses. Three populations of single IFN-γ^+ cells, single IL-2^+ cells or dual IFN-γ^+/IL-2^+ $CD8^+$ T cells were calculated independently. As shown in Fig. 3A, no significant association wasobserved between single IFN-γ^+ (r=0.0827, P=0.335) or single IL-2^+ $CD8^+$ T cell responses (r=0.0843, P=0.326) and viral loads. Though a positive correlation trend was observed between dual IFN-γ^+/IL-2^+ $CD8^+$ T cell responses and viral loads, it did not reach statistical significance (r=0.160, P=0.0601). Similarly, no significant association was observed between those $CD8^+$ T cell responses and CD4 counts (P=0.994, 0.933, and 0.810 for association of single IFN-γ^+, single IL-2^+ or dual IFN-γ^+/IL-2^+ $CD8^+$ T cell responses with CD4 counts) (Fig. 3B).

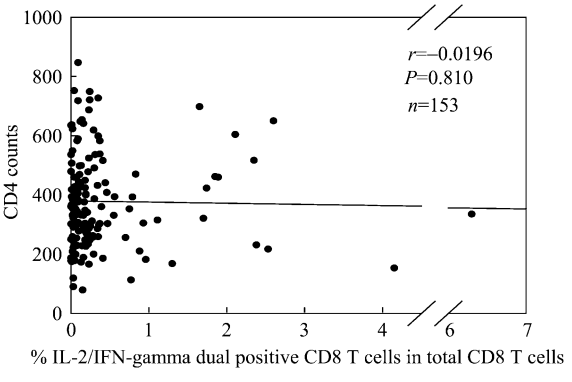
We further examined the association of the breadth of $CD8^+$ T cell responses with viral loads (Fig. 3C) or CD4 counts (Fig. 3D). No significant association was observed between the breadth of single IFN-γ^+ (r=0.113, P=0.188) or dual IFN-γ^+/IL-2^+ $CD8^+$ T cell responses (r=0.07, P=0.414) with viral loads.On the contrast, the breadth of single IL-2^+ $CD8^+$ T cell response was significantly correlated with viremia (r=0.242, P=0.00427). In order to be consistent with this observation, a negative association trend was identified between the breadth of single IL-2^+ $CD8^+$ T cell responses and CD4 counts though it did not reach statistical significance (r=–0.134, P=0.0992) (Fig. 3D). No significance was reached between the breadth of single IFN-γ^+ (r=0.0246, P=0.762) or dual IFN-γ^+/IL-2^+ $CD8^+$ T cell responses (r=–0.00544, P=0.947) with CD4 counts.

A

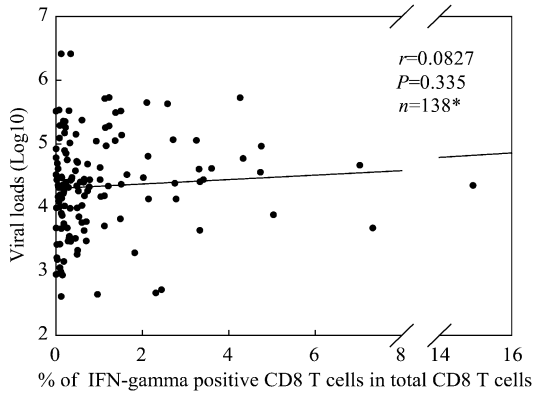


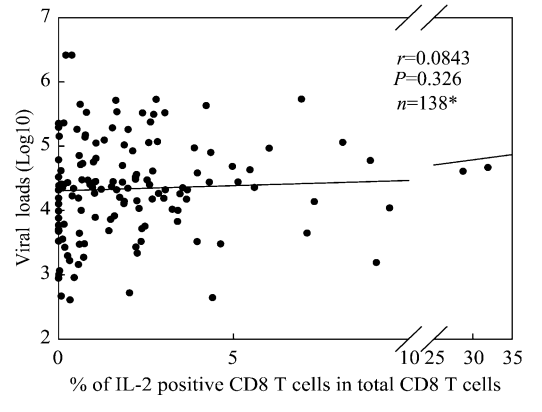

r=–0.00686
P=0.933
n=153
CD4 counts
% of IL-2 positive CD8 T cells in total CD8 T cells


r=0.160
P=0.0601
n=138*
Viral loads (Log10)
% of IL-2/IFN-gamma dual positive CD8 T cells in total CD8 T cells

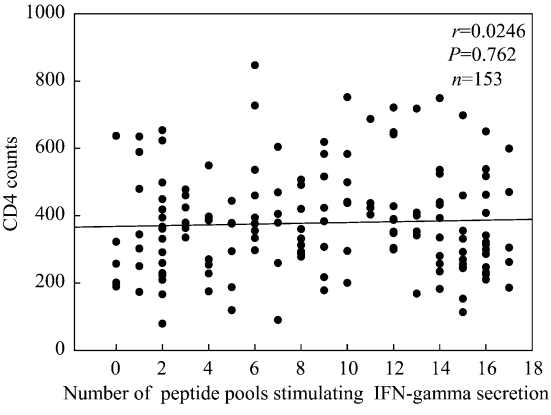

r=–0.0196
P=0.810
n=153
CD4 counts
% IL-2/IFN-gamma dual positive CD8 T cells in total CD8 T cells

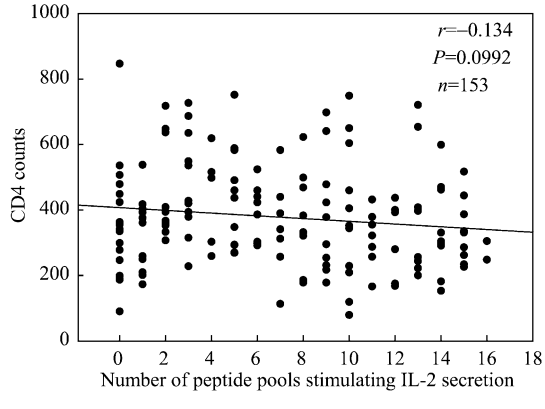
B

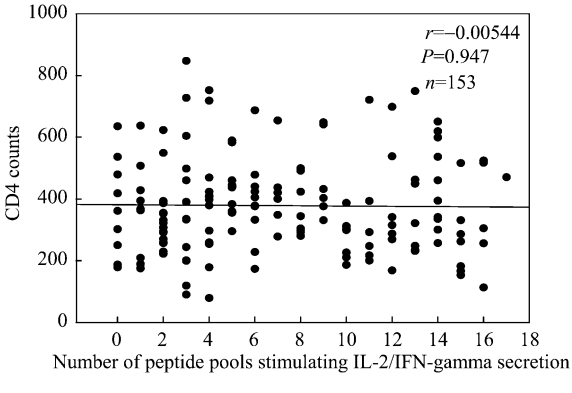

r=0.0827
P=0.335
n=138*
Viral loads (Log10)
% of IFN-gamma positive CD8 T cells in total CD8 T cells


r=0.0843
P=0.326
n=138*
Viral loads (Log10)
% of IL-2 positive CD8 T cells in total CD8 T cells

C


r=0.0246
P=0.762
n=153
CD4 counts
Number of peptide pools stimulating IFN-gamma secretion


r=–0.134
P=0.0992
n=153
CD4 counts
Number of peptide pools stimulating IL-2 secretion


r=–0.00544
P=0.947
n=153
CD4 counts
Number of peptide pools stimulating IL-2/IFN-gamma secretion

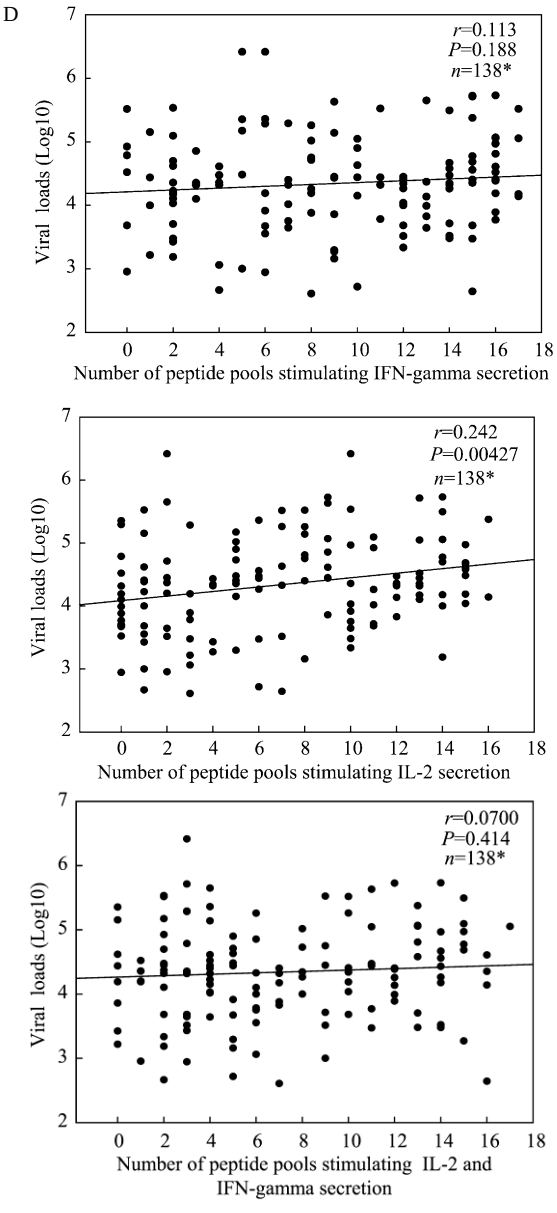


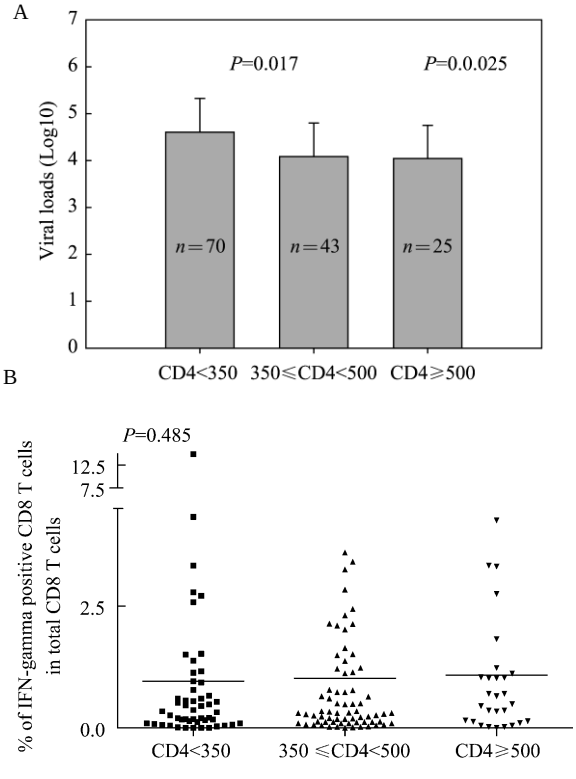
FIG. 3. Correlation analysis between the frequency/breadth of HIV-1 specific CD8 T cell responses and CD4 count or plasma viral load Specific IL-2/IFN-γ responses against each peptides of HIV-1 full genome peptides were summed up and correlations between log viral load/CD4 count and frequency of CD8 T cell responses were calculated through Pearson Product Moment Correlation test; correlations between breadth of CD8 T cell responses and CD4 count or viral load were calculated through Spearman Rank Order Correlation test. Significant direct correlation was only observed between the breadth of HIV-1 specific IL-2 secretion and plasma viral load (3d). *The plasma viral loads of 15 patients were undectable.

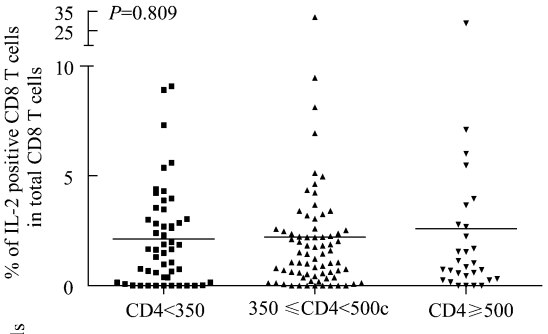
HIV-1 Specific CD8$^+$ T-cell Responses in Groups with Different CD4 Counts

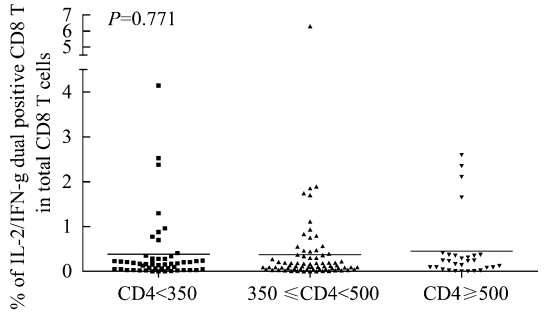
All the 153 patients were divided into three strata according to CD4 count: CD4≥500 cells/μL; 350 cells/μL≤CD4<500 cells/μL; CD4<350 cells/μL. Comparisons of HIV-1 specific CD8 T cell response frequency/breadth showed that no significant difference existed among the three groups (data shown in Fig. 4).

Correlation Analysis between HIV-1 Specific CD8 T cell Response Frequency/Breadth and CD4 Count or Plasma Viral Load Showed Different Correlation Pattern in the Three CD4 Strata

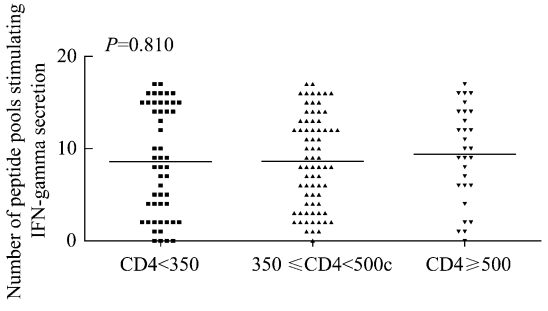
As for patients with CD4<350 cells/μL, no significant correlation was identified between frequency/breadth of CD8 T cell responses and CD4 count/viral load; as for patients with 350 cells/μL≤CD4<500 cells/μL, a significantly direct correlation was only observed between the breadth of IL-2$^+$IFN-γ$^+$ CD8 T cells and CD4 count; however, as for patients with CD4≥500 cells/μL, more correlations were observed between these variables (detailed data shown in Table 2): the frequency of IL-2$^+$IFN-γ$^+$ CD8

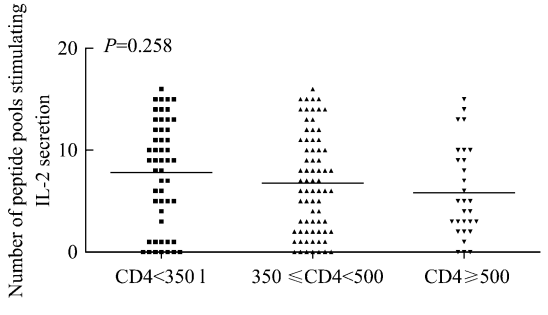


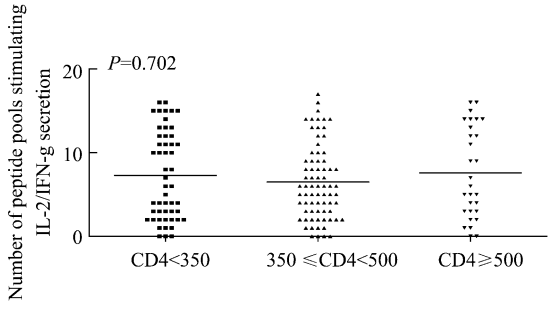

P=0.809
% of IL-2 positive CD8 T cells in total CD8 T cells
CD4<350
350 ≤CD4<500c
CD4≥500

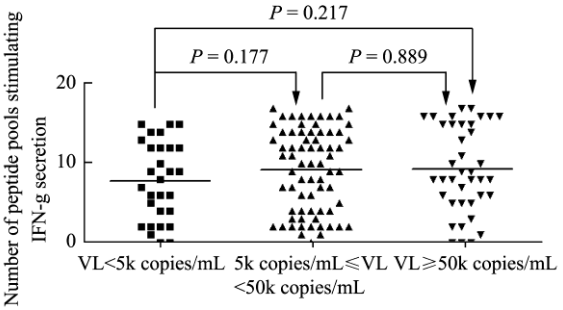

P=0.771
% of IL-2/IFN-g dual positive CD8 T cells in total CD8 T cells
CD4<350
350 ≤CD4<500
CD4≥500

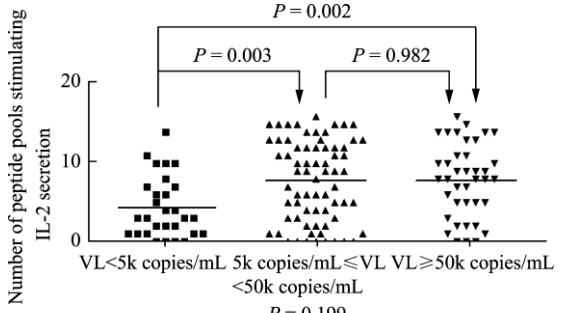
C

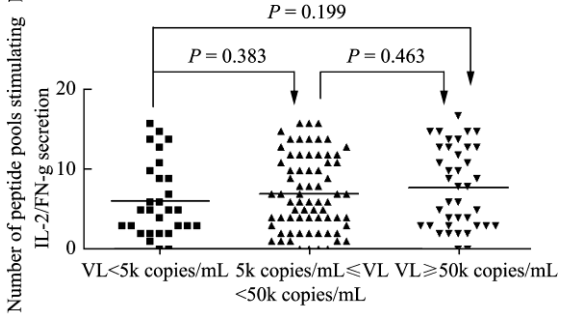

P=0.810
Number of peptide pools stimulating IFN-gamma secretion
CD4<350
350 ≤CD4<500c
CD4≥500

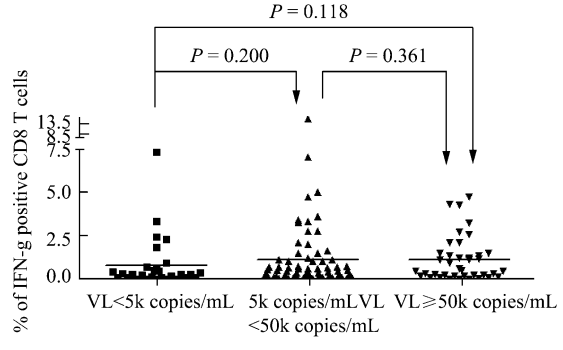

P=0.258
Number of peptide pools stimulating IL-2 secretion
CD4<350 l
350 ≤CD4<500
CD4≥500

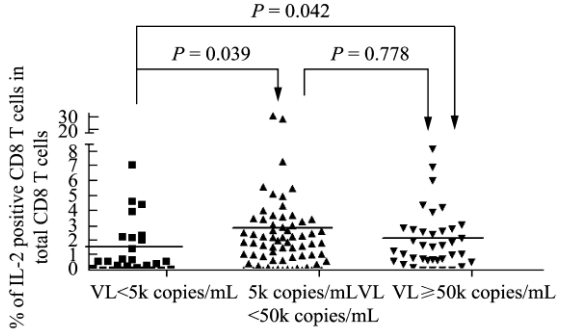

P=0.702
Number of peptide pools stimulating IL-2/IFN-g secretion
CD4<350
350 ≤CD4<500
CD4≥500


P = 0.217
P = 0.177
P = 0.889
Number of peptide pools stimulating IFN-g secretion
VL<5k copies/mL
5k copies/mL≤VL <50k copies/mL
VL≥50k copies/mL


P = 0.002
P = 0.003
P = 0.982
Number of peptide pools stimulating IL-2 secretion
VL<5k copies/mL
5k copies/mL≤VL <50k copies/mL
VL≥50k copies/mL


P = 0.199
P = 0.383
P = 0.463
Number of peptide pools stimulating IL-2/FN-g secretion
VL<5k copies/mL
5k copies/mL≤VL <50k copies/mL
VL≥50k copies/mL


P = 0.118
P = 0.200
P = 0.361
% of IFN-g positive CD8 T cells
VL<5k copies/mL
5k copies/mLVL <50k copies/mL
VL≥50k copies/mL


P = 0.042
P = 0.039
P = 0.778
% of IL-2 positive CD8 T cells in total CD8 T cells
VL<5k copies/mL
5k copies/mLVL <50k copies/mL
VL≥50k copies/mL

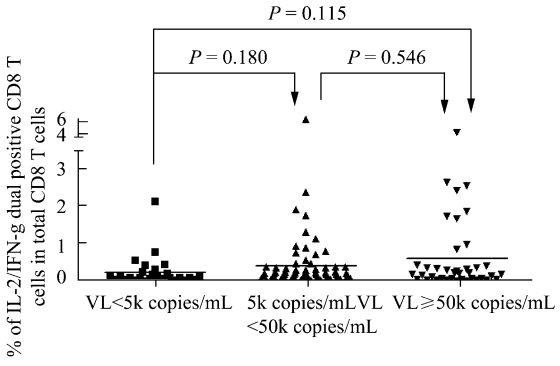


FIG. 4. Frequency/breadth comparisons of HIV-1 specific CD8 T cell responses among different CD4 count strata All the 153 patients were divided into three strata according to CD4 count: CD4≥500 cells/μL; 350 cells/μL≤CD4<500 cells/μL; CD4<350 cells/μL. No significantly different CD8 T cell reponse was found. Multiple comparisons among three CD4 strata were performed by the method of Kruskal-Wallis One Way Analysis of Variance on Ranks. The plasma viral loads of 15 patients were undectable.

T cells directly associated with viral load, the breadth of IL-2^+ CD8 T cells directly associated with viral load, and both the frequency and breadth of IFN-γ^+ CD8 T cells directly associated with viral load.

DISCUSSION

It's almost putative that acute HIV-1 infection is marked by rapid viral load increasing and rapid CD4 T cells depletion, but the dynamics of CD4 T cell and viral load and their relationships with HIV-1 specific CD8 T cell responses in the chronic infection phase remain confusing[21]. In nonhuman primates, transient depletion of CD8 T cells in chronically SIV-infected rhesus macaques leads to a rapid increase in viral replication[22-24], which implies specific CD8 T cell responses playing vital roles in the control of plasma viral load. But for humans, conflicted data have been generated before[8-16].

Here, we presented our data generated from an untreated, mainly HIV-1 subtype-B'(2 of 153 patients were uncertain) infected FBDs to elucidate the relationship that lies between HIV-1 specific IL-2 and/or IFN-γ secreting CD8 T cells and disease progression. On the whole, an inverse correlation was observed between CD4 count and plasma viral load, which was consistent with current knowledges of AIDS progession. Intensive analysis showed that correlations between specific CD8 T cell responses and CD4 count/viral load varied according to different CD4 count strata (detailed data shown in Table 2). There are many reasons that may affect the correlations between HIV-1 specific CD8 T cell responses and CD4 count or viral load, such as different targeting proteins and different cohorts, but we argue that different CD4 count levels would be definitely one of the important reasons.

Previous studies indicated that the correlation between Gag-specific CD8 T cell reponse and disease progression might depend on disease status[25]. In our study, CD4 count levels associated with correlation patterns were also found in different CD4 count strata: no significant correlation existed between variables in patients with CD4<350 cells/μL; only the breadth of IL-2/IFN-γ dual secreting CD8 T cell was found to be directly correlated with CD4 count in patients with 350 cells/μL≤CD4<500 cells/μL; more direct correlations were observed in patients with CD4≥500 cells/μL(Table 2). It seemed that HIV-1 specific CD8 T cells could be activated more easily in patients with CD4≥500 cells/μL than in patients with CD4<350 cells/μL. Why does this happen? Perhaps because high level of cognate CD4 T cells may help CD8 T cells to maintain high functional intactness, and this kind of intactness might play a vital role in the control of disease progression. In fact, previous studies had already demonstrated that multi-functions of HIV-1 specific CD8 T cell were kept better in long time no progressors than in progressors[19], although we still don't know what leads to the difference.

Besides, our results also showed that HIV-1 specific CD8 T cells responses against Pol were highest, not Gag (data were shown in Fig. 2), and we think reasonably that Pol is more conserved than Gag, although it is inconsistent with previous studies[26-28].

Taken together, this study illustrated the relationship between HIV-1 specific CD8 T cell responses and disease progression at a relatively large scale (153 patients). Our results indicated that the correlations of HIV-1 specific CD8 responses with clinical disease progression is not universally consistent throughout the entire course of chronic HIV-1 infection. Under different CD4 count levels, HIV-1 specific CD8 T cell response might play different roles in chronic HIV-1 infection.

Finally, several limitations of this study should be noted. It is a cross-sectional and not a longitudinal study, which necessitates cautious interpretation of the findings. In addition, only IL-2 and IFN-γ were assayed, which might not be enough to represent the multifunction of CD8 T cells. Inspite of those, our findings elucidated that different correlation patterns between specific CD8 T cells responses and disease progression did exist under different CD4 count levels during chronic HIV infection. Now, it's badly needed to clarify how the difference come into formation, and this will undoubtly do great help for our understanding of HIV pathogenesis and rational design of therepeutic HIV vaccines.

TABLE 2.

Correlations between the Frequency/Breadth of CD8 T cell Response and CD4 Count/Viral Load in Three CD4 Strata

$IL\text{-}2^{+}IFN\text{-}\gamma^{+}$	Frequency		Breadth	
CD4<350 cells/μL				
CD4 Count,cells/μL(*n*=73)	*r*=-0.00411	*P*=0.972	*r*=0.0978	*P*=0.409
Viral Load,copies/mL(*n*=70)*	*r*=-0.000580	*P*=0.996	*r*= -0.0716	*P*=0.555
350 cells/μL≤CD4<500 cells/μL				
CD4 Count,cells/μL(*n*=51)	*r*=0.214	*P*=0.132	*r*=0.322	*P*=0.0216
Viral Load,copies/mL(*n*=43)#	*r*=0.115	*P*=0.463	*r*= 0.161	*P*= 0.302
CD4≥500 cells/μL				
CD4 Count,cells/μL(*n*=29)	*r*=-0.0910	*P*=0.639	*r*=-0.153	*P*=0.423
Viral Load,copies/mL(*n*=25)Δ	*r*=0.447	*P*=0.0251	*r*= 0.276	*P*= 0.180
$IL\text{-}2^{+}$				
CD4<350 cells/μL				
CD4 Count,cells/μL(*n*=73)	*r*=0.0882	*P*=0.458	*r*=-0.0338	*P*=0.776
Viral Load,copies/mL(*n*=70)*	*r*=-0.0746	*P*=0.539	*r*= 0.170	*P*= 0.158
350 cells/μL≤CD4<500 cells/μL				
CD4 Count,cells/μL(*n*=51)	*r*=0.124	*P*=0.385	*r*=0.204	*P*=0.149
Viral Load,copies/mL(*n*=43)#	*r*=0.101	*P*=0.521	*r*=0.215	*P*=0.164
CD4≥500cells/μL				
CD4 Count,cells/μL(*n*=29)	*r*=-0.223	*P*=0.245	*r*=0.107	*P*=0.576
viral Load,copies/mL(*n*=25)Δ	*r*=0.273	*P*=0.186	*r*=0.410	*P*=0.0416
$IFN\text{-}\gamma^{+}$				
CD4<350 cells/μL				
CD4 Count,cells/μL(*n*=73)	*r*=0.0177	*P*=0.882	*r*=0.121	*P*=0.309
Viral Load,copies/mL(*n*=70)*	*r*=-0.0336	*P*=0.783	*r*=-0.0401	*P*=0.741
350 cells/μL≤CD4<500 cells/μL				
CD4 Count,cells/μL(*n*=51)	*r*=0.0295	*P*=0.837	*r*=0.0391	*P*=0.784
Viral Load,copies/mL(*n*=43)#	*r*=0.0542	*P*=0.730	*r*=0.299	*P*=0.0517
CD4≥500cells/μL				
CD4 Count,cells/μL(*n*=29)	*r*=-0.103	*P*=0.596	*r*=-0.0317	*P*=0.869
Viral Load,copies/mL(*n*=25)Δ	*r*=0.556	*P*=0.00389	*r*=0.399	*P*=0.0482

Note. The correlations between frequency and CD4 count/viral load were calculated using Pearson Moment Product Correlation test; the correlations between breadth and CD4 count/viral load were calculated by Spearman Rank Order Correlation test and $P<0.05$ was considered to be significant.*The plasma viral loads of 3 patients were undetectable. # The plasma viral loads of 8 patients were undetectable. ΔThe plasma viral loads of 4 patients were undetectable.

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