LETTER TO THE EDITOR

HIV-1-SPECIFIC CD8+ T CELLS DO NOT CORRELATE WITH VIRAL LOAD IN HIV-1-POSITIVE PATIENTS

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HIV-1-specific CD8+ T cells play an important role in the control of HIV-1 infection. During the infection, HIV-1-specific CD8+ T cells eliminate HIV-1-infected cells and suppress the virus replication by various mechanisms (*1, 2*).

These include cytotoxic killing of target cells dependent on cell-to-cell contact or production of different cytokines and chemokines with antiviral activity, e.g., interferon gamma (IFN-γ), interleukin 2 (IL-2), lymphotoxins etc. It is hypothesized that a strong cytotoxic immune response is responsible for a delay in the progression of HIV-1 infection. Better understanding of the role of HIV-specific CD8+ T cells could improve a long-term follow-up of HIV-1-positive patients and help to introduce new treatment strategies, including adjuvant immunotherapy with IL-2 or other cytokines (*1*). Furthermore, the characterization of intensity, diversity and dynamics of the HIV-1-specific CD8+ T cell response is an important part in evaluation of therapeutic vaccines (*3*).

Major histocompatibility complex (MHC) tetramers, used recently for detection of CD8+ T cells, is a complex of four recombinant MHC class I molecules bound to an antigen and streptavidin molecule (*4*). During incubation with CD8+ T cells, the antigen in the complex is attached to antigen-specific T-cell receptor (TCR). As the tetramers are labeled with fluorochrome, antigen-specific CD8+ T cells can be visualized and detected by flow cytometry.

The aim of this study was to determine the frequency and diversity of HIV-1 Gag- and Pol-specific CD8+ T cells in naïve as well as HAART-treated HIV-1-positive patients using MHC tetramers directed against respective antigens. Besides, viral load, in particular the number of HIV-1 copies was estimated. This prospective study was conducted in accordance with the Helsinki Declaration as revised in 1996. Sixty-three HIV-1-positive patients (sex ratio M/F 49/14, median age 35 years) were enrolled in the study between June 2005 and June 2006 based on their HLA-A2 haplotype as determined by flow cytometry. According to the Centers for Disease Control and Prevention classification, 37 patients were classified in the stage A1/A2 of HIV-1 infection, 10 patients in the stage B1/B2, and 16 patients as having AIDS (CDC A3, B3, and C). Out of 63 patients, 38 were treated with highly active antiretroviral therapy (HAART), while 25 were not (naïve patients). CD4+ and CD8+ T-cell counts were determined in EDTA-treated peripheral blood samples by flow cytometry using a FACSCalibur™ apparatus (Becton Dickinson, USA) (*5*). The frequencies of HIV-1 Gag-specific and Pol-specific CD8+ T cells were assayed using commercial HLA-A*0201 tetramer complexes with

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Abbreviations: HAART = highly active antiretroviral therapy; HIV-1 = Human immunodeficiency virus 1; IFN-γ = interferon gamma; IL-2 = interleukin 2; MHC = major histocompatibility complex; TCR = T-cell receptor

Gag (aa 77–85, SLYNTVATL) and Pol (aa 476–484, ILKEPVHGV) epitopes, respectively. Both complexes were labeled with phycoerythrin (Beckman Coulter, Marseille, France). Monoclonal antibodies anti-CD3 PerCP and anti-CD8 FITC (BD Biosciences, Germany) were used for staining CD8+ T cells. After staining, the analysis was performed immediately using three-color flow cytometry and the Cellquest™ software. The frequency of epitope-specific CD8+ T cells was expressed as a percentage from total CD8+ T cells and only the values exceeding the threshold (0.05%) were considered significant. Plasma HIV-1 RNA load was determined by a quantitative PCR (Roche Diagnostic Systems, Switzerland) with a detection limit of 50 RNA copies per ml.

The results showed that the naïve group of patients had medians of CD4+ and CD8+ T cell counts of 387 cells/μl (range 11–1176) and 1078 cells/μl (range 347–3304), respectively, while the HAART group of patients had corresponding values of 464 cells/μl (range 91–1678) and 891 cells/μl (range 391–2438), respectively. Thus, there was no significant difference between the medians of CD4+ and CD8+ T cell counts of the two groups ($p > 0.05$).

As expected, viral load was significantly higher in naïve patients compared to HAART-treated patients (22,500 vs. 50 copies/ml, $p < 0.01$). Interestingly, the CD4+ T cell count and the CD4+/CD8+ ratio correlated with viral load in HAART-treated patients ($r = -0.39$, $p < 0.02$; $r = -0.45$, $p < 0.01$, respectively), but not in naïve patients. The results of the analysis of epitope-specific CD8+T cells (the table) showed lack of correlation between viral load and the frequencies of Gag- or Pol-specific CD8+ T cells in both groups of patients.

This finding is of interest in view of those of previous prospective study (*6*), which documented a decrease in HIV-1-specific CD8+ T cell count in patients after HAART introduction. This difference could be due to the fact that the groups of naïve and HAART-treated patients in our study consisted of different individuals. Regarding the relationship of the frequency of HIV-1-specific CD8+ T cells to viral load, previous reports brought quite controversial results. The studies describing correlations between the viral load and the specific CD8+ T cell response often suffered from a restricted number of epitopes and a relatively low number of patients. Busseyne *et al*. (*7*) have observed a negative correlation between Gag-, Nef-, and Pol-specific cytotoxic CD8+ T cell counts and plasma viral load in children over 5 years. Ogg *et al*. (*8*) have attempted to explain the above phenomenon by the suppression of the virus by cytotoxic CD8+ T cells by the abovementioned mechanisms and not *vice versa*. In contrast, larger, more comprehensive studies inclusive of our investigating individuals at different stages of disease have failed to find such an association (*9, 10*). A possible explanation of the lack of correlation between Gag- and Pol-specific CD8+ T cells and viral load, which was observed in our study, could be also the detection of CD8+ T cells with less efficient responses to the virus. As shown in previous studies comparing different techniques of analysis of HIV-1-specific responses, the MHC tetramer-binding assay detects more HIV-1-specific CD8+ T cells than do other methods (*11, 12*). Therefore, the subpopulations of MHC tetramers-stained CD8+ T cells that are not effective in the control of viral replication, e.g., memory cells or functionally impaired cells, may superimpose on the effective cells and thus hide their association with viral load.

In conclusion, these results suggest that the frequencies of Gag- and Pol-specific CD8+ T cells detected by MHC tetramers are not associated with the control of viral replication during chronic HIV-1 infection both in naïve and HAART-treated patients. Therefore, future studies characterizing functional status of these cells are needed to improve our understanding of the role of HIV-1-specific CD8+ T cells in the protection against HIV-1 infection.

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HIV-1-positive patients	No. (%) of patients with CD8+ T cells specific to		Frequency[a] of CD8+ T cells specific to				Plasma HIV-1 RNA[b]
	Gag	Pol	Gag		Pol		
Naïve (n = 25)	12 (48)	6 (24)	0.34	(0.05–1.88)	0.09	(0.05–0.39)	22,500 (40–6,500,000)
HAART-treated (n = 38)	13 (34)	7 (18)	0.19	(0.05–1.59)	0.06	(0.05–0.34)	50 (50–132,000)

[a]Mean (range) percentage from total CD8+ T cells.
[b]Median (range) of HIV-1 RNA copies per ml.

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