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Residual Viral Replication during Antiretroviral Therapy Boosts Human Immunodeficiency Virus Type 1-Specific CD8$^+$ T-Cell Responses in Subjects Treated Early after Infection

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Human immunodeficiency virus type 1 (HIV-1)-infected subjects treated early after infection have preserved HIV-1-specific CD4$^+$ T-cell function. We studied the effect of highly active antiretroviral therapy (HAART) on the frequency of HIV-1-specific CD8$^+$ T cells in patients treated during early ($n = 31$) or chronic ($n = 23$) infection. The degree of viral suppression and time of initiation of treatment influenced the magnitude of the CD8$^+$ T-cell response. HIV-1-specific CD8$^+$ T cells can increase in number after HAART in subjects treated early after infection who have episodes of transient viremia.

Highly active antiretroviral therapy (HAART) can reduce levels of human immunodeficiency virus type 1 (HIV-1) in plasma to below the limit of detection. However, viral replication persists, as suggested by ongoing viral sequence evolution (14, 43), the presence of two-long-terminal-repeat episomal circles (36), the expression of viral mRNA in lymphoid cells and peripheral blood mononuclear cells (PBMCs) (16), the persistence of proviral DNA (10, 17), and the presence of low but detectable levels of viral mRNA and infectious virions in the plasma of infected subjects (9, 10, 42). Depending on the magnitude of residual viral replication during HAART, viral sequence evolution and drug resistance may result, thereby contributing to disease progression (24). Although the source of the residual virus is unknown, possibilities include latently infected CD4$^+$ T cells and de novo production from cellular or anatomic reservoirs protected from optimal levels of antiretroviral agents.

The relationship between antigen load and viral replication is complex (18), and the effect of residual viral replication on HIV-1-specific CD8$^+$ T-cell responses is not well understood. Antiviral CD8$^+$ T-cell responses correlate inversely with levels of plasma HIV-1 RNA in some subjects and usually decrease

TABLE 1. Demographic, virologic, and immunologic characteristics of subjects with early or chronic HIV-1 infection[a]

Group	Age (yrs)	M/F	Ethnicity	Approx duration of infection (pre-HAART)	Data obtained: Before HAART: HIV-1 RNA log (copies/ml)	Before HAART: CD4$^+$ T cells (cells/mm^3)	Before HAART: CD8+ T cells (cells/mm^3)	During HAART: CD4$^+$ T cells (cells/mm^3)	During HAART: CD8$^+$ T cells (cells/mm^3)	During HAART: Time to suppression BD (days)
Early infection ($n = 31$)	36	30/1	18 white 7 black 6 Hispanic	2 mo	5.6	503	1,117	708	745	126
Chronic infection ($n = 23$)	36	23/0	20 white 1 black 1 Hispanic 1 other	2.1 yr	5.3	381	706	53.7	769	135

[a] Except for ethnicity, numerical entries are mean values. Drug combinations used to treat subjects with early infection were zidovudine (AZT)-lamivudine (3TC)-abacavir (ABC)-amprenavir or ABC-3TC-amprenavir-indinavir. Drug combinations used to treat subjects with chronic infection were AZT-3TC-nelfinavir or AZT-3TC-ABC-amprenavir. CD4$^+$ and CD8$^+$ T-cell counts during HAART were averages of all counts available 150 days after initiation of HAART. M, male; F, female; BD, below limit of detection.

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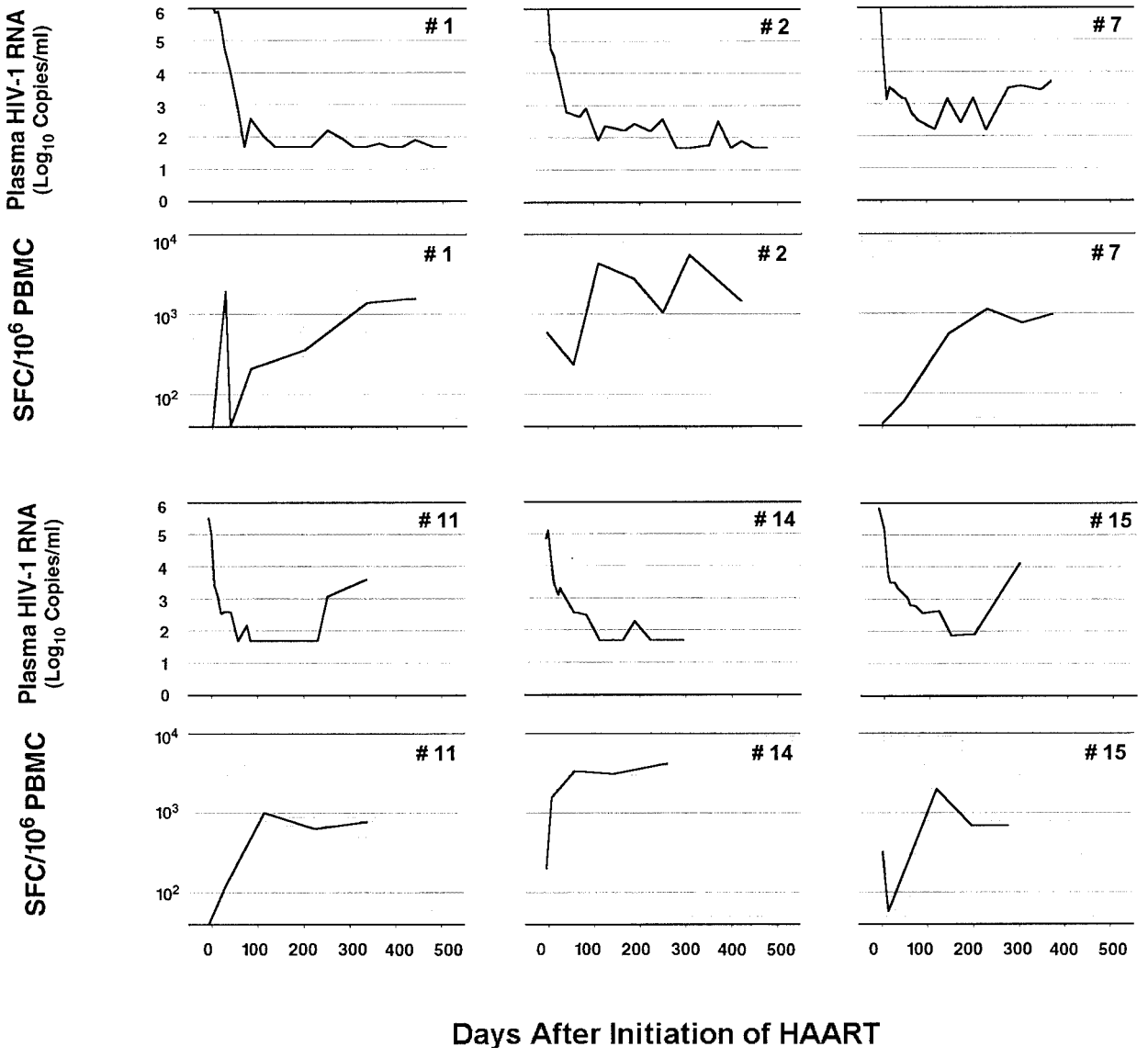

FIG. 1. Longitudinal measurements of plasma viral load and HIV-1-specific $CD8^+$ T-cell responses in six subjects with early HIV-1 infection who had at least two viremic episodes per year while on HAART. Total $CD8^+$ T-cell responses are expressed as spot-forming cells (SFC) per million PBMCs and represent the sum of responses against Env, Gag, Pol, and Nef.

in response to HAART (7, 8, 13, 19, 25–27, 30, 32, 34, 39, 41). However, antiviral $CD8^+$ T-cell responses can remain constant or even increase after initiation of HAART (2, 8, 25, 30, 32, 38). Whether responses are functional and capable of responding to antigen remains controversial (1, 3, 5, 11–13, 21, 35, 40).

To determine the effects of low-level virus replication on HIV-1-specific $CD8^+$ T-cell responses, we undertook a longitudinal study of subjects treated during early HIV-1 infection ($n = 31$) and subjects treated during the chronic phase of infection ($n = 23$). All were enrolled in HAART trials. The clinical and virologic characteristics of some of these subjects have been described elsewhere (33). The demographic, virologic, and immunologic characteristics of our subjects are shown in Table 1. The mean age of the subjects and the time required to suppress plasma virus load to <50 copies/ml on two consecutive clinical visits did not differ significantly between the groups. However, different patterns of viral load suppression within the groups were observed after initiation of HAART.

Plasma samples were assayed for HIV-1 RNA with a reverse transcriptase PCR assay (Roche Diagnostics, Alameda, Calif.) (lower limit of detection, 50 copies/ml) or a branched-DNA assay (Chiron, Emeryville, Calif.) (lower limit of detection, 50 copies/ml). Most subjects exhibited stable suppression of HIV-1 plasma viral load after initiation of HAART. Some subjects on HAART had intermittent viremic episodes, defined as time points at which the plasma HIV-1 load was detectable after having been stably suppressed below detection limits. In others, such suppression was not achieved and ongoing viremia was observed. In the latter subjects, viremic episodes were counted after 150 days on HAART, the time by which viremia had been stably suppressed in the majority of

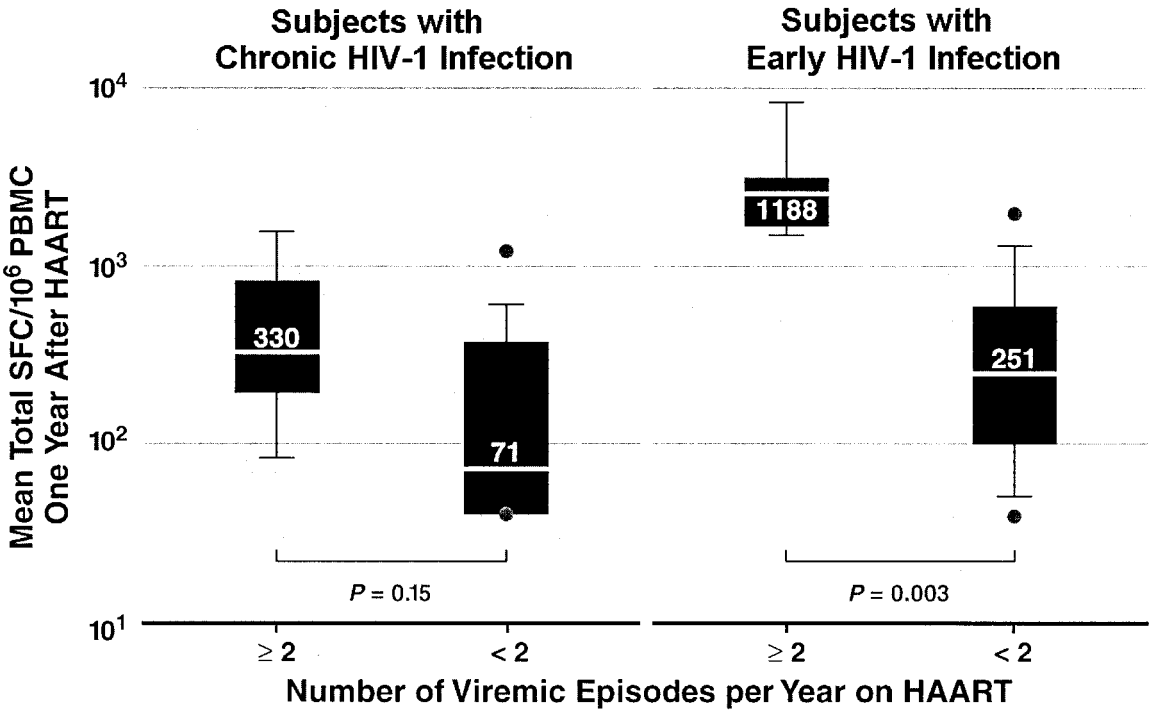

FIG. 2. Box plot comparisons of total HIV-1-specific $CD8^+$ T-cell responses after 1 year of HAART in subjects treated during early or chronic HIV-1 infection who had less than two or two or more viremic episodes per year. Median values are shown within the boxes. Bars, 10th and 90th percentiles; dots, 5th and 95th percentiles. *P* values were determined by rank-sum *t* test. SFC, spot-forming cells.

subjects. The subjects were divided into those with less than two and those with two or more viremic episodes per year while on HAART. A recent study has shown that the occurrence of at least two intermittent viremic episodes per year is associated with an increase in the half-life of the latent reservoir of HIV-1 (33). Twenty-five patients treated during early infection and 15 treated during chronic infection had fewer than two viremic episodes per year, whereas six treated during early infection and eight treated during chronic infection had two or more viremic episodes per year. Plasma viral profiles of patients treated during early infection who had at least two viremic episodes per year are shown in Fig. 1.

HIV-1-specific $CD8^+$ T-cell responses were measured with a recombinant vaccinia virus-based gamma interferon enzyme-linked immunospot (ELISPOT) assay as described elsewhere (22), with minor variations. PBMCs were infected with recombinant vaccinia viruses expressing the HIV_{IIIB} proteins Env-gp160, Gag-p55, Pol, and Nef (Therion Biologics, Cambridge, Mass.). The negative control was a vaccinia virus with a deletion in the thymidine kinase gene, where recombinant genes were inserted (Therion Biologics), and the positive control was phytohemagglutinin (Sigma, St. Louis, Mo.) at 10 μg/ml. $CD8^+$ T cells are the predominant cell type producing gamma interferon in this assay (15, 22, 37).

Immune responses were measured longitudinally during HAART. Interestingly, some members of each group had increases in HIV-1-specific $CD8^+$ T-cell responses while on HAART (Fig. 1). PBMC samples obtained closest to 1 year after initiation of HAART were used for direct cross-sectional comparison of HIV-1-specific $CD8^+$ T-cell responses.

Among patients treated during early HIV-1 infection, the total HIV-1-specific $CD8^+$ T-cell response was greater in the six subjects who averaged two or more viremic episodes per year while on HAART than in those in whom HIV-1 replication was relatively well suppressed (Fig. 2). A similar trend was noted among patients treated during chronic infection, but the difference was not statistically significant. The total HIV-1-specific $CD8^+$ T-cell response increased in only three of eight subjects treated during chronic infection who had two or more viremic episodes per year while on HAART (data not shown). In subjects treated during early HIV-1 infection with at least 300 days of immunologic follow-up, a positive correlation was found between total HIV-1-specific as well as Pol-specific $CD8^+$ T-cell response and number of viremic episodes ($P = 0.05$ and 0.03, respectively).

We propose an antigenic-threshold model for the relationship between HIV-1-specific $CD8^+$ T-cell responses and residual viral replication. Augmentation of memory $CD8^+$ T-cell responses requires sufficient levels of antigen, functional T-helper-cell responses, and intact antigen presentation networks (6, 20, 29, 31). HIV-1 infection appears to disturb both the antigen presentation networks and the T-helper-cell responses (2, 4, 23, 28), and the disturbances may be greater in subjects treated during chronic infection than in those treated during early infection (2, 6). These disturbances would affect the ability of $CD8^+$ T cells to respond to antigen. In our study, only subjects treated during early infection had significant increases in the magnitude of HIV-1-specific $CD8^+$ T-cell responses associated with viremic episodes. Thus, subjects treated during early infection may have a lower antigenic threshold because

their immune responses are better preserved, as reflected in their higher T-helper-cell responses and intact antigen presentation networks. These findings may partly explain why some subjects have increases in HIV-1-specific $CD8^+$ T-cell responses after initiating HAART.

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