

CYTOTOXIC-T-CELL RESPONSES, VIRAL LOAD, AND DISEASE PROGRESSION  
IN EARLY HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTIONLUWY MUSEY, M.D., JAMES HUGHES, PH.D., TIMOTHY SCHACKER, M.D., THERESA SHEA, P.A.C., LAWRENCE COREY, M.D.,  
AND M. JULIANA McELRATH, M.D., PH.D.**ABSTRACT**

**Background** Early in human immunodeficiency virus type 1 (HIV-1) infection there is a decline in viral replication that has been attributed to host immunity, but the components of this response, particularly the ability of cytotoxic T lymphocytes to control viral burden and influence the outcome of disease, are poorly understood.

**Methods** We prospectively studied 33 patients with primary HIV-1 infection for HIV-specific activated cytotoxic T lymphocytes and memory cytotoxic T lymphocytes and compared these lymphocyte responses with changes in viral load and clinical status over the subsequent 18 to 24 months.

**Results** Soon after infection, activated HIV-specific cytotoxic T lymphocytes, mediated primarily by CD8<sup>+</sup> cells, were detected in 17 of 23 patients (74 percent). Memory cytotoxic T lymphocytes were found in 6 of 6 patients tested (100 percent) during the first three months of infection and in 17 of 21 patients (81 percent) tested during the first six months. The frequencies of memory cytotoxic T lymphocytes varied markedly over time, but overall they declined over the first 6 to 8 months and then stabilized over the next 12 to 18 months. The patients with higher frequencies of Env-specific memory cytotoxic T lymphocytes had a median level of plasma HIV-1 RNA about one third that of the patients with lower frequencies (median number of RNA copies per milliliter, 22,000 vs. 62,000;  $P=0.006$ ). Patients with low frequencies of Env-specific memory cytotoxic T lymphocytes (or none) in early infection had a more rapid decline to less than 300 CD4<sup>+</sup> cells per cubic millimeter ( $P=0.05$ ).

**Conclusions** In early HIV-1 infection, the induction of memory cytotoxic T lymphocytes, particularly those specific for Env, helps control viral replication and is associated with slower declines in CD4<sup>+</sup> cell counts. Host cytolytic effector responses appear to delay the progression of HIV-1 disease. (N Engl J Med 1997; 337:1267-74.)

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**W**ITHOUT antiretroviral therapy, most people with human immunodeficiency virus type 1 (HIV-1) infection will have the acquired immunodeficiency syndrome (AIDS) within a decade. Many clues about the pathogenesis of HIV-1 have come from the study of patients with unusually rapid or unusually slow progression of disease. Accumulating evidence

indicates that both host and viral factors affect disease progression.<sup>1-7</sup> Several studies, mostly cross-sectional in design, have shown that host immunity to HIV-1 may be related to disease progression.<sup>8-11</sup> In particular, HIV-1-specific cytotoxic T lymphocytes decrease in frequency over time, and persons with detectable cytotoxic T lymphocytes tend to have stable clinical disease. In contrast, more recent studies show that higher plasma levels of HIV-1 RNA are the strongest independent predictors of progression to AIDS and death.<sup>12-14</sup>

Primary infection is typically associated with initially high levels of plasma HIV-1 RNA that subsequently decline. HIV-1-specific cytotoxic T lymphocytes can appear early, even before seroconversion, and their emergence may coincide with a decline in viral load.<sup>15,16</sup> Exceptions have been found,<sup>17</sup> however, and it has been argued that the decline in viremia is largely attributable to the lack of available CD4<sup>+</sup> target cells for infection.<sup>18</sup> After approximately six months of infection, plasma viremia reaches a quasi-steady-state level,<sup>12-14</sup> and it has been hypothesized that host immunity must have a critical role in achieving that equilibrium.<sup>19,20</sup> Thus, the critical events governing the variability in disease progression must include both viral replication and immune responses, and it is likely that these may be determined in early infection.

We undertook a prospective study of 33 patients with primary HIV-1 infection to determine the extent to which the responses of cytotoxic T lymphocytes induced during this critical early period are associated with the level of viral replication over the first one to two years and their relation to the subsequent course of disease.

**METHODS****Study Patients**

We studied the first 33 consecutive persons enrolled at the Primary HIV Infection Clinic of the University of Washington.<sup>21</sup> Patients at risk who had HIV-related symptoms or tested HIV-positive for the first time on routine testing were referred from the

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university clinics, AIDS-prevention programs, jail facilities, and physicians in the community. A time of infection was assigned for each patient that represented the first day of clinical illness in 31 patients (94 percent) and the halfway point between a negative and a positive serologic test for HIV-1 in 2 patients (6 percent). Five healthy, HIV-1-seronegative volunteers served as controls in the assays of cytotoxic T lymphocytes. The Human Subjects Review Board of the University of Washington approved all aspects of the study.

Patients were screened for HIV-1 antibodies as previously described.<sup>21</sup> Plasma HIV-1 RNA levels were determined by the branched-chain DNA amplification method<sup>22</sup>; the lower level of sensitivity of the assay was 10,000 copies per milliliter. The HIV-1 titer in peripheral-blood mononuclear cells was measured by quantitative cell microculture.<sup>23</sup> T-cell subgroups were counted by flow cytometry.<sup>21</sup>

### Assays of Cytotoxic T Lymphocytes

Vectors containing recombinant vaccinia virus were used to express HIV-1 or control gene products. The recombinant vaccinia virus encoding HIV-1<sub>LAI</sub> *env* (vPE-16),<sup>24</sup> HIV-1<sub>LAI</sub> *gag* (vDK-1),<sup>25</sup> HIV-1<sub>LAI</sub> *pol* (vRT),<sup>26</sup> and *lacZ* (vSC-8, used as a control)<sup>27</sup> were obtained through the AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). The recombinant vaccinia virus encoding the HIV-1<sub>LAI</sub> gene products Env, Gag, and Pol (vEGP) was kindly provided by D. Panicali (Therion Biologics, Cambridge, Mass.).

Activated cytotoxic T lymphocytes were measured in the first consecutive 21 volunteers and in 6 other volunteers enrolled soon thereafter, on the basis of the availability of autologous B-lymphoblastoid cell lines. Fresh peripheral-blood mononuclear cells and CD4- and CD8-enriched T cells were used as effector cells. To enrich the cell population for T-cell subgroups and remove natural killer cells, the peripheral-blood mononuclear cells were incubated with anti-Leu-11 (CD16) and either anti-Leu-2A (CD8) or anti-Leu-3A (CD4) monoclonal antibodies (Becton Dickinson, San Jose, Calif.), and were then negatively selected after incubation with goat antimouse IgG-coated beads (Dynal, Lake Success, N.Y.). The purity of the T-cell subgroups, as determined by flow cytometry, ranged from 92 to 98 percent.

Autologous Epstein-Barr virus (EBV)-transformed B-lymphoblastoid cell lines were established from donor peripheral-blood mononuclear cells<sup>28</sup> and infected with recombinant vaccinia virus. K562 cells (American Type Culture Collection CCL243) served as targets to measure the lytic activity of natural killer cells. Target cells labeled with chromium-51 and effector cells were distributed into 96-well round-bottomed plates (Costar, Cambridge, Mass.) in triplicate, and the chromium-release assay was performed.<sup>29</sup> The spontaneous release of chromium-51 was less than 25 percent in all experiments. HIV-1-specific lysis was determined by calculating the difference between the percentage of lysis of target cells expressing the HIV-1 gene product and that of the target cells infected with vSC-8; lysis of 10 percent or more was considered positive. The responses were considered negative if the HIV-1 and natural-killer lytic activities in the undepleted peripheral-blood mononuclear cells were both greater than 10 percent but HIV-specific lysis in the populations enriched with CD4, CD8, or both was less than 10 percent.

The frequencies of precursor cytotoxic T lymphocytes were measured by limiting-dilution assay in 30 consecutively enrolled volunteers, including 6 enrolled within the first 90 days after infection and 21 enrolled in the first 6 months. Adherent autologous monocytes were infected with vEGP, irradiated with ultraviolet and gamma radiation, and used as stimulator cells.<sup>29</sup> Serial dilutions of fresh peripheral-blood mononuclear cells in RPMI with 10 percent heat-inactivated human AB serum (Biocell, Rancho Dominguez, Calif.) were plated in 96-well round-bottomed plates in 24-well replicates. Stimulator cells (1000 cells), irradiated autologous peripheral-blood mononuclear cells (50,000 cells), and 20 U of human recombinant interleukin-2 (Chiron, Em-

eryville, Calif.) were added to each well. The cultures were incubated at 37°C in 5 percent carbon dioxide and provided with fresh medium on days 3 and 7. On day 10, the cells from each well were divided equally into four wells and tested to determine the lysis of 5000 radiolabeled autologous target cells previously infected with vPE-16, vDK-1, vRT, or vSC-8. Sixteen wells of each of the four infected target cells were incubated with medium, and 16 wells were incubated with 5 percent Triton-X, to determine the spontaneous and maximal release of chromium, respectively. After a four-hour incubation at 37°C, the supernatants were harvested and the release of chromium was measured. The spontaneous release was less than 20 percent of the maximal release, with a standard deviation of less than 15 percent in the replicate wells. In wells associated with lysis that exceeded the mean spontaneous release by 3 SD, the results were considered positive. The frequencies of memory cytotoxic T lymphocytes were estimated from the initial number of responder cells at which 37 percent of the wells were negative for cytotoxicity on the basis of the single-hit Poisson model.<sup>30</sup> The calculations were performed on the basis of the chi-square minimization method<sup>31</sup> with software<sup>32</sup> kindly provided by Dr. C. Orosz (Ohio State University, Columbus).

### Statistical Analysis

Descriptive statistics (medians, ranges, and percentages of patients with positive results) were used to summarize the data. When the measures of cytotoxic T lymphocytes were plotted against the time since infection, all the observations in one person were connected by dashed lines to show the within-person and between-person variability. The overall trends were characterized by a solid, smooth line generated by the loess procedure.<sup>33</sup>

Because of restrictions in the available volume of blood, measurements of frequencies of memory cytotoxic T lymphocytes and viral load or CD4+ counts in the same blood sample were available for only one third of the data set. Therefore, to explore the relation between memory cytotoxic T lymphocytes and the viral load or CD4+ count, we paired each observation of memory cytotoxic T lymphocytes with the closest measurement of viral load or CD4+ cells, within a range of three weeks. This increased the percentage of paired measurements to 86 percent. Spearman's rank-correlation coefficient was used to describe the strength of the association between the frequency of memory cytotoxic T lymphocytes and the viral load or CD4+ count. A bootstrap procedure (in which persons rather than observations were resampled) was used to assess the significance of the correlation coefficients.<sup>34</sup> Generalized estimating equations were used to compare log RNA levels above and below the median frequency of memory cytotoxic T lymphocytes, or 5 per million peripheral-blood mononuclear cells.

Patients for whom measurements of memory cytotoxic T lymphocytes were available within the six-month period after infection (19 of the 30 patients for whom data on cytotoxic T lymphocytes were available) were classified as having either high or low frequencies of Env-specific memory cytotoxic T lymphocytes. The frequency was high if the first measurement was above the smoothed line for trend, and low if the measurement was on or below that line. Kaplan-Meier methods were then used to compute the time to the first CD4+ cell count of less than 300 cells per cubic millimeter for the patients in these two groups, and a log-rank test was used to compare the two curves. All calculations of P values were two-tailed.

## RESULTS

### Study Patients

Thirty-three adults with primary HIV-1 infection (median age, 33 years; one patient was female) were studied over the first two years of their infection. The demographic and clinical characteristics of this group were similar to those of the larger cohort pre-

viously described.<sup>21</sup> The median time of enrollment was 55 days after HIV-1 infection, and the median CD4 count was 568 cells per cubic millimeter (Table 1). No patient reported use of antiretroviral drugs at enrollment, and only 15 percent received treatment during the subsequent months. Ninety-four percent of the patients with acute infection had symptoms (Table 1). The median interval from the onset of symptoms to the first analysis of cytotoxic T lymphocytes was 61 days.

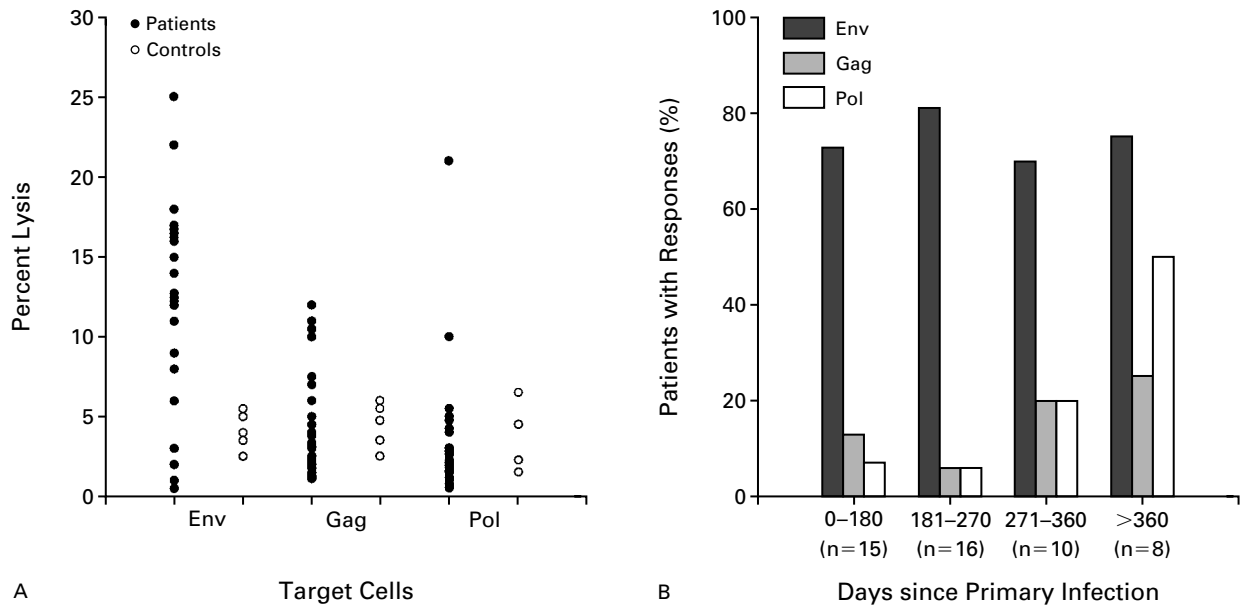
**HIV-Specific Activated Cytotoxic T Lymphocytes**

Fresh peripheral-blood mononuclear cells obtained from 23 patients soon after presentation (median, 61 days after infection) were tested for their ability to recognize target cells expressing HIV-1 gene products. Activated HIV-specific cytotoxic T lymphocytes were detected in 17 of these patients (74 percent) (Fig. 1A). The HIV-specific lysis ranged from 10 to 26 percent among responding patients at an effector:target cell ratio of 100:1, and it was less than 10 percent in the five HIV-1-seronegative control do-

**TABLE 1.** CLINICAL VARIABLES IN THE 33 PATIENTS WITH PRIMARY HIV-1 INFECTION.

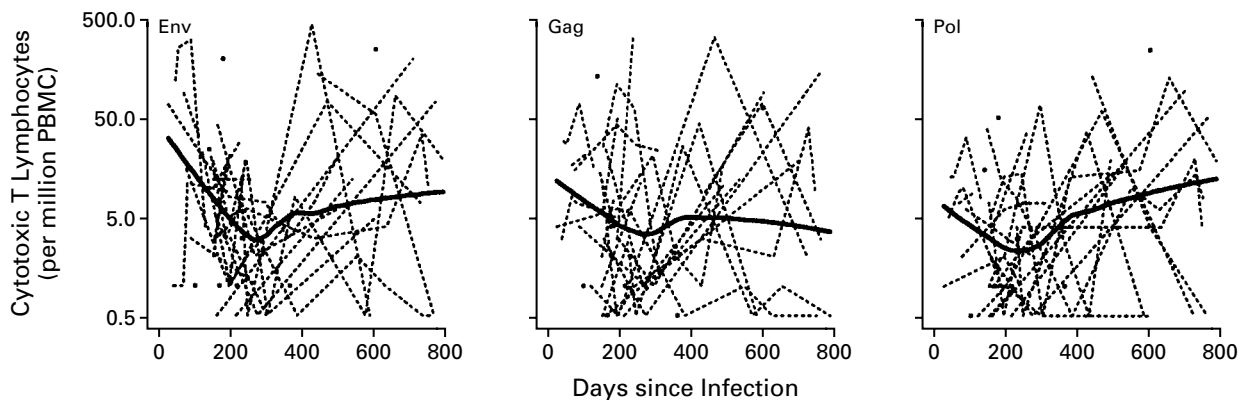
VARIABLE	VALUE
Days since infection	
Median	55
25th–75th percentile	32–101
CD4 count — cells/mm <sup>3</sup>	
Median	568
25th–75th percentile	429–667
CD8 count — cells/mm <sup>3</sup>	
Median	1035
25th–75th percentile	715–1311
Symptoms associated with primary infection	
— no. of patients (%)*	
None	2 (6)
Mild	5 (15)
Moderate	10 (30)
Severe	16 (48)
Days from initial symptoms to first analysis of memory cytotoxic T lymphocytes	
Median	61
25th–75th percentile	30–84

\*The classification of symptoms was as defined by Schacker et al.<sup>21</sup>



**Figure 1.** Responses of Activated Cytotoxic T Lymphocytes to Specific HIV-1 Gene Products.

Panel A shows the responses of activated cytotoxic T lymphocytes in 23 patients with primary HIV-1 infection who were tested a median of 61 days after the infection and in 5 HIV-uninfected controls. Freshly isolated effector peripheral-blood mononuclear cells were tested without in vitro stimulation to assess the lysis of autologous target cells infected with vPE-16 (Env), vDK-1 (Gag), vRT (Pol), or vSC-8 (control) at an effector:target ratio of 100:1. Panel B shows the proportion of patients with primary HIV-1 infection who had activated cytotoxic-T-lymphocyte responses to autologous target cells expressing HIV-1 Env, Gag, or Pol during four periods over the course of infection. The number of patients studied in each period is shown below the graph.



**Figure 2.** Longitudinal Analysis of Memory Cytotoxic T Lymphocytes during the 800 Days after Infection.

Each data point represents a measurement of the frequency of cytotoxic T lymphocytes (per million peripheral-blood mononuclear cells [PBMC]) against autologous target cells expressing HIV-1 Env, Gag, and Pol. The dashed lines connect three or more measurements in each patient. The solid lines showing overall trends were drawn through the data with use of the loess procedure (see the Methods section).

nors (Fig. 1A). The most common gene product to be recognized initially was HIV-1 Env, found in 94 percent of the patients with positive responses; by contrast, HIV-1 Gag and Pol were recognized less frequently, in 29 percent and 12 percent, respectively (Fig. 1A).

Activated cytotoxic T lymphocytes were mostly mediated by CD8<sup>+</sup> effector cells, observed in 9 of 19 patients (47 percent) at an effector:target ratio of 25:1. The CD8<sup>+</sup> effectors primarily recognized Env-expressing targets in these patients (in eight patients, or 42 percent); they recognized Gag-expressing (in three patients, or 16 percent) and Pol-expressing (in one patient, or 5 percent) targets less often. CD4<sup>+</sup> effector cells were also detected in 4 of 18 patients (22 percent). The natural-killer-specific lysis was less than 10 percent among effector-cell populations with either CD8<sup>+</sup> or CD4<sup>+</sup> HIV-1-specific cytotoxicity. Furthermore, low-level lysis of mismatched target cells was easily distinguishable from the lysis of autologous targets expressing HIV-1 Env in four patients (data not shown), indicating that the cytotoxic activities were unlikely to represent antibody-dependent cellular cytotoxicity but instead represented major-histocompatibility-complex-restricted cytotoxic T lymphocytes.

The frequency of patients with activated cytotoxic-T-lymphocyte effector cells was similar when the patients were studied at intervals of three to six months (Fig. 1B). The predominant activated cytolytic responses were again Env-specific: over the one-to-two-year period, 70 to 80 percent had detectable Env-specific cytotoxic T lymphocytes, whereas less than 50 percent had Gag-specific or Pol-specific responses (Fig. 1B). Among patients in whom concurrent measurements of activated and memory

cytotoxic T lymphocytes were made, activated cytotoxic T lymphocytes were detected only when memory responses were present.

#### Frequencies of HIV-Specific Memory Cytotoxic T Lymphocytes

The frequencies of HIV-1-specific memory cytotoxic T lymphocytes reached peak levels during primary infection and declined over the next 200 to 300 days (Fig. 2). All six patients tested during the first 90 days of infection had Env- and Gag-specific responses. When examined at intervals of three to six months, over 80 percent had detectable HIV-1-specific cytotoxic T lymphocytes. The frequencies of memory cytotoxic T lymphocytes per million peripheral-blood mononuclear cells ranged from 0 to 446 for Env, 0 to 333 for Gag, and 0 to 364 for Pol (Fig. 2). By contrast, the frequencies of memory cytotoxic T lymphocytes specific for HIV-1 Env, Gag, and Pol were less than 0.1 per million in peripheral-blood mononuclear cells from five healthy HIV-seronegative donors on repeated testing. The precursor frequencies measured in assays with the control targets infected with the vSC-8 were less than 3 memory cytotoxic T lymphocytes per million peripheral-blood mononuclear cells in all patients studied. The coefficient of variation for the frequencies of memory cytotoxic T lymphocytes against the same HIV-1 gene product was less than 12 percent (range, 3 to 11 percent). Repeated measurements of frequencies over time in a given person revealed considerable variability, as Figure 2 shows. More often, cytotoxic-T-lymphocyte precursors specific for one gene product predominated, particularly those for HIV-1 Env. In some cases, the predominant gene product recognized early was replaced by another over time (data not shown).

### Correlation of Memory Cytotoxic T Lymphocytes with Viral Load

To determine whether the cytolytic responses in patients with primary infection correlated with the concurrent viral load, we paired the log<sub>10</sub>-transformed frequencies of memory cytotoxic T lymphocytes with measurements of HIV-1 RNA in plasma and peripheral-blood-mononuclear-cell-associated infectious HIV-1 from blood collected the same day or within a range of three weeks (Table 2). There was an overall inverse correlation between the frequencies of Env-specific memory cytotoxic T lymphocytes and plasma HIV-1 RNA ( $P=0.02$ ) and infectious virus ( $P=0.1$ ). A similar trend was found between the frequencies of Gag-specific memory cytotoxic T lymphocytes and infectious virus ( $P=0.06$ ) but not plasma HIV-1 RNA ( $P=0.22$ ) (Table 2). When the data were stratified into three time periods (less than 6 months, 6 to 12 months, and more than 12 months after infection), the strongest inverse correlation, albeit not a statistically significant one, between Env-specific memory cytotoxic T lymphocytes and viral load was seen after 12 months of infection.

To ascertain the magnitude of the effect of cytotoxic T lymphocytes on viral load throughout the study period, median plasma HIV-1 RNA levels (either concurrent ones or the closest within three weeks) were compared with the frequencies of memory cytotoxic T lymphocytes that fell either above or at or below the overall median level of memory cytotoxic T lymphocytes (5 per million peripheral-blood mononuclear cells) (Table 3). The number of copies of HIV-1 RNA per milliliter was significantly lower in the group with higher frequencies of Env-specific cytotoxic T lymphocytes (median, 22,000) than in those with lower frequencies (median, 62,000;  $P=0.006$ ). Thus, higher frequencies of Env-specific memory cytotoxic T lymphocytes were associated with, on average, one third the level of plasma RNA that was observed in patients with lower frequencies.

### Correlation of Cytotoxic T Lymphocytes with CD4+ Counts and Disease Progression

We performed a similar analysis comparing concurrent measurements of frequencies of memory cytotoxic T lymphocytes and CD4+ cell counts (Table 2). There was a trend toward higher frequencies of Env-specific but not Gag- or Pol-specific memory cytotoxic T lymphocytes correlating with overall higher CD4+ cell counts ( $P=0.09$ ).

We next studied the relation between frequencies of memory cytotoxic T lymphocytes and disease progression, as evidenced by declining CD4+ cell counts. We divided the patients into two groups according to whether the response of memory cytotoxic T lymphocytes was high or low in the first six

**TABLE 2.** RANK CORRELATION BETWEEN THE FREQUENCY OF PRECURSOR CYTOTOXIC T LYMPHOCYTES AND THE VIRAL LOAD AND CD4+ COUNT.\*

VARIABLE AND INTERVAL SINCE INFECTION	NO. OF OBSERVATIONS	CYTOTOXIC T LYMPHOCYTES		
		ENV-SPECIFIC	GAG-SPECIFIC	POL-SPECIFIC
correlation coefficient				
Plasma RNA				
Overall study	79	-0.29†	-0.20	-0.02
<6 mo	29	-0.08	-0.32‡	-0.04
6-12 mo	28	-0.30	-0.20	-0.03
>12 mo	22	-0.41	-0.14	-0.08
HIV-1 titer in PBMC				
Overall study	79	-0.22‡	-0.26‡	0.17
<6 mo	28	0.01	0.004	0.27
6-12 mo	27	-0.11	-0.33	0.17
>12 mo	24	-0.57‡	-0.45‡	0.02
CD4+ count				
Overall study	79	0.25‡	0.07	-0.10
<6 mo	30	-0.03	-0.13	-0.23
6-12 mo	27	0.29	-0.05	0.04
>12 mo	22	0.44	0.32	-0.09

\*The CD4+ counts and measurements of viral load (HIV-1 titers in peripheral-blood mononuclear cells [PBMC] and plasma RNA levels as determined by branched-chain DNA amplification) were those obtained closest to the time the precursor cytotoxic T lymphocytes were measured, within a range of three weeks. The resulting data were log-transformed for this analysis of correlation coefficients.

† $P=0.01$  to  $0.05$ .

‡ $P=0.05$  to  $0.10$ .

months after infection and compared the groups' CD4+ counts over time (see the Methods section). After 18 months, 8 of the 19 patients in whom measurements of memory cytotoxic T lymphocytes were available (42 percent) had declines in their CD4+ counts to less than 300 cells per cubic millimeter (Fig. 3). Among these 19 patients, 80 percent of those with initial Env-specific responses maintained CD4+ counts greater than 300 cells per cubic millimeter, as compared with only 44 percent of those without such responses ( $P=0.05$ ). The majority of the difference was apparent within the first six months of infection. After 18 months, the patients whose initial frequencies of Env-specific memory cytotoxic T lymphocytes were higher had on average 90 more CD4+ cells per cubic millimeter than those with lower frequencies, although the difference was not statistically significant.

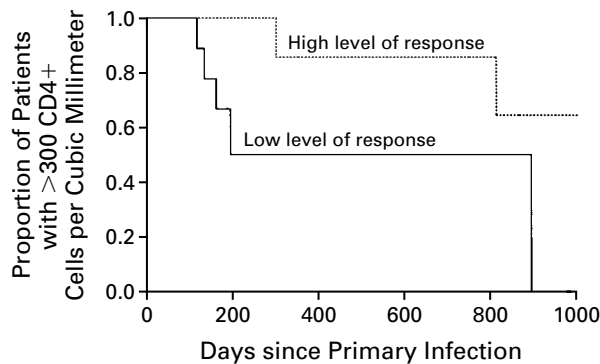
### DISCUSSION

Although several studies have suggested that cytolytic responses play a key part in controlling HIV-1 infection, this study provides longitudinal data on the frequency and kinetics of the cytotoxic-T-lymphocyte response after HIV-1 infection, as well as the temporal relation between cytotoxic T lymphocytes, viral load, and the decline in CD4+ cells. Our

**TABLE 3.** PLASMA HIV-1 RNA LEVELS IN PATIENTS STRATIFIED ACCORDING TO THE OVERALL FREQUENCY OF PRECURSOR CYTOTOXIC T LYMPHOCYTES (pCTL).\*

SPECIFICITY OF LYMPHOCYTES	FREQUENCY OF LYMPHOCYTES		P VALUE
	≤5 PER MILLION PBMC	>5 PER MILLION PBMC	
	median no. of RNA copies/ml (25th–75th percentile)		
Env pCTL	62,000 (19,000–103,000)	22,000 (<10,000–53,000)	0.006
Gag pCTL	55,000 (12,000–103,000)	25,000 (12,000–53,000)	0.16
Pol pCTL	35,000 (10,000–72,000)	49,000 (14,000–81,000)	0.47

\*Plasma RNA levels were measured by branched-chain DNA amplification within three weeks before or after the measurement of cytotoxic T lymphocytes. The patients were stratified according to whether their frequencies were above or at or below the overall median frequency of precursor cytotoxic T lymphocytes (5 per million peripheral-blood mononuclear cells [PBMC]).



**Figure 3.** Kaplan–Meier Analysis of Cumulative Rates of Progression to a CD4+ Count of 300 Cells or Fewer per Cubic Millimeter in 19 Patients with High and Low Levels of Response of Cytotoxic T Lymphocytes to HIV-1 Env in the Six Months after Infection.

In this analysis, the patients were stratified according to whether the first measurement of the Env-specific cytotoxic-T-lymphocyte response was above or below the smooth trend line shown in Figure 2.

study clearly shows that in early HIV-1 infection there is a profound induction of virus-specific cytotoxic T lymphocytes and that the levels of these responses decrease gradually and stabilize within the first year of infection. This pattern resembles the overall trends in viral load but occurs slightly later. In addition to the fluctuations we observed in the overall frequencies of memory cytotoxic T lymphocytes, there was remarkable variation in the individual responses when measurements were repeated over time.

To assess the magnitude and kinetics of memory cytotoxic T lymphocytes in early infection, we used a limiting-dilution assay that did not stimulate detectable *in vitro* primary cytotoxic T lymphocytes, averted the concurrent expansion of nonspecific ef-

factor cells, and was reproducible when the same specimen was measured repeatedly. Comparative studies in our laboratory indicate that this method results in frequencies of memory cytotoxic T lymphocytes as much as 1 to 2 log lower than previously reported methods.<sup>9,10,35</sup> Although we restricted our analysis to the recognition of epitopes of HIV-1<sub>LAI</sub> expressed on target cells, the response patterns might have been different if we had used epitopes from more divergent or autologous strains.

This study demonstrates the predominance of Env-specific cytotoxic T lymphocytes in early infection, a finding consistent with previous reports.<sup>16,36,37</sup> These results suggest that in early infection HIV-1 envelope epitopes may be immunodominant and that over time, in association with virus variation and escape, the repertoire of cytotoxic T lymphocytes may broaden as other epitopes exert greater pressure on the immune system. The evolution of virus-specific cytotoxic T lymphocytes actually parallels the patterns of viral diversity that have been described in homosexual men (the principal risk group in this study), in which the early viral populations are relatively homogeneous within the envelope region.<sup>38</sup> Thus, Env-specific cytotoxic T lymphocytes may contribute the major effector responses in patients soon after the acquisition of HIV-1 infection.

Our findings indicate that higher frequencies of HIV-1 Env-specific and to a lesser extent Gag-specific cytotoxic T lymphocytes correlate with lower levels of plasma HIV-1 RNA and peripheral-blood-mononuclear-cell-associated infectious virus. When we studied specific periods after infection to understand when this effect is strongest, the correlation was not found in the early period (up to six months after infection), when typically there are marked changes in measurements of both memory cytotoxic T lymphocytes and viral load, but it became more apparent as time passed. Although the magnitude of

the response of cytotoxic T lymphocytes in reducing viral load was less than that achievable with potent antiretroviral therapy, we believe a reduction in plasma HIV-1 RNA by a factor of nearly three is not trivial. Moreover, such an effect, if sustained, can have prognostic implications with respect to rates of CD4 decline and disease progression.<sup>13</sup>

These analyses do not prove that the cytolytic effector cells detected by an *in vitro* assay are actually destroying virus-infected cells *in vivo*. However, there is evidence in other viral diseases that such activity does correlate with *in vivo* antiviral responses or the disease course,<sup>39-41</sup> through either direct lysis of infected cells or the release of antiviral cytokines. Why cytotoxic T lymphocytes directed specifically to the HIV-1 envelope are associated with lower viral loads is not known, but perhaps these lymphocytes have greater avidity for virus-infected cells. Alternatively, the Env-specific cytotoxic T lymphocytes may be mediated by both CD4+ and CD8+ effector cells,<sup>29</sup> the combination of which may provide two distinct pathways with which to recognize and attack virus-infected cells.

Our findings indicate that virus-specific cytotoxic T lymphocytes may contribute to the control of early HIV-1 infection by reducing the viral load and slowing the progression of disease. Given the wide fluctuations in cytotoxic T lymphocytes and viral load in a given person, particularly in the early months of infection, the correlation of cytotoxic-T-lymphocyte responses with viral load and the CD4 count is best seen longitudinally rather than cross-sectionally. Although the strongest effect of cytotoxic T lymphocytes appears later in the course of infection, future studies in patients identified very soon after their exposure to HIV-1 should give more insight into the effect of the early induction of cytotoxic T lymphocytes on the long-term progression of disease.

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