Longitudinal Analysis of Human Immunodeficiency Virus Type 1–Specific Cytotoxic T Lymphocyte Responses: A Predominant Gag-Specific Response Is Associated with Nonprogressive Infection

Oscar Pontesilli, Michèl R. Klein,* Susana R. Kerkhof-Garde, Nadine G. Pakker, Frank de Wolf, Hanneke Schuitemaker, and Frank Miedema

To establish correlates of protective immunity during human immunodeficiency virus type 1 (HIV-1) infection, the frequencies of circulating cytotoxic T lymphocyte (CTL) precursors (p) directed against 4 HIV-1 gene products (reverse transcriptase, gag, nef, and env) were evaluated in HIV-1–infected homosexual men who progressed to AIDS and in long-term survivors over time. For both groups, HIV-1–specific CTL responses had similar kinetics and magnitude. At maximum expansion, HIV-1–specific CTLp had a median frequency of 0.2% mononuclear cells in both progressors and long-term survivors, with peaks of 0.5% and 2%, respectively. Long-term survivors maintained the established CTLp pool and presented a persistently predominant gag-specific response. The fraction and, to a lesser extent, the frequency of gag-specific CTLp were inversely correlated with virus load. In progressors, general T cell function and measurable HIV-1–specific CTLp frequencies dropped simultaneously, suggesting a further loss of virus control due to the ensuing immunodeficiency.

Cytotoxic T lymphocytes (CTL) are widely regarded as the most efficient component of the immune system in controlling viral infections [1]. In human immunodeficiency virus type 1 (HIV-1) infection, the temporal relationship between the early recruitment of virus-specific CTL and the reduction of initial viremia has been interpreted as active control of HIV-1 replication by the immune response [2–5]. In addition, vigorous and broadly directed HIV-1–specific CTL responses coinciding with low numbers of circulating HIV-1–infected CD4 T cells are detected in most asymptomatic persons [6–8], suggesting that HIV-1–specific CTL may help to prevent viral replication.

In contrast to the beneficial role of HIV-1–specific CTL, it is well established that most HIV-1–infected persons eventually develop AIDS, indicating that antiviral CTL responses do not provide absolute protection. In addition, it has been suggested that HIV-1–specific CTL might even be the major cause of progressive immunodeficiency, since CTL eliminate HIV-1–infected immunocompetent CD4 T cells and macrophages [9, 10] and mediate inflammatory reactions in some AIDS-related disorders [11, 12].

Detailed longitudinal evaluations of the relationship between dominant HIV-1–specific CTL responses and markers of disease progression are still lacking. The current knowledge was mainly derived from cross-sectional studies [13, 14] and by longitudinal analysis of CTL specific for selected viral antigens [8, 15]. Therefore, we quantified the circulating precursors of CTL (CTLp) directed against 4 major gene products of HIV-1 (gag, reverse transcriptase [RT], env, and nef) over time in parallel with determination of CD4 T cell counts, general T cell function, and virus load in patients who rapidly progressed to AIDS and compared these findings with those of subjects with prolonged asymptomatic HIV-1 infection.

Materials and Methods

Study population. For this study, both rapid progressors to AIDS and long-term survivors of HIV-1 infection were selected from participants of the Amsterdam Cohort study on HIV-1 infection and AIDS in homosexual men [16]. Recruitment for the cohort study was started in October 1984. After enrollment in the study, participants visited the Municipal Health Service in Amsterdam every 3 months. We collected clinical, epidemiologic, and social data for participants by standardized questionnaires and by physi-
cal examination. Blood samples were obtained for standard immuno-logic and virologic tests, and both sera and viable cells were cryopreserved.

By June 1995, 46 patients of 120 cohort participants with known date of HIV-1 seroconversion (calculated as midpoint between the last negative and the first positive HIV-1 antibody test) had progressed to AIDS according to the 1987 CDC classification [17, 18] (median time to AIDS, 4.2 years; range, 0.5–9.5). For this study, 6 progressors were selected (H39, H424, H450, H493, H1211, and H1215) because they were nearest to the group’s median time to AIDS and had samples available for the entire follow-up period. The selected subjects developed AIDS within 3.1 and 6.0 years from seroconversion (median, 4.3) and did not receive antiretroviral therapy before AIDS diagnosis, with the exception of patient H1215 who was treated with zidovudine and zalcitabine beginning 20 and 4 months before AIDS diagnosis, respectively.

Long-term survivors were participants who met the following criteria: documented HIV-1 seropositivity for ≥9 years, absence of AIDS-defining conditions (by the 1993 CDC case definition [19]), mean CD4 T cells >400/μL during both years 8 and 9 of follow-up, and no use of antiretroviral drugs. By January 1996, 21 cohort participants fit the above criteria for long-term survivors. Six long-term survivors (H16, H90, H337, H448, H658, and H709) were randomly selected from this group. Chemokine receptor (CCR5) genotype was later done, and uneven distribution in the selected group compared with that of the whole group of long-term survivors was ruled out (table 1) [20]. Median follow-up after HIV-1 seroconversion or seroprevalent entry into the cohort study was 10.7 years (range, 9.2–10.9).

Clinical data and HLA class I type of study patients are summarized in table 1. CD4 T cell counts of all study subjects are shown in figures 1 and 2.

We previously studied patients H90, H709, and H450 [8]. Analysis of their gag-specific CTL responses was repeated in parallel to env-, RT-, and nef-specific responses in this setting.

**Recombinant (r) vaccinia viruses (VV).** rVV were constructed from the Copenhagen isolate of VV. The following rVV-expressing single genes of HIV-1 LAI [23] were used for this study: TG.1144 (gag) [24], TG.4163 (RT) [7], TG.3183 (env) [25], TG.1147 (nef) [26], and control-rVV 186poly, containing no specific insert. All constructs were provided by M. P. Kieny (Transgêne, Strasbourg, France) and by Y. Rivière (Institut Pasteur, Paris).

**In vitro restimulation and expansion of HIV-1-specific CTL.** HIV-1-specific CTL were expanded in vitro by antigen-specific stimulation as previously described [8, 27]. In brief, peripheral blood mononuclear cells (PBMC) isolated and cryopreserved at different times during the study were thawed and resuspended in complete medium (RPMI 1640; Life Technologies, Breda, Netherlands) containing antibiotics and heat-inactivated pooled human serum (10%) from 8–10 healthy HIV-1-seronegative blood donors. Eight serial dilutions of PBMC ranging from 26,000 to 246 cells/well were seeded in 12 replicate wells in 96-well round-bottom microtiter plates. Preliminary experiments with mixed populations of stimulator cells separately infected with 2 rVV or stimu-

Table 1. Clinical data for HIV-1–infected long-term survivors and progressors.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>HLA class I*</th>
<th>CCR5 genotype†</th>
<th>HIV-1 seroconversion status‡</th>
<th>Age§ (N)</th>
<th>SI/NSI</th>
<th>Event¶</th>
<th>Follow-up**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long-term survivors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>H16</td>
<td>A2,29; B44,51; Cw2,w5</td>
<td>WT/Δ32</td>
<td>I</td>
<td>29</td>
<td>NSI</td>
<td>—</td>
<td>&gt;10.4</td>
</tr>
<tr>
<td>H90</td>
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<td>WT/WT</td>
<td>I</td>
<td>41</td>
<td>NSI</td>
<td>—</td>
<td>&gt;10.8</td>
</tr>
<tr>
<td>H337</td>
<td>A23,−; B49,50; Cw5,w7</td>
<td>WT/Δ32</td>
<td>II</td>
<td>40</td>
<td>NSI</td>
<td>—</td>
<td>&gt;10.7</td>
</tr>
<tr>
<td>H448</td>
<td>A1,2; B7,57; Cw6,w7</td>
<td>WT/Δ32</td>
<td>II</td>
<td>45</td>
<td>NSI</td>
<td>—</td>
<td>&gt;9.8</td>
</tr>
<tr>
<td>H658</td>
<td>A1,2; B6,61; Cw2,w7</td>
<td>WT/WT</td>
<td>I</td>
<td>37</td>
<td>NSI</td>
<td>—</td>
<td>&gt;9.2</td>
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<td>H709</td>
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<td>WT/WT</td>
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<td>29</td>
<td>NSI</td>
<td>—</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td><strong>Progressors</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>H39</td>
<td>A1,2; B8,44; Cw5,w7</td>
<td>WT/WT</td>
<td>I</td>
<td>36</td>
<td>SI</td>
<td>CAO</td>
<td>3.2</td>
</tr>
<tr>
<td>H424</td>
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<td>WT/WT</td>
<td>I</td>
<td>37</td>
<td>NSI</td>
<td>CAO</td>
<td>3.6</td>
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<tr>
<td>H450</td>
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<td>WT/Δ32</td>
<td>I</td>
<td>29</td>
<td>SI</td>
<td>KS</td>
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<td>A1,2; B8,35; Cw3,w7</td>
<td>WT/WT</td>
<td>I</td>
<td>46</td>
<td>NSI</td>
<td>KS</td>
<td>3.3</td>
</tr>
<tr>
<td>H1211</td>
<td>A2,29; B4,−; Cw4,−</td>
<td>WT/WT</td>
<td>I</td>
<td>26</td>
<td>NSI</td>
<td>CM</td>
<td>5.9</td>
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<tr>
<td>H1215</td>
<td>A1,2; B7,8; Cw7,−</td>
<td>WT/WT</td>
<td>I</td>
<td>23</td>
<td>SI</td>
<td>CAO</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* HLA class I typing by standard serologic methods [21] (done by N. M. Lardy, Dept. of Transplantation Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB]).
† CC chemokine receptor 5 (CCR5) polymorphism determined by polymerase chain reaction with sequence-specific primers: wt/wt, homozygous wild type; wt/Δ32, heterozygous wild type (i.e., 1 allele with 32-bp deletion in CCR5) (done by A. M. De Roda Husman, Dept. of Clinical Viro-Immunology, CLB).
‡ Known date of HIV-1 seroconversion, I; seropositive at entry in cohort study, II.
§ Age (years) at HIV-1 seroconversion or first seropositive visit.
¶ Biologic virus phenotype [22]: non-syncytium–inducing (NSI) vs. syncytium–inducing (SI).
¶¶ AIDS diagnosis according to 1987 CDC classification [18]: CAO, *Candida albicans* esophagitis; KS, Kaposi’s sarcoma; CM, cryptococcal meningitis.
** Time (years) between HIV-1 seroconversion or seroprevalent entry and AIDS diagnosis for progressors or October 1995 for long-term survivors.
Figure 1. HIV-1–specific CTL responses and virus load in long-term survivors. Top panels, frequencies of gag-, reverse transcriptase (RT)-, nef-, and env-specific CTL precursors (CTLp). Lower panels, HIV-1 RNA in serum and frequency of circulating CD4 T cells productively infected with HIV-1. Vertical bars indicate SE as calculated from maximum of 30 iterative computer simulations [33]. Superimposed thick lines represent CD4 T cells. PBMC, peripheral blood mononuclear cells.
Figure 2. HIV-1–specific CTL responses and virus load in progressors. Top panels, frequencies of gag-, reverse transcriptase (RT)-, nef-, and env–specific CTL precursors (CTLp). Lower panels, HIV-1 RNA in serum and frequency of circulating CD4 T cells productively infected with HIV-1. Vertical bars indicate SE as calculated from maximum of 30 iterative computer simulations [33]. Superimposed thick lines represent CD4 T cells. PBMC, peripheral blood mononuclear cells.
ator cells infected with rVV expressing 2 HIV-1 gene products resulted in underestimation of CTL specificity at lower frequency (data not shown). Therefore, we preferred separate PBMC stimulation. The number ($n_i$) of replicates in each culture group was reduced from 24, as described [8], to 12 to allow the simultaneous analysis of up to 6 PBMC samples from each patient, using 4 separate stimulator cell populations (1 for each HIV-1 gene product). Coefficient of variation increases from 15% to 22% when $n_i$ is reduced from 24 to 12. In this way, considering a 95% confidence interval (CI) equal to ±2 SD, 2 frequencies ($f_i > f_j$) will be significantly different when $f_i - 0.44f_i > f_j + 0.44f_j$. This is solved as $f_i > 2.57f_j$.

In a series of 217 CTLp frequency determinations in 22 PBMC samples from 4 patients, the average (±SD) 95% CI (one-sided) was 43.2% ± 5.9%.

To each well, 10$^4$ fixed stimulator cells and an equal number of feeder cells were added. Stimulator cells were autologous Epstein-Barr virus–transformed B lymphoblastoid cell lines (B-LCL) infected with 5 MOI of rVV expressing single genes of HIV-1LAI and fixed with 1% (weight/vol) paraformaldehyde for 15 min at room temperature followed by incubation with 0.2 M glycine in PBS for 15 min. Feeder cells were generated prior to initiation of the experiments by polyclonal activation and expansion of PBMC obtained during each subject’s early asymptomatic period as previously described [28]. Autologous feeder cells from a single PBMC sample were irradiated (30 Gy) and used for all samples from the same patient.

Microcultures were maintained for 15 days at 37°C with 5% CO$_2$. At days 2 and 9, cultures were fed with complete medium containing recombinant interleukin-2 (rIL-2; 360 IU/mL final concentration; provided by R. Rombouts, Chiron Benelux, Amsterdam). At day 7 they were restimulated with 10$^4$ fixed stimulator cells and rIL-2. On day 15, wells were split and effector cells were tested for cytotoxicity.

**Cytotoxicity assays.** Standard $^{51}$chromium-release assays were done as previously described [8, 27]. In brief, autologous B-LCL were infected with 5 MOI of rVV expressing single HIV-1 gene products or control-rVV 186poly and labeled with 100 μCi of Na$^2$CrO$_4$ (Amersham International, Amersham, UK) for 16 h. A total of 4 × 10$^3$ target cells were added to each effector cell–containing well. After 4 h, supernatants were harvested and radioactivity was counted by gamma counter (Cobra-II; Canberra Packard Benelux, Groningen, Netherlands). Spontaneous $^{51}$Cr-release was always <15% of maximum release. Specific lysis was calculated as 100 × [(experimental release – spontaneous release)/ (maximum release – spontaneous release)]. Wells were considered positive when the $^{51}$Cr-release exceeded 10% specific lysis. Statistical analysis was done as described by Strijbosch et al. [29].

CTLp frequencies were expressed as number of CTLp/10$^6$ PBMC. CTLp frequencies determined with control targets were subtracted to obtain CTLp frequencies for each specific antigen. Total HIV-1–specific CTLp of each blood sample were estimated by computing the sum of CTLp frequencies specific for gag, RT, env, and nef.

**Polyclonal T cell function.** In vitro T cell reactivity was measured in whole blood lymphocyte cultures as described [30]. In brief, 1:10 diluted heparinized venous blood was cultured in 3 replicate wells of round-bottom microtiter plates in the presence of CD3 monoclonal antibodies (Mabs) or phytohemagglutinin (PHA). The proliferative response was measured after 4 days by $[^3]$H]thymidine incorporation. T cell reactivity was expressed as counts per minute. Samples from 5 healthy HIV-1–negative blood donors were included in each assay as controls. Results were expressed as percentage of the mean T cell reactivity of controls.

**Virologic markers.** Serum HIV-1 RNA levels were determined as previously described [31] using a single-tube quantitative HIV-1 RNA nucleic acid sequence-based amplification assay with electrochemiluminescent-labeled probes (NASBA HIV-1 RNA QT; Organon Teknika, Böxtel, Netherlands). The virologic set point was calculated as the average of ≥3 HIV-1 RNA determinations within 0.5 log difference from the lowest determination after the initial decline but before the late rise (if applicable).

The frequency of circulating CD4 T cells productively infected with HIV-1 was determined using clonal virus isolation procedures as described [32]. In brief, 12,500–25,000 PBMC from HIV-1–infected persons were cocultivated in 48 replicate wells with 10$^5$ 2-day PHA-stimulated PBMC from HIV-1–seronegative blood donors. HIV-1 replication was monitored by screening culture supernatants for p24 production using a p24 capture ELISA. Statistical analysis of positive wells was done as described by Strijbosch et al. [29]. Virus load was expressed as TCID/10$^6$ CD4 T cells.

Biologic phenotype of HIV-1 viruses was determined as described [22]. In brief, 10$^6$ PBMC from HIV-1–infected patients were cocultivated with MT-2 cells to determine the viral phenotype. Cultures were monitored microscopically to check for syncytium formation.

**Statistics.** Correlations between pairs of variable parameters were analyzed by Spearman’s rank correlation test. For comparison of parameters at cross-sections, the Mann-Whitney U test was used.

**Results**

**HIV-1–specific CTL responses and virus load in long-term survivors.** HIV-1–specific CTL responses were observed in all long-term survivors; however, there were large quantitative differences among subjects (figure 1). Total CTL activity, calculated as the sum of gag-, RT-, nef-, and env-specific CTLp frequencies at each time point, ranged from 73 to 22,221 TCID/10$^6$ PBMC. A common feature of total HIV-1–specific CTL responses was its persistence at virtually constant levels throughout the follow-up. Maximum CTL activity was usually reached in the fourth year of infection, although this occurred later in some subjects. All 4 HIV-1 gene products were seen in all 6 long-term survivors.

Virus load (the frequency of circulating CD4 T cells productively infected with HIV-1) was <10 TCID/10$^6$ CD4 T cells in the 6 long-term survivors, with the exception of late samples from H16 and H337 (17 and 70–143 TCID/10$^6$ CD4 T cells, respectively). Serum HIV-1 RNA levels were always below the detection limit (10$^3$ copies/mL) in H90 and, after an initial 2.32 log reduction, in H658. In the long-term survivor with the highest HIV-1–specific CTL response (H448), there was a
progressive increase of HIV-1 RNA to a maximum of $10^{4.62}$ copies/mL. However, the number of circulating infected cells remained low (4–8 TCID/10^6 CD4 T cells) during the study period (figure 1). Serum viral RNA levels were variably detectable in the other 3 long-term survivors with relatively low HIV-1–specific CTL responses (median $10^{3.72}$ copies/mL; range, $<10^3$–$10^6.56$; figure 1). Primary viruses isolated from the 6 long-term survivors were always of the non-SI (syncytium-inducing) phenotype.

**HIV-1–specific CTL responses and virus load during progression to AIDS.** CTL activity against the 4 HIV-1 gene products tested was also observed in the progressors studied, except for nef- and env-specific CTL, which were undetectable in subjects H1211 and H39, respectively.

Two apparently distinct patterns of HIV-1–specific CTL responses in relation to virus load were observed. In the 2 progressors (H39 and H493) with the shortest interval from HIV-1 seroconversion to AIDS (3.2 and 3.3 years, respectively), vigorous HIV-1–specific CTL responses were observed (peak values, 5188 and 2413 total HIV-1–specific CTLp/10^6 PBMC). These were maintained to AIDS diagnosis. Shortly thereafter, HIV-1–specific CTL responses significantly deteriorated. Increasing numbers of HIV-1–infected CD4 T cells occurred early in both patients in the face of already established and vigorous HIV-1–specific CTL responses (figure 2). In patient H39, this was associated with emergence of SI variants as early as 18 months after seroconversion. Serum RNA levels were always high in these 2 patients ($>10^{4.46}$ copies/mL). Thus, lack of virus control was observed in progressors H39 and H493 despite HIV-1–specific CTL responses that were comparable in magnitude and breadth to those in persons with more protracted asymptomatic infection.

In the other patients, HIV-1–specific CTL activity increased after seroconversion, peaking at 17–48 months. Peak values were 964–2392 total HIV-1–specific CTLp/10^6 PBMC (median, 1307). In 3 of these patients, initial reduction (0.6–2.0 log) of viral RNA levels was seen in association with peak CTL activity. The fourth patient in this group (H450) had virtually undetectable viral RNA for the first 6 months after HIV-1 seroconversion. Four of 5 monthly samples produced negative results, and serum obtained at month 5 contained $10^{2.28}$ copies/mL. The initially low virus load may explain this patient’s relatively slow expansion of HIV-1–specific CTL. HIV-1 RNA levels stabilized in each patient at relatively high virologic set points (range, $10^{4.08}$–$10^{5.72}$ copies/mL). In these 4 patients, HIV-1–specific CTLp frequencies declined before AIDS diagnosis to values 12- to 23-fold lower than peak values. Decline of HIV-1–specific CTL responses was invariably associated with loss of CD4 T cells and steep increases in the frequency of productively infected CD4 T cells (figure 2). SI viruses were detected in association with increasing numbers of infected CD4 T cells in patients H450 and H1215 at 4 and 12 months before AIDS diagnosis, respectively. Thus, increasing numbers of circulating productively infected cells were observed only after profound reduction of HIV-1–specific CTL activity occurred.

**Composition of HIV-1–specific CTL responses and correlations with virus load.** Although all subjects mounted a CTL response against the 4 HIV-1 gene products tested, there were large variations in antigen specificity. The extent of the response to each HIV-1 protein was quantified as the percentage of the total CTLp frequency at each time point and related to virus load at that time. Correlations were made for 68 pairs of CTL and RNA load measures and for 56 pairs of CTL and numbers of circulating infected CD4 T cells. For the latter, 36 paired determinations were done on the same sample; the other 20 were obtained from the nearest samples obtained within a 6-month interval. When all determination pairs were considered, total CTLp were not significantly correlated with virus load. However, when individual specificities were considered, both the frequency and the percentage of gag-specific CTL negatively correlated with HIV-1 RNA load ($r = -0.342$, $P = .005$, respectively). When we limited the analysis to the 21 determination pairs obtained within 24 months after seroconversion (or seroprevalent entry in 2 cases), a more significant negative correlation was seen between the percentage of gag-specific CTL and both HIV-1 RNA and circulating infected CD4 T cells ($r = -0.55$, $P = .01$; $r = -0.639$, $P = .006$, respectively). The negative association between gag-specific CTL responses and virus load was reflected in persistently predominant gag-specific responses in 4 of the 6 long-term survivors. In progressors, even when an initially predominant gag-specific response was found, it was not maintained over time (figure 3). The differences in the composition of the HIV-1–specific CTL responses could not be related to patient HLA type. When the 2 patient groups were compared at the time of each subject’s highest HIV-1–specific CTLp frequency, the percentage of gag-specific CTLp was the only immune parameter significantly higher in long-term survivors. The 2 groups differed also for the set point of HIV-1 RNA and to a lesser extent for numbers of CD4 T cells (table 2).

**Deterioration of immune functions and disease progression.** Declining CD4 T cells and deterioration of polyclonal T cell reactivity are hallmarks of progression to AIDS [34]. Kinetics of in vitro T cell reactivity to polyclonal stimuli and total HIV-1–specific CTLp in the patients progressing to AIDS are shown in figure 4. Sequential decline of CD3- and PHA-induced T cell reactivity, respectively, was observed during progression to AIDS, confirming previous observations [35]. Maintenance of steady HIV-1–specific CTLp frequency appeared to be related to preserved reactivity to PHA. In all 6 progressors, no substantial loss of CTL activity was seen as long as PHA-induced T cell response was 30% above that of controls, a threshold level previously established as predictive for rapid disease progression [34]. Analysis of 26 paired determinations of total HIV-1–specific CTLp and either CD3 MAb- or PHA-induced T cell reactivity in the period after maximal CTL
Figure 3. Relative composition of HIV-1–specific CTL precursors (CTLp) at 3 comparable time points in long-term survivors and progressors: 3 months (progressors only) and 2, 4, and 9 years (long-term survivors only), on average, from seroconversion or seroprevalent entry. RT, reverse transcriptase.

Responses in progressors revealed a highly significant positive correlation between HIV-1–specific CTLp frequency and T cell reactivity to PHA in vitro ($r_s = .752; P < .001$) and, to a lesser extent, between HIV-1–specific CTLp frequency and CD3 MAb-induced T cell proliferation ($r_s = .569; P < .005$).

Discussion

The relevance of CTL responses in limiting HIV-1 replication is unclear, even though the accumulation of mutations within putative viral epitopes of CTL recognition suggests efficient pressure exerted by the immune system on the virus [36]. We attempted to define characteristics of the HIV-1–specific CTL response that could be responsible for the limitation of viral replication by comparing balanced groups of long-term survivors and persons with relatively rapid progression to AIDS chosen within a homogeneous population of HIV-1–infected subjects. We found that virtually all HIV-1–infected subjects mounted specific CTL responses against all major viral proteins. The persistence and in some cases the continuous expansion for 6 or 7 years of the circulating pool of HIV-1–specific CTLp distinguished the long-term survivors from the progressors studied. This suggests that even in the presence of low virus load, continuous antigenic stimulation maintains a large pool of specific T lymphocytes and, in agreement with other observations [8, 13, 37–40], that immune control may contribute to maintenance of the asymptomatic phase of HIV-1 infection.

HIV-1–specific CTL response has been assigned a crucial role in reducing the initial viremia on the basis of temporal relationships in kinetics [2] and a weak inverse correlation between env-specific CTLp and HIV-1 RNA load [5]. However, the significant difference in virologic set point between the 2 groups analyzed in the present study can hardly be explained by the vigor of the CTL responses. Although the progressors in our study had a relatively short AIDS-free interval,
the magnitude of HIV-1–specific CTL responses, as shown by the frequency of cells reacting with conserved epitopes in the HIV-1\textsubscript{LAI} sequence, was strikingly similar to that observed in long-term survivors and reached its maximum significantly earlier than in long-term survivors. Differences in specificity may account for the heterogeneous outcome. The long-term survivors had a persistently predominant gag-specific response that was not seen in the progressors. This predominance may be interpreted either as reflecting the recognition of conserved epitopes that the virus cannot mutate without disadvantage or as the simultaneous recognition of several epitopes in the same protein. The detection of CTL responses to rev and tat exclusively in long-term survivors, although quantitatively small, could be interpreted in the same fashion \cite{41}. Epitope variation related to higher virus replication rate, however, must be considered as a possible cause of rapid changes in the predominant CTL specificity and, given the readout system, which is based on a single antigen sequence, may account for discrepancies in the response patterns seen with individual HIV-1 proteins.

Although the gag-specific CTL response is common in most HIV-1–infected subjects \cite{7, 8, 13}, the simultaneous response to the major viral proteins shown in this study demonstrates that its relative predominance might be a key aspect of a protective immune response. It is of interest that in 4 long-term survivors the predominant gag-specific CTL response persisted for several years and in 3 it was later replaced by a different response without clear changes in patient status (figure 3). These observations are somewhat similar to those of a group of HLA-B\textsuperscript{27}\textsuperscript{+} subjects who exhibited a CTL response to a single epitope in gag and only after the late emergence of an escape mutant experienced uncontrolled viral replication, decline of circulating CTLp, and disease progression \cite{42}. The continuing follow-up of our subjects who had highly diversified CTL responses will clarify whether changes in the specificity of the CTL response are accompanied by clinical deterioration. gag-specific T cell responses have often been related to a more favorable course of infection, when CTL \cite{43, 44} and lymphoproliferative responses \cite{45} are analyzed. A recent report of strong p24-stimulated in vitro lymphoproliferative responses in peripheral blood of 2 long-term HIV-infected survivors and in 3 subjects treated with highly active antiretroviral therapy early after infection has revived interest in this issue and suggests a possible protective role \cite{46}.

Differences in specificity of the CTL response could be explained by genetic background, but no such association was found in our study subjects. Even though progressor H424 and long-term survivor H658 were fully matched for CCR5 genotype, HLA type (table 1), and TAP alleles (not shown) \cite{47}, the duration of their HIV-1–specific CTL responses was strikingly different, indicating that viral properties or other host cofactors are likely involved.

The deterioration of HIV-1–specific CTL responses observed during progression to advanced HIV-1 infection was not clearly related to patient clinical status. In 2 progressors, we observed high serum HIV-1 RNA levels and increasing numbers of infected cells in the face of strong and ongoing

### Table 2. Comparison of long-term survivors and progressors at the time that peak HIV-1 cytotoxic T lymphocyte precursor (CTLp) frequencies were measured.

<table>
<thead>
<tr>
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<th>Long-term survivors*</th>
<th>Progressors*</th>
<th>(P^*)</th>
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</thead>
<tbody>
<tr>
<td>Median time (months) at peak HIV-1 CTLp frequency</td>
<td>65 (29–85)</td>
<td>19 (5–47)</td>
<td>(&lt;.01)</td>
</tr>
<tr>
<td>Mean no. of CD4 T cells/(\mu)L</td>
<td>710 ± 115</td>
<td>498 ± 154</td>
<td>(.03)</td>
</tr>
<tr>
<td>Mean CD3 monoclonal antibody response (% of controls)</td>
<td>89 ± 55</td>
<td>91 ± 77</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Mean phytohemagglutinin response (% of controls)</td>
<td>99 ± 49</td>
<td>102 ± 34</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Mean HIV-1 RNA set point (log copies/mL)</td>
<td>3.43 ± 0.46</td>
<td>4.51 ± 0.47</td>
<td>(.01)</td>
</tr>
<tr>
<td>Median infected CD4 T cells/(10^6) CD4 T cells</td>
<td>2.5 (0–75)</td>
<td>15 (0–38)</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Median HIV-1–specific CTLp frequencies (/(10^6) PBMC)</td>
<td>2015 (140–22,221)</td>
<td>2019 (964–5188)</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Total</td>
<td>1123 (83–15,426)</td>
<td>281 (61–940)</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Reverse transcriptase (RT)</td>
<td>382 (52–1138)</td>
<td>482 (134–3905)</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Nef</td>
<td>248 (3–2561)</td>
<td>480 (0–912)</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Env</td>
<td>282 (0–3463)</td>
<td>233 (0–1112)</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Mean HIV-1–specific CTL responses (% of total)</td>
<td>49.9 ± 17.8</td>
<td>17.2 ± 10.5</td>
<td>(.01)</td>
</tr>
<tr>
<td>Gag</td>
<td>19.1 ± 11.8</td>
<td>42.6 ± 32.4</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>RT</td>
<td>11.1 ± 6.1</td>
<td>19.5 ± 17.3</td>
<td>(\text{NS})</td>
</tr>
<tr>
<td>Nef</td>
<td>19.8 ± 16.2</td>
<td>20.7 ± 18.5</td>
<td>(\text{NS})</td>
</tr>
</tbody>
</table>

\(\text{Note. NS, not significant.}\)

\(\text{* Data are shown as (range) or ± SD.}\)

\(\text{¹ Mann-Whitney U test.}\)
HIV-1–specific CTL responses. In the remaining progressors, a steep rise in HIV-1–infected CD4 T cells and progression to AIDS occurred only after HIV-1–specific CTL activity diminished. Loss of HIV-1–specific CTL was observed in close association with deterioration of general T cell function and numbers of CD4 T cells. In our study, the in vitro detection of HIV-1–specific CTL activity appeared to be strictly associated with the maintenance of T cell reactivity to PHA. In agreement with previous studies [34, 35], the deterioration of the T cell reactivity to PHA occurred relatively late and was preceded by a loss of CD3 MAb-induced T cell reactivity. It is therefore conceivable that, although elevated CTLp were still detectable under “optimal” in vitro stimulation conditions, in vivo defective T cell activation impaired effector functions, allowing increasing numbers of circulating infected cells.

This study adds to the growing body of evidence that HIV-1–specific CTL may help limit HIV-1 replication, thereby allowing maintenance of the asymptomatic phase of infection. The association of a dominant gag-specific CTL response with long-term survival should be taken into account in the design of preventive and, given the possibilities opened by current highly active therapies [48], of therapeutic vaccines.

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References


