Evolution of Cytotoxic T Lymphocyte Responses to Human Immunodeficiency Virus Type 1 in Patients with Symptomatic Primary Infection Receiving Antiretroviral Triple Therapy

Marc Dalod, Martine Harzic, Isabelle Pellegrin, Béatrice Dumon, Bruno Hoen, Daniel Sereni, Jean-Christophe Deschemin, Jean-Paul Levy, Alain Venet, and Elisabeth Gomard*

The impact of highly active antiretroviral treatment (HAART) on anti–human immunodeficiency virus (HIV) cytotoxic T lymphocytes (CTL) was studied in 17 patients with recent symptomatic HIV-1 primary infection receiving triple combination therapy. Anti-HIV CTL were initially detected in 15 patients. In 6, CTL disappeared rapidly and persistently after initiation of therapy. Most of them had a rapid and sustained decrease in plasma HIV RNA to undetectable levels. Conversely, in 6 other patients, CTL remained detectable, which was associated with a less efficient control of viral replication. In 3 others, CTL disappeared only transiently, without clear correlation with the virologic profile. Altogether, despite individual variations, there was a positive correlation between viral replication and anti–HIV-1 cytotoxicity in most subjects, suggesting that the persistence of viral antigens is the main determinant for the maintenance of CTL activity. This raises the question of the potential benefit of anti-HIV CTL induction by immunotherapy in acute seroconverters treated by HAART.

The early events in human immunodeficiency virus (HIV) primary infection are critical determinants of the ultimate progression toward AIDS, as indicated by the faster evolution of infection in symptomatic seroconverters than in asymptomatic ones [1–3]. The level of the initial peak of viral replication is not a reliable predictor of disease progression, while the plasma HIV RNA level becomes a good indicator of evolution toward AIDS at ~6 months after infection, when an apparent steady state of viral replication has been established [4, 5]. This suggests that the rate of progression toward AIDS in a given individual results from early interactions between viral replication and immunologic responses [3, 5, 6]. Antibodies may contribute to the clearance of viruses from plasma during this period [6]. Neutralizing antibodies are apparently not involved [6–8], but other mechanisms, such as antibody-dependent cellular cytotoxicity, might be important [9]. NK cells could also be involved in the control of viral replication in the first few days after infection, but very little is known about this activity in primary infection. The CD8 T lymphocyte responses are probably the major component of the immune response involved in controlling viral replication during primary infection. Both anti–HIV-1 cytotoxic T lymphocyte (CTL) activity and inhibition of viral replication have been detected early after infection and correlated with a decrease in plasma virus load [10–14]. Furthermore, the recent observations of rapid selection of CTL-escaping virus early after infection demonstrate that anti-HIV CTL exert a strong selective pressure on virus variants and are therefore probably efficient in lysing infected cells in vivo from the early phases of infection onwards [15–18]. Nevertheless, it is still not clear whether a strong CTL response to a primary HIV infection results in an effective control of viral replication or whether it is only indicative of a massive viral replication. Significant CTL responses have been found associated with decreased virus loads [10–13], while poor or delayed CTL responses have been associated with rapid progression in a few cases [11, 12, 17]. Conversely, there have also been reports of early strong CTL responses followed by rapid progression [9, 12, 16, 18, 19] and of delayed responses associated with very low virus loads and slow progression [6, 10, 13, 16, 18]. Therefore, there must be a subtle balance between viral replication and CTL responses from early primary infection onwards. The lack of...
CTL activity may therefore be associated with a poor prognosis (with high virus load) or with a good prognosis (with low virus load) [20]. The qualitative nature of the CTL response must also be taken into account. The long-term persistence of efficient CTL is likely to depend on the initial breadth of the repertoire mobilized [5, 19–21] and perhaps on less activation of each CTL clone [19, 22].

Effective reduction of viral replication by drug treatment as early as possible after infection could avoid the occurrence of events critical for the development of HIV-1–related pathogenesis [23–25]: the shift from non–syncytium-inducing to cytopathogenic syncytium-inducing isolates, the appearance of immune and drug escape mutants, and the onset of chronic immune activation, which could lead to the exhaustion of CTL clones [5, 19, 26, 27] or to the development of CD8–mediated immune pathogenesis [27–30]. On the other hand, highly active antiretroviral treatment (HAART) could drastically diminish HIV-specific CTL responses or even abrogate them if persisting antigen is needed for memory maintenance. It is therefore important to study the evolution of anti-HIV CTL in patients treated early after acute primary HIV-1 infection by triple combination antiretroviral therapy, which was recently shown to be very effective in reducing virus load [31]. This report describes the follow-up of CTL responses and plasma HIV RNA levels in 17 persons with acute HIV primary infection treated with lamivudine, zidovudine, and ritonavir.

Materials and Methods

Subjects. Patients were enrolled into the Agence Nationale de la Recherche sur le Sida 053 clinical trial of zidovudine, lamivudine, and ritonavir combination in symptomatic primary HIV-1 infection. To be enrolled, patients had to present with at least two symptoms of acute primary infection within the 4 weeks before inclusion and to meet at least two of the following biologic criteria: positive p24 antigenemia, no more than three bands on Western blot, negative or weakly positive ELISA test for HIV-1 at the time of inclusion. These subjects were given triple combination therapy, consisting of zidovudine (500 mg/day), lamivudine (300 mg/day), and ritonavir (1200 mg/day) [32]. Each subject was assigned a three-figure number as a code name (table 1). Day 0 refers to the start of treatment, which occurred 7–48 days after the onset of acute retroviral syndrome. Plasma HIV RNA and CD4 and CD8 T cells were monitored on days 0, 3, 7, 14, and 28, monthly up to month 3–4, and then every 3 months (table 1). Anti-HIV CTL were tested on day 0 and at months 2, 4, 7, 12, and 18.

HIV RNA measurement in plasma. Plasma was separated within 2 h of blood sampling, aliquoted, and immediately stored at −80°C. HIV-1 RNA levels were measured by a quantitative reverse transcription–polymerase chain reaction assay (AmpliCor HIV-1 monitor; Roche Molecular Systems, Neuilly sur Seine, France) [33]. The lower limit of detection for this assay was 200 copies/mL. Samples with RNA levels <200 copies/mL were ultracentrifuged for a 10-fold concentration and retested by the Ultra Direct Assay, with a lower limit of quantification of 20 copies/mL (Roche). However, the sensitivity of this Ultra Direct Assay is only reliable for RNA levels >50 copies/mL. Therefore, samples with values <50 copies/mL were considered undetectable.

Generation of anti–HIV-1 polyclonal cell lines. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation (Lymphocyte Separation Medium; Flow, Irvine, UK) from freshly drawn heparinized venous blood. They were used after freezing and thawing. PBMC (10^6/mL) were set up in culture with autologous phytohemagglutinin-activated lymphocytes (2 × 10^5/mL) prepared as described elsewhere [34]. The cells were incubated for 3 days in RPMI 1640 supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, nonessential amino acids, 1 mM sodium pyruvate, 10 mM HEPES buffer, and 10% heat-inactivated fetal calf serum and then maintained for 2–3 weeks at 10^5/mL in medium supplemented with 10 U/mL human recombinant interleukin-2 (Boehringer, Mannheim, Germany) before use as effector cells in the chromium-release test.

Chromium-release test. The target cells used were autologous lymphoblastoid cell lines obtained by transforming PBMC with Epstein-Barr virus (EBV-LCL). They were infected for 18 h at 37°C with 5 pfu/cell wild type vaccinia virus (Copenhagen strain) or various recombinants from the same strain encoding the Env (VAC/Env), Gag (VAC/Gag), Pol (VAC/Pol), or Nef (VAC/Nef) proteins of HIV-1LAI, as described [34]. The EBV-LCL were then labeled with 100 µCi of [51Cr]Na2CrO4 (Amersham International, Amersham, UK) for 1 h and washed twice before use as target cells. The test was done in microculture plates by incubating various concentrations of effector cells and 5 × 10^3 target cells in RPMI 1640 supplemented with 10% fetal calf serum for 4 h at 37°C. The supernatants were then harvested, and the chromium released was measured in a gamma counter. The spontaneous release was 10%–20% of the total Cr incorporated. Specific chromium release was calculated as 100 × [(experimental release – spontaneous release)/(total Cr incorporated – spontaneous release)]. HIV-specific cytotoxic activity was considered to be positive when the specific chromium release was 10% higher than the values obtained with wild type vaccinia virus for two different effector-to-target ratios. Cytotoxic activity was expressed in lytic units, calculated for 10^5 effector cells, as 10^5/(5000 × E:T30%), where E:T30% is the effector-to-target ratio that gives 30% specific lysis of 5000 target cells. E:T30% was calculated by semilogarithmic interpolation of the curve giving the percentage of specific lysis as a function of the E:T ratio if statistically correct (R² > .75). Values were considered to be significant only when they were >3.5.

Results

CTL activity was detected before treatment in 15 of the 17 seroconverters tested (figure 1). Various CTL specificities were found, Env recognition being the predominant one (in 11 patients), followed by Nef, Gag, and Pol (in 6, 6, and 3, respectively). Seven patients recognized several proteins (3 patients reacted against two proteins and 4 against three proteins). Eight patients had CTL specific for only one protein (Env in 5 of them). No patient reacted against all four proteins tested.

In the 2 patients with no detectable cytotoxic activity at day 0, CTL remained undetectable throughout the study (patients 125 and 131). Both patients showed a dramatic decrease in
Table 1. Evolution of plasma HIV RNA levels in acute HIV-1 seroconverters receiving antiretroviral triple combination therapy and relation with compliance.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Delay</th>
<th>D0</th>
<th>M4</th>
<th>M7</th>
<th>M9</th>
<th>M12</th>
<th>M15</th>
<th>Time of undetectability</th>
<th>Duration of undetectability</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>17</td>
<td>430,948</td>
<td>475</td>
<td>200</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>131</td>
<td>44</td>
<td>44,864</td>
<td>63</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>116</td>
<td>38</td>
<td>6650</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>250</td>
<td>&gt;200</td>
</tr>
<tr>
<td>134</td>
<td>24</td>
<td>13,121</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>138</td>
<td>11</td>
<td>57,053</td>
<td>75</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>57</td>
<td>&gt;483</td>
</tr>
<tr>
<td>144</td>
<td>15</td>
<td>879,540</td>
<td>161</td>
<td>881</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>91</td>
<td>&gt;456</td>
</tr>
<tr>
<td>127</td>
<td>20</td>
<td>431,697</td>
<td>123</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>257</td>
<td>&gt;168</td>
</tr>
<tr>
<td>137</td>
<td>45</td>
<td>766,004</td>
<td>56</td>
<td>76</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>206</td>
<td>&gt;337</td>
</tr>
<tr>
<td>112</td>
<td>46</td>
<td>94,009</td>
<td>436</td>
<td>—</td>
<td>58</td>
<td>134</td>
<td>—</td>
<td>260</td>
<td>&gt;310</td>
</tr>
<tr>
<td>114</td>
<td>14</td>
<td>129,442</td>
<td>2331*</td>
<td>28,145*</td>
<td>47,545*</td>
<td>3073</td>
<td>—</td>
<td>456</td>
<td>&gt;82</td>
</tr>
<tr>
<td>115</td>
<td>22</td>
<td>2,382,540</td>
<td>991</td>
<td>300</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>170</td>
<td>&lt;257</td>
</tr>
<tr>
<td>119</td>
<td>26</td>
<td>10,709,763</td>
<td>200</td>
<td>1070†</td>
<td>3058†</td>
<td>852†</td>
<td>1048†</td>
<td>Never</td>
<td>0</td>
</tr>
<tr>
<td>132</td>
<td>18</td>
<td>42,077</td>
<td>12,690†</td>
<td>—</td>
<td>—</td>
<td>200†</td>
<td>744†</td>
<td>58†</td>
<td>&lt;184</td>
</tr>
<tr>
<td>124</td>
<td>14</td>
<td>1,290,726</td>
<td>—</td>
<td>8923†</td>
<td>—</td>
<td>84†</td>
<td>Lost†</td>
<td>56†</td>
<td>&lt;124</td>
</tr>
<tr>
<td>129</td>
<td>16</td>
<td>383,070</td>
<td>672†</td>
<td>241†</td>
<td>616†</td>
<td>168†</td>
<td>628†</td>
<td>315</td>
<td>&lt;41</td>
</tr>
<tr>
<td>104</td>
<td>15</td>
<td>355,729</td>
<td>51</td>
<td>964†</td>
<td>13,629†</td>
<td>8503†</td>
<td>52,669†</td>
<td>Never</td>
<td>0</td>
</tr>
<tr>
<td>139</td>
<td>18</td>
<td>137,849</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>62</td>
<td>&gt;443</td>
</tr>
</tbody>
</table>

NOTE. Plasma HIV-1 RNA levels are expressed in copies/mL and are given at following time points: day 0 (D0) and months 4, 7, 9, 12, and 15 (M4, M7, M9, M12, and M15); — indicates undetectable plasma HIV RNA levels (<50 copies/mL). Time of undetectability is first day of undetectability of plasma HIV RNA; duration of undetectability is interval in days between first time of undetectability and subsequent first time of reappearance of HIV RNA in plasma if ever. Delay between first appearance of symptoms of HIV-1 primary infection and initiation of treatment (in days). Deviations from strict compliance with triple combination therapy are indicated with symbols below:

- Poor observance of treatment.
- Interruption of antiprotease, strict observance of double combination therapy.
- Interruption of treatment or patient lost to follow-up (Lost).

plasma HIV RNA to persistently undetectable levels from month 6 and month 2 onwards, respectively (table 1).

Early and persistent disappearance of anti-HIV CTL was observed in 6 patients (patients 116, 134, 144, 127, and 137; figure 1) in the course of antiretroviral therapy. They were undetectable as early as at the first point of follow-up (month 2) in 4 patients (138, 116, 134, and 144) and by month 4 in the remaining 2 (137 and 127). In all of these patients, CTL remained undetectable throughout the observation period, up to 18 months after initiation of treatment.

The median initial virus load of these 6 patients was 244,375 copies/mL (range, 6650–879,540) (table 1). By month 4, plasma HIV RNA was undetectable in 2 of these 6 patients (116 and 134) and had decreased to between 50 and 200 copies/mL in the remaining 4 (median, 99; range, 56–161). Thereafter, HIV RNA plasma levels further decreased below the 50-copy threshold and remained undetectable until the end of follow-up in these 6 patients.

Since plasma HIV RNA became rapidly undetectable in most of these patients, the lack of detectable CTL observed in the evolution could have been due to insufficient amount of virus for in vitro restimulation of autologous cells. Therefore, we looked for CTL activity by cultivating PBMC sampled at months 4 or 7, when no CTL were found, with, as stimulators, their autologous cells taken on day 0 when viremia was at its maximum. This did not change the pattern of CTL responses in 3 of the 4 patients tested, as illustrated with patient 137 in figure 2. Thus, even large doses of stimulating viruses failed to produce any anti-HIV CTL activity from PBMC samples obtained after several months of efficient treatment. Conversely, the CTL response observed with PBMC taken on month 2, when viremia was clearly detectable, could be efficiently restimulated by autologous cells taken on month 4, when the plasma HIV RNA was dramatically reduced to <200 copies/mL. On the whole, these results strongly suggest that there is a drastic decrease or even a lack of memory CTL in these patients who had a rapid decrease in viral replication to undetectable levels.

In 6 other patients (112, 114, 115, 119, 132, and 124), anti-HIV CTL were constantly detectable during the study period (figure 1). However, some fluctuations were observed in the CTL response, both quantitatively and qualitatively. In patients 114 and 119, the intensity of the anti-HIV cytotoxic activity decreased in the course of treatment, with disappearance of the reactivity against Nef in patient 119. Conversely, in patient 115, both the intensity and the breadth of the anti-HIV cytotoxic activity tended to increase over time, with appearance of CTL directed against Pol and Env after 2 months and 1 year of treatment, respectively (figure 1). Significant CTL activity has constantly been observed in patient 124, although the specificities recognized had changed: Env-specific CTL were detected initially (day 0) and disappeared later, whereas Gag-specific...
Figure 1. Comparative evolutions of plasma HIV RNA levels and anti-HIV CTL activities in HIV-1 seroconverters receiving highly active antiretroviral treatment. Anti-HIV effector cells were generated by polyclonal in vitro restimulation of peripheral blood mononuclear cells sampled on day 0 and months 2, 4, 7, 12, and 18 with phytohemagglutinin blasts derived from same sample. Cytotoxic activity was determined against autologous Epstein-Barr virus lymphoblastoid target cells infected with wild type or recombinant vaccinia viruses expressing Env, Gag, Pol, and Nef proteins of HIV-1LAI. Values were considered significant only when $\geq 3.5$ lytic units. For clarity, only symbols for proteins recognized at least once by patients’ CTL are indicated, but all 4 proteins were tested. HIV-1 RNA levels were assayed in plasma samples by amplicor HIV-1 monitor test “200 copies” and Ultra Direct Assay when values were $\leq 200$ copies/mL. Sensitivity of Ultra Direct Assay (50 copies/mL) is shown as dotted line. Values under threshold were considered undetectable and arbitrary set at 20 copies/mL.

CTL appeared on month 2 and were always found afterwards. We confirmed by CD8 and CD4 T cell depletion that both anti-Env and anti-Gag CTL were CD8 T cells. In addition, we could not detect anti-Env activity in cultures of PBMC sampled at month 7 stimulated by autologous cells sampled at day 0, which suggests once again the loss of anti-Env memory CTL.

Plasma viral RNA has been almost always detectable in these 6 patients (table 1). However, individual variations were also observed in the virologic profile: in 2 patients (114, 119), HIV RNA plasma levels had been consistently high above threshold until 1 year after initiation of therapy; in 2 other patients (124 and 132), virus load decreased to undetectable
Impact of Tritherapy on Anti-HIV CTL

levels at month 2 and 7, respectively, but rebounded after a short period. It is noteworthy that in all 4 patients, the less efficient control of viral replication appears correlated with a poor compliance with therapy. This is particularly the case for patients 124 and 132, who transiently interrupted their treatment around month 7 and 4, respectively (table 1). Indeed, patient 124 was lost to follow-up at month 9. The 2 remaining patients (112 and 115) appeared to have a better control of the virus load. Both were compliant with therapy (table 1). As soon as month 1, HIV RNA plasma levels of patient 112 were <200 copies/mL and remained so throughout the study period except at one time point (month 4). However, most of the plasma samples of patient 112 were quantitated above 50 copies/mL of HIV RNA by the Ultra Direct Assay, suggesting the persistence of a low but continuous ongoing viral replication. In patient 115, the virus load became undetectable by month 9 and has remained so thereafter. However, the decrease was only slowly progressive, with values >1000 copies/mL up to month 4.

In the last 3 patients, we observed a transient disappearance of CTL activity (patients 129, 104, and 139; figure 1). In patient 129, the reactivity against Pol disappeared at month 2, while that against Gag progressively decreased, with complete disappearance at month 7. However, CTL directed against both proteins reappeared at month 12. In this patient, plasma HIV RNA remained clearly detectable, >200 copies/mL, for the whole period of observation (except at month 12). In patients 104 and 139, anti-HIV CTL activity was undetectable at two consecutive time points but reappeared thereafter. In patient 104, the CTL directed against Env, Gag, and Nef disappeared at month 2, when plasma HIV RNA was decreased to ~200 copies/mL. At month 7, the CTL activity reappeared but only directed against Gag. An increase in HIV RNA plasma levels was also observed, concurrent with the withdrawal of ritonavir because of side effects. A nearly similar pattern of CTL evolution was observed in patient 139, but with a complete switch in specificity: actually the anti-Nef CTL detected initially disappeared, while CTL specific for Gag were detected at month 12 (figure 1). However, his virologic profile was completely different from that of patient 104, since plasma HIV RNA levels had become and remained undetectable from month 2 up to the end of follow-up, at month 15.

Discussion

Cytotoxic lymphocytes were found in 15 of the 17 patients tested during acute primary infection. Such an early activity has been already described [10–13, 35]. The frequency of response is somewhat higher than that reported in a large series of recently infected persons [35]. However, this work describes cytotoxic CD4 and CD8 effector cells expressing lysis of HIV-specific targets without any in vitro stimulation. The protocol that we used allows the detection of CD8 memory CTL, and the different technical approaches may explain the observed differences. The predominant recognition of Env has been previously reported, but other proteins are frequently recognized, as currently observed later in HIV infection, and as we have previously reported in untreated persons during primary infection [10]. The 2 patients who did not have any CTL activity before treatment were not different from the 15 responders in terms of virus load or delay between the onset of first symptoms and the first blood sampling (table 1).

Cytotoxic activity remained negative in the 2 patients in which it was lacking at time of inclusion. In these patients, HIV-1 RNA had become undetectable from month 7 and month 9 onward (table 1). In the course of treatment, CTL activity disappeared in 6 patients who did initially respond. This disappearance occurred early, and CTL remained undetectable. Conversely, CTL were constantly detectable in 6 other patients. In 2 additional patients, CTL disappeared at the first two time points during follow-up and reappeared later, while in the last patient, CTL persisted with a declining activity, to disappear completely at a single time point and reappear thereafter.

Neither the initial virus load nor the delay between the onset of acute retroviral syndrome and the initiation of treatment were correlated with the maintenance or the loss of CTL activity (table 1). Neither was there a clear correlation between T lymphocyte counts and virus load or CTL activity. Nevertheless, most patients had a moderate to high increase in CD4:CD8 ratio, beyond 1 when plasma HIV RNA levels decreased to <200 copies/mL. The normalization of the CD4:CD8 ratio after treatment with a combination of three reverse transcriptase inhibitors was also reported recently in seroconverters [24] but...
was not observed in subjects treated later in the course of HIV infection [36].

Our results suggest that a critical factor associated with sustained disappearance of anti-HIV CTL is the efficiency of control of viral replication. Indeed, by month 4, plasma HIV RNA levels were drastically decreased to <200 copies/mL in the 6 patients in whom CTL activity disappeared completely and even to <50 copies/mL in subjects 116 and 134 (table 1). Furthermore, all 6 patients had a sustained control of viral replication, since none showed reappearance of virus in plasma for the whole period of observation. Conversely, viral replication appeared less controlled in most patients who maintained CTL activity (table 1). This is particularly the case for 4 of them (114, 119, 124, 132). Compliance with therapy appears a key factor for the control of virus load. Indeed, compliance was achieved in the 6 patients who showed disappearance of CTL activity but not in 4 of the 6 patients with persistently detectable CTL (114, 119, 124, 132). In patients 124 and 132, the rebound of the virus load following cessation of therapy after an initial decrease of HIV RNA plasma levels further strengthens this point.

However, on an individual basis, the patterns of virologic and immunologic profiles may vary substantially. For instance, patients 112 and 139 presented with persistently low virus loads and yet maintained CTL activity. Similarly, in patients 119 and 124, the initial control of the virus load was identical in kinetics to that observed in those who lost their CTL activity (figure 1), but CTL activity was constantly observed. We verified and confirmed by Heteroduplex Mobility Assay that these patients were infected with virus belonging to clade B, so their virus load was not underestimated by the Amplicor HIV-1 monitor test, which has been shown to recognize poorly some HIV clades [37].

The profile of patient 112 appears particularly interesting. This patient was initially considered as a good responder to therapy, since his virus load had become undetectable early in therapy (<200 copies/mL), from month 2 up to the end of follow-up, which was consistent with good compliance. However, when his samples were quantitated with the Ultra Direct Assay, HIV RNA was almost always detected at low levels slightly above 50 copies/mL. This suggests low but ongoing viral replication in this patient. This is further strengthened by a persistent high provirus load, in contrast to patients 134 and 125, as assessed by HIV intracellular DNA analysis (data not shown). This virologic profile may explain the persistence of the CTL activity in this subject. The maintenance of sustained
viral replication in lymphoid organs or other cryptic sites [38, 39] may also account for sustained anti-HIV cytotoxic activity despite very low or undetectable plasma viremia. Indeed, a significant heterogeneity in the response of lymphoid organs to a 6-month period of antiretroviral therapy has been recently reported, even when >99.9% of virus was cleared in most patients [31]. The presence of anti-HIV CTL without detectable plasma HIV RNA could then be an indicator of less efficient control of viral replication in the lymphoid organs. The clearance of virus from lymphoid organs remains the main goal to achieve, and this question appears currently critical. The follow-up of patients with particular virologic and immunologic profiles, and especially their virologic and immunologic evaluation at the lymphoid tissue level, would therefore be of great interest to further analyze this point.

Patients 144 and 115 have apparent similar virologic profiles. However, CTL activity disappeared in the former and remained detectable in the latter. As previously discussed, a difference in virus load within their lymphoid organs may account for this discrepancy. However, their virologic profiles are identical only in terms of kinetics. Indeed, the virus load of patient 115 was always greater than that of patient 144, with a >1-log difference at each time point from day 3 after treatment up to month 4. This delayed response to therapy with a slow rate of decrease in HIV RNA levels may also be responsible for the persistence of CTL activity.

The relationship between virus load and CTL activity appears more complex in 2 of the 3 patients who displayed a transient disappearance of CTL activity. In patient 104, the anti-HIV CTL activity and the plasma HIV RNA levels evolved in close parallel: anti-HIV CTL disappeared at month 2 and 4 when HIV plasma RNA levels were at their lower values, <200 copies/mL, and anti-Gag CTL reappeared concurrently with the increase in plasma HIV RNA levels when this patient stopped ritonavir. By contrast, the evolution of virus loads did not parallel that of CTL activity in the 2 other patients. Patient 129 maintained significant levels of HIV RNA in plasma when CTL activity disappeared transiently. In patient 139, the initial response was directed only against the Nef protein. This response completely disappeared, while a Gag-specific response appeared much later in the evolution, even though the plasma HIV RNA had been undetectable for 9 months.

In summary, although individual patterns may be observed, there was a good correlation between the sustained decrease in plasma HIV RNA to undetectable levels and the disappearance of CTL activity. These results suggest that a certain amount of virus production is required to maintain CTL activity, as already described in asymptomatic patients with high CD4 T cell counts, low virus loads, and no detectable CTL [30, 40, 41]. It is noteworthy that some of these asymptomatic patients further developed anti-HIV cytotoxic responses during evolution when virus burdens increased [40, 41]. Therefore, the view that CTL activity controls HIV-1 replication, and that a lack of CTL is associated with increased virus burden, is probably too simplistic. A subtle balance should exist between viral replication and CTL responses. In consequence, the lack of CTL activity may be associated either with a poor prognosis (with high virus load) or with a good prognosis (with low virus load) [20].

An important question to be addressed by future studies is whether the down-regulation of HIV-specific CTL induced by HAART during primary infection could have any consequence for future evolution. The absence of any detectable CTL, even in response to large doses of stimulating viruses, suggests that most patients in whom CTL activity decreased actually lack memory CTL. This abrogation of anti-HIV memory CTL could be problematic in case of cessation of therapy or escape of virus from antiretroviral drugs, since these patients will have to mount a new anti-HIV immune response as in a de novo infection. However, the reappearance of Gag-specific CTL in patient 104, when the cessation of ritonavir led to an increase in virus load, may indicate the existence of CTL memory in this subject. In fact, the reappearance of CTL from memory CTL, or the induction of new effectors, cannot be proven in such experiments, and only analysis of the optimal epitopes recognized or T cell receptor CDR3 sequencing could clarify this point. Evolution in patients 124 and 139 argues in favor of emergence of new CTL populations after a transient CTL disappearance.

It is noteworthy that anti-HIV CTL never completely disappeared from patients at later stages of HIV infection who were undergoing antiretroviral triple therapy, even in those with decrease of plasma HIV RNA to <200 copies/mL (unpublished data). These differences in the evolution of the CTL response to triple therapy depending on the stage at which treatment is initiated may result from different parameters. The duration and the intensity of CTL stimulation by active viral replication may be an important factor for the maintenance of memory cells, as already suggested for subject 115. The amount of intracellular RNA, intracellular proviral DNA, and the virus load within the lymphoid organs are other factors that may vary in different ways under therapy according to the stage of infection. The understanding of the mechanisms underlying the maintenance or the disappearance of memory CTL after therapy at different stages of the disease clearly requires further investigation.

These results also raise the question of the potential benefit of an additional immunotherapy in patients with efficient reduction of plasma HIV RNA in the course of antiretroviral triple combination therapy. The appearance of HIV-specific T helper activities has been recently reported in seroconverters receiving HAART [42], and we have observed a similar phenomenon in another cohort of patients during primary infection (data not shown). These HIV-specific CD4 T lymphocytes would be of importance for efficient reactivation or de novo induction of anti-HIV CTL by immunization [42]. It is interesting to note, however, that the appearance of HIV-specific CD4 helper T cells does not seem to be sufficient to sustain a strong CTL response when the antigenic mass has already drastically diminished.
Finally, together with the data on the appearance of HIV-specific helper T cells [42] and on the waning of antibody responses [24], our observations of the increase in the CD4/CD8 ratio and of CTL disappearance in heavily treated seroconverters emphasize once more how different the effects of triple combination therapy may be depending on the stage of the infection. The best strategy to contain evolution of HIV-1 infection in seropositive persons must really be, as D. D. Ho proposed 2 years ago [23]: “Time to hit HIV, early and hard.”

Acknowledgments

We thank all the people who agreed to participate in this study and provided repeated blood samples; Laurianne Beauvais (Glaxo-Wellcome Laboratories) and Jean-Pierre Chauvin (Abbott France), who provided the reverse transcriptase and protease inhibitors; Jean-Gérard Guillet and Hervé Fleury for support; all of the hospitals throughout France that took part in this study; and Corinne Carpentier and Caroline Lascoux for hematologic and virologic data centralization. The English text was edited by Owen Parkes.

References


