

Functional T Cell Reconstitution and Human Immunodeficiency Virus-1-Specific Cell-Mediated Immunity during Highly Active Antiretroviral Therapy

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Lymphoproliferative responses (LPRs) to recall antigens (Ags) and human immunodeficiency virus type 1 (HIV-1) Gag and frequencies of circulating HIV-1-specific cytotoxic T lymphocyte precursors (CTLps) were measured in 12 patients undergoing highly active antiretroviral therapy (HAART) after long-standing HIV-1 infection. LPRs to at least 1 recall Ag became detectable or increased in all patients during HAART. No significant LPRs to Gag-p24 were observed, whereas 4 of 8 patients tested presented with Gag-p17-specific LPRs. HIV-1-specific CTLp frequencies became measurable or increased early during therapy in 6 of 10 patients tested and were maintained or decreased thereafter. Increasing HIV-1-specific CTLp frequencies were seen only in association with partial HAART failure in 1 patient. In conclusion, restoration of CD4⁺ T lymphocyte responsiveness to recall Ags is achieved during HAART. The data provide evidence for limited HIV-1-specific CD4⁺ memory T cells during advanced HIV-1 infection and suggest that both CD4⁺ and CD8⁺ HIV-1-specific T cells are poorly stimulated when viral load is suppressed.

Current antiretroviral therapy (ART) has greatly modified the poor prognosis of human immunodeficiency virus type 1 (HIV-1) infection. Treatment with combinations of drugs that block two crucial steps of the virus's life cycle, reverse transcription and protein cleavage, is commonly referred to as highly active ART (HAART). These powerful treatment regimens can reduce viral load both in the circulation and in lymphoid tissue and significantly increase circulating CD4⁺ T cell numbers [1–4]. Successful HAART is thus likely to achieve viral

latency, although virus persists in reservoirs [5, 6]. From this perspective, irreversible damage induced during untreated HIV-1 infection and the occurrence of viral resistance or drug toxicity may determine the survival of treated patients. It is, therefore, of great clinical importance to establish whether the recently available regimens of HAART achieve functional immune recovery that confers protection from late infectious complications.

Moreover, assuming that a functional immune system is also effective in limiting HIV-1 replication and spread, it is reasonable to speculate that improvement of T cell immunity might be an additional factor involved in the control of HIV-1 during therapy. In this view, the combination of HIV-1-specific immunotherapy and HAART has been proposed to maximally exploit the amelioration of immune conditions and overcome the paucity of viral antigen (Ag) occurring during therapy [7].

Initial interpretations of the transient increase in CD4⁺ T cell number observed during monotherapy with a protease inhibitor suggested that the natural T cell homeostasis was re-established and that lymphocytes were driven to proliferate toward normal levels [8, 9]. Transient functional improvement was also detected in lymphocyte responses to mitogens and Ags, but the short duration of drug effect on viral load halted any significant benefit to the treated patients [10]. The analysis of patients experiencing persistent viral load reduction because of effective drug combinations revealed a different scenario. Early lymphocyte repopulation appears to be caused mostly by rapid

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Informed consent was obtained from all participants. The protocols were approved by the Scientific Advisory Board of the Dutch AIDS Treatment Evaluation Center and by the institutional review boards of the participating centers.

Part of the study was performed within the Amsterdam Cohort studies on HIV infection and AIDS.

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Table 1. Characteristics of patients selected from study NUCB2019 at entry.

Study no.	Age (years)	CDC ^a	Treatment ^b	Pre-HAART illnesses	T cell function ^c			
					CD4 ⁺ T cells (cells/ μ L)	CD3 (%)	PHA (%)	HIV-1 RNA (log ₁₀ copies/mL)
20492	43	A	Imm.	Crohn's disease	403	20	73	4.59
20444	43	B	Imm.	Diarrhea >1 month	323	23	19	5.10
20478	50	B	Del.	Diarrhea >1 month	270	2	3	5.03
20484	38	B	Imm.	Oral candidosis	137	3	7	6.09
20506	28	C	Imm.	Toxoplasmosis, oral candidosis	93	16	46	5.89
20482	34	A	Del.	None	80	3	7	5.41
20489	30	C	Del.	Tuberculosis, multidermatomal zoster	57	1	1	5.42
20490	40	B	Imm.	Oral candidosis	40	3	6	6.36

NOTE. HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus type 1.

^a 1993 Centers for Disease Control (CDC) classification [24].

^b Imm., simultaneous start of 3 drug administration; del., lamivudine and zidovudine were added after 3 weeks of zidovudine monotherapy.

^c T cell reactivity to CD3 monoclonal antibody and phytohemagglutinin (PHA) expressed as % of controls.

recirculation of CD4⁺ T cells, predominantly of the memory phenotype [11, 12], that show little degree of proliferation, as assessed by the expression of cell cycle-associated Ki-67 nuclear Ag [13]. Preliminary observations suggested that, nonetheless, memory T cell function, measured as *in vitro* lymphoproliferative responses (LPRs) to mycobacterial and cytomegalovirus (CMV) Ags, barely detectable before therapy, was at least partially restored [11].

To establish the degree and persistence of putatively protective immunity and the potential immune control on HIV-1 itself, we combined the analysis of T cell responses to recall and microbial Ags and to HIV-1 Ags in patients with moderately advanced HIV-1 infection who were undergoing HAART. The appearance of stable recall LPRs comparable with those detected in retrospective samples from an earlier, asymptomatic state and the detection of moderate, often transient, HIV-1-specific cytotoxic T lymphocyte (CTL) responses in some treated patients are the major findings reported and discussed here.

Materials and Methods

Patients and treatment. Twelve patients naive for ART were selected on the basis of HIV-1 plasma viremia reduction >2.5 log RNA copies/mL upon initiation of ART. Eight of them participated in the NUCB2019 study [4] and received either immediate triple therapy—a combination of zidovudine (3TC; 150 mg 2 \times /day), lamivudine (3TC; 150 mg 2 \times /day), and zidovudine (ZDV; 300 mg 2 \times /day)—or zidovudine alone for 3 weeks and then the triple combination (delayed triple therapy). The remaining 4 patients were chosen among HIV-1-infected homosexual men participating in the Amsterdam Cohort (ACH) studies on HIV infection and AIDS [14] to compare immune responses in samples obtained during long-term follow-up of asymptomatic infection with those measured during HAART. One of the ACH patients (1095) participated in the NUCB2019 study under code 20472, and the remaining patients initially received a combination of 3TC (150 mg 2 \times /day) and stavudine (d4T; 40 mg 2 \times /day) or ZDV (200 mg 3 \times /day); indinavir (800 mg 3 \times /day) was added when plasma viremia exceeded the value of 500 copies/mL [15].

Plasma viremia. Plasma HIV-1 RNA levels were measured by use of a commercial quantitative reverse transcriptase (RT) polymerase chain reaction kit (Amplicor HIV Monitor Test; Roche Molecular Systems, Branchburg, NJ) with an average lower detection level of 2.4 log₁₀ copies/mL or, for most pretherapy retrospective determinations, by use of a nucleic acid sequence-based amplification assay (NASBA HIV-1 RNA QT; Organon Teknica, Boxtel, The Netherlands) with a lower detection limit of 10³ copies/mL of serum.

***In vitro* lymphocyte proliferation assays.** *In vitro* T cell reactivity to mitogens was measured in fresh whole blood lymphocyte cultures as previously described [16]. Briefly, 1:10 diluted heparinized venous blood was cultured in 3 replicate wells of round-bottom microtiter plates in the presence of CD3 monoclonal antibody (mAb) or phytohemagglutinin (PHA). LPR was measured after 4 days by [³H]-thymidine incorporation. T cell reactivity was expressed as counts per minute (cpm). Samples from 5 healthy HIV-1-negative blood donors were included in each assay as controls. Results were expressed as a percentage of the mean T cell reactivity of controls.

As previously described [17], Ag-specific LPRs were measured retrospectively, culturing 10⁵ thawed peripheral blood mononuclear cells (PBMC) in 3–6 replicate round-bottom microtiter wells for 7 days in 0.1 mL of RPMI-1640 supplemented with antibiotics and 10% human pooled serum in the presence of one of the following Ags: (1) *Candida albicans* type A mannan-depleted broken yeast Ags (Accurate, Westbury, NY), extensively dialysed (1:100 final dilution); (2) tetanus toxoid (TT; RIVM, Bilthoven, The Netherlands), final concentration 30 Lf/mL; (3) *Mycobacterium tuberculosis* purified protein derivative (PPD; Statens Serum Institute, Copenhagen, Denmark), final concentration 10 μ g/mL; or (4) *Escherichia coli*-expressed recombinant Gag-p24 and Gag-p17 of HIV-1 (British Biotech Pharmaceutical Ltd., Oxford, United Kingdom), final concentration 6 μ g/mL. [³H]-thymidine (specific activity, 5 mCi/mmol) was added for the last 20 h of culture, and the incorporated radioactivity was measured in a β -scintillation counter. Results were expressed as net cpm, that is, the difference between median cpm in the Ag-containing cultures and median cpm in cultures in medium alone, and as stimulation index (LSI), that is, the ratio between median cpm in the Ag-containing cultures and median cpm in cultures in medium alone. Positive LPRs were

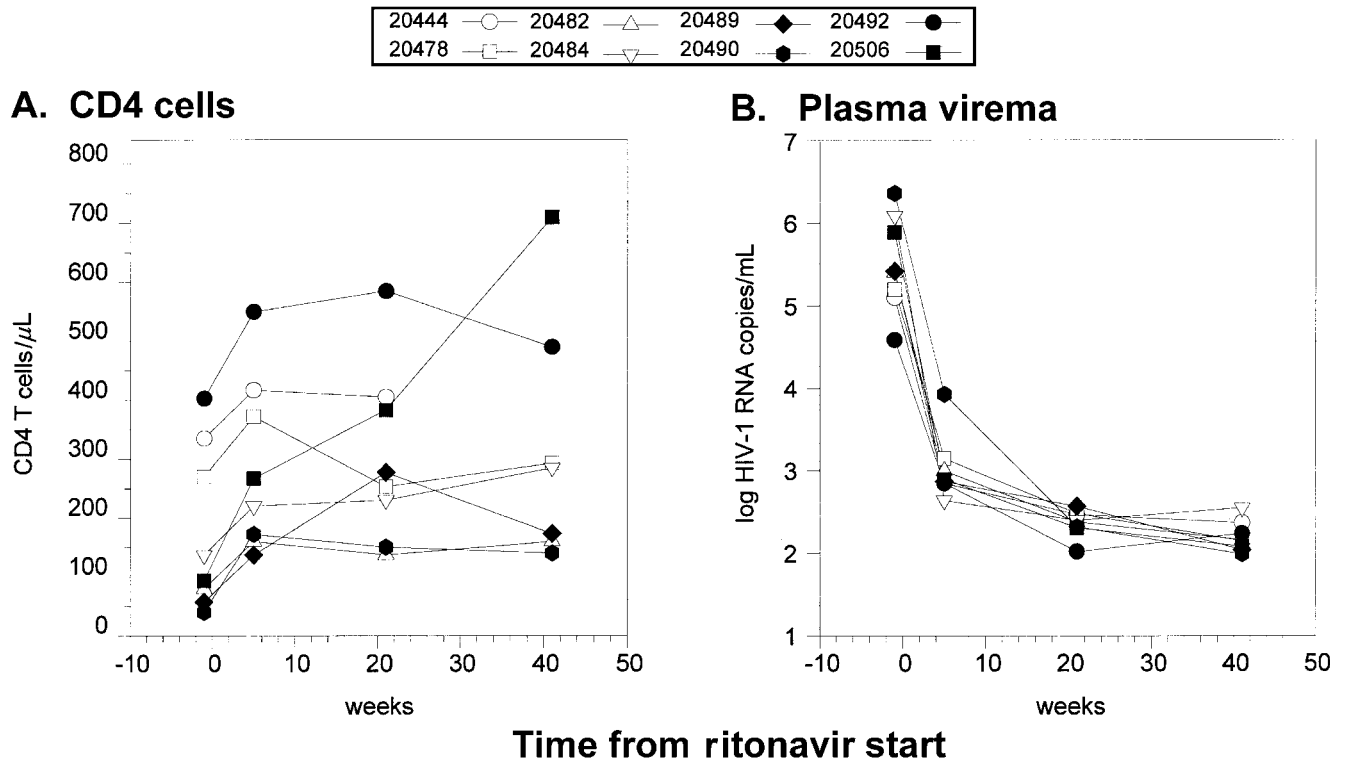


Figure 1. *A*, CD4⁺ T cell numbers and *B*, human immunodeficiency virus type 1 (HIV-1) RNA plasma load measured at the time of T cell response assessment in 8 patients enrolled in the NUCB2019 study [4] of triple drug combination (ritonavir, lamivudine, and zidovudine). See patients' characteristics at study entry in table 1.

scored when both net cpm were >2 SD from the mean of unstimulated PBMC cultures (280 cpm) and the LSI was >3 .

HIV-1-specific CTL responses. Recombinant vaccinia viruses (rVVs) TG.1144 [18] and TG.4163 [19], which express HIV-1_{LAI} *gag* and HIV-1_{LAI} RT, respectively, were kindly provided by Dr. Y. Rivière (Institut Pasteur, Paris, France) and Dr. M. P. Kieny (Transgène S.A., Strasbourg, France). Vaccinia virus 186poly containing no insert was used as a control. HIV-1-specific CTL precursors (CTLps) were expanded in vitro by Ag-specific stimulation as previously described [20–22]. Frequencies of CTLps specific for each HIV-1 product were determined by use of standard methods of limiting dilution analysis [23]. Briefly, PBMC were thawed and resuspended in RPMI-1640 supplemented with antibiotics and 10% pooled human serum. Eight serial dilutions, ranging from 246 to 26,000 PBMC/well, were seeded in 24 replicate wells in 96-well round-bottom microtiter plates. Stimulator cells were autologous Epstein-Barr virus-transformed B-lymphoblastoid cell lines (B-LCLs) infected with rVVs expressing the individual HIV-1 gene products and subsequently fixed with paraformaldehyde. To each well, 10^4 fixed stimulator cells and an equal number of irradiated feeder cells were added. Feeder cells were previously generated by Leucoagglutinin A (Sigma Chemicals, St. Louis) stimulation of autologous cryopreserved PBMC. Microcultures were maintained for 15 days at 37°C and 5% CO₂. At days 2 and 9, cultures were fed with medium containing human recombinant interleukin (rIL)-2 (360 IU/mL final concentration, kindly provided by Dr. R.

Rombouts; Chiron Benelux, Amsterdam), and on 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 in a standard ⁵¹chromium-release assay with autologous B-LCLs infected with each HIV-1 gene product-expressing rVV separately or with 186poly. Specific lysis was calculated with the formula $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Wells were considered positive when the ⁵¹Cr release exceeded 10% specific lysis. CTLp frequencies were expressed as the number of CTLps/ 10^6 PBMC. HIV-1-specific CTLp frequencies were computed as differences between CTLp frequencies determined with HIV-1 gene-expressing rVV-infected targets and those determined with control targets.

Statistical analysis. Comparison of paired data from patients at different times of follow-up was performed by Wilcoxon's signed rank test.

Results

Immune reconstitution during HAART. A group of 8 patients enrolled in the NUCB2019 study was selected on the basis of viral load reduction >2.5 log HIV-1 RNA copies/mL persisting for up to 9 months from start of therapy. Baseline CD4⁺ T cell numbers, plasma viremia levels, and T cell reactivity to PHA and CD3 mAb are reported in table 1. Immune para-

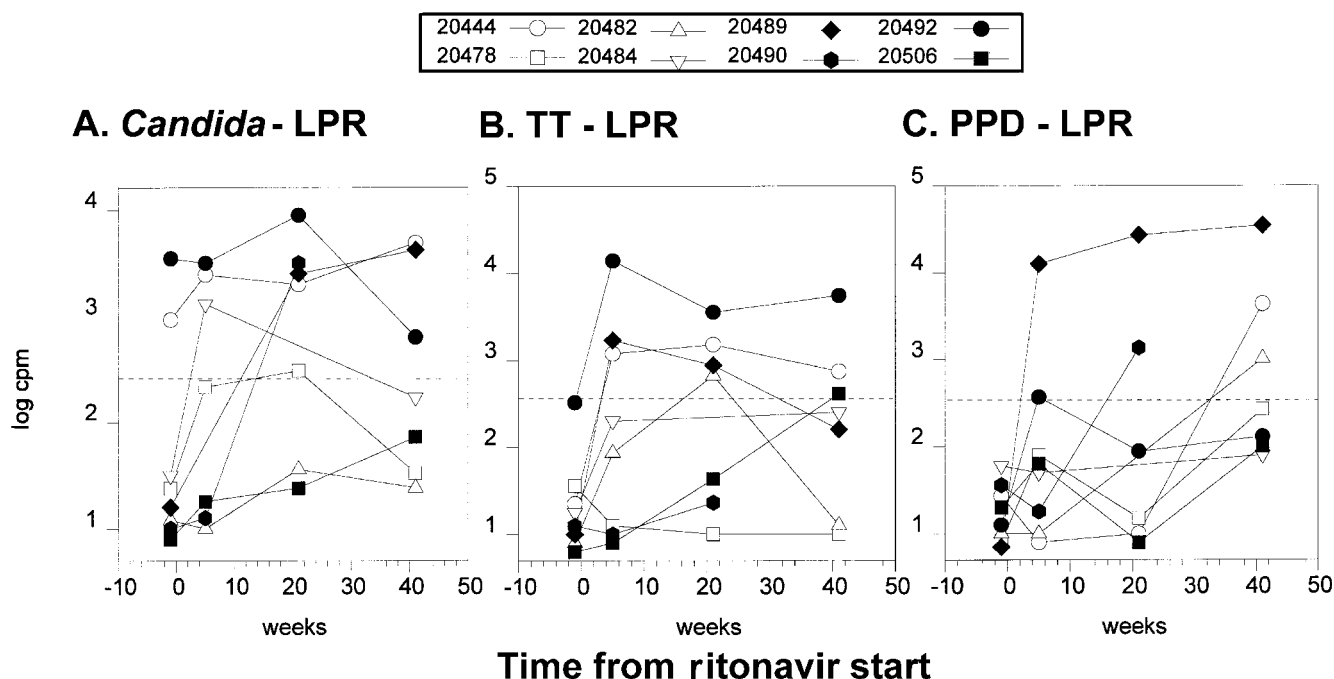


Figure 2. Lymphoproliferative response (LPR) to recall antigens before and during 9 months of highly active antiretroviral therapy in 8 patients enrolled in the NUCB2019 study [4]. Results are expressed as net counts per minute (cpm). Horizontal dashed lines represent 2 SD from the mean cpm in unstimulated cultures, taken as assay detection limit. TT, tetanus toxoid; PPD, purified protein derivative.

meters were analyzed in samples collected during the 2 weeks preceding HAART administration (t0) and, on average, after 5 weeks (month 1), 21 weeks (month 4), and 41 weeks (month 9) of therapy. As shown in figure 1, all patients showed an increase in CD4⁺ T cell numbers >100 cells/μL, together with rapid decline of plasma viremia, approaching undetectable levels after 1 month of therapy and remaining below or occasionally just above the assay quantification limit thereafter.

We studied responses to *Candida* Ags as a typical and common opportunistic agent, TT as a prototypic vaccine Ag unlikely to be encountered during the follow-up, and *M. tuberculosis* PPD as an example of a pathogen. The responses, measured in cultures of thawed PBMC from each patient, are shown in figure 2. Before HAART was begun, measurable LPRs to *Candida* were found in the 2 patients with the highest baseline CD4⁺ T cell counts. During therapy, LPRs to *Candida* were detected in 5 patients. Among them, the 2 patients with pre-HAART *Candida*-specific responses showed increased responses during therapy. In a paired-data statistical analysis, a significant difference in comparison with baseline values of LPRs to *Candida* was found at week 21 ($P = .018$) but not at week 41, when LPRs in 3 of the 5 responders were no longer detectable or were greatly reduced. Responses to TT followed a similar pattern, being barely detectable in only 1 patient before therapy and increasing from week 5 of therapy to reach statistically significant difference from baseline values at week

21 ($P = .043$). In total, 5 patients showed improved LPRs to TT during HAART, together with improved responses to *Candida* in 3. As for *Candida* Ags, such responses were no longer detectable in 2 patients at week 41. LPRs to PPD, totally undetectable before therapy, were seen in 4 patients during HAART and were significantly different from baseline values only at week 41 ($P = .018$). Only patient 20489, who had a history of active tuberculosis, showed vigorous PPD-specific LPRs starting from week 5 onwards. Appearance of LPR to at least 1 recall Ag was seen in all 8 patients during HAART and appearance of LPR to at least 2 of the 3 Ags tested in 5 patients. A more detailed description of LPRs and immune parameters in 4 of these patients is included in a separate report [25].

HIV-1-specific T cell responses. As representative of HIV-1-specific CD4⁺ T lymphocyte responses, the LPR to recombinant HIV-1-Gag proteins was measured. As shown in figure 3A, no p24-specific LPRs were detected before HAART, and only 1 patient (20444) showed a significant response (LSI = 4.7) at week 21. LPRs specific to p17 were also undetectable before HAART and appeared in 4 patients, at either week 5 or 21, with LSIs of 4.1–5.9 (figure 3B).

HIV-1-specific CTLp frequencies were measured in the 6 patients included in the study for whom B-LCL could be established (figure 4). HIV-1-Gag and RT were chosen as representative specificities, given the results of our previous lon-

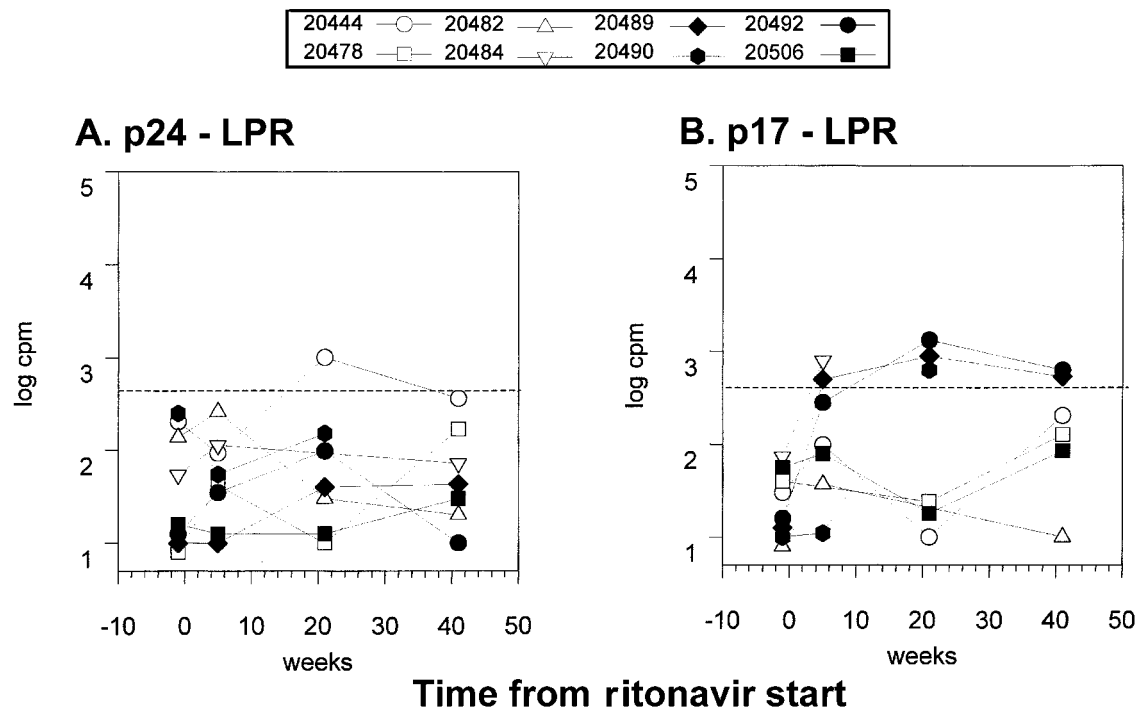


Figure 3. Lymphoproliferative response (LPR) to human immunodeficiency virus type 1 Gag-p24 (*A*) and Gag-p17 (*B*), expressed as net counts per minute (cpm), before and during 9 months of highly active antiretroviral therapy in 8 patients enrolled in the NUCB2019 study [4]. Horizontal dashed lines represent the assay detection limit.

gitudinal evaluation [22]. In that study, Gag- and RT-specific CTLps were the most abundant and were detected in all patients, representing a suitable parameter to evaluate specific T cell function. No CTL activity was detected in pretherapy samples. Although a statistically significant difference with baseline values was not reached, Gag-specific CTLp frequencies ranging from 23 to 80 CTLps/ 10^6 PBMC were found in 3 patients at weeks 5 and 21, in association with detectable RT-specific CTL activity in 2 (13–29 CTLps/ 10^6 PBMC). Only 1 patient (20478) had detectable HIV-1-specific CTLps, against both Gag- and RT-expressing targets, in PBMC obtained after 9 months of therapy.

Longitudinal analysis of T cell responses during untreated asymptomatic HIV-1 infection and during treatment. To establish the degree of T lymphocyte reconstitution obtained during therapy, retrospective PBMC samples from 4 patients enrolled in the Amsterdam Cohort of homosexual men were studied, and recall Ag- and HIV-1-specific responses during therapy were compared with those measured during untreated infection with limited immune impairment. CD4⁺ T cell numbers, viral load, recall Ag-specific LPRs, and HIV-1-specific CTLp frequencies in these patients are shown in figure 5, upper-lower panels. All of these patients started therapy with plasma viremia >5.5 log RNA copies/mL and reduced CD3

mAb T cell reactivity (<20% of that of controls in 3 of them and <40% in patient ACH6085).

Patient ACH68 had relatively stable and elevated numbers of circulating CD4⁺ T cells that doubled with therapy. Interestingly, this patient had undergone splenectomy as treatment for thrombocytopenia several years before HAART. Immediately after splenectomy, a significant p24-specific LPR (LSI = 6.6) was detected. During HAART, the p24-specific LPR did not reach significant levels (LSI < 2), whereas the response to *Candida* increased, approaching the levels of early infection. Gag-specific CTL activity, barely detectable before HAART, did not increase thereafter. The patient had a diagnosis of non-Hodgkin's lymphoma after 3 months of dual therapy, and indinavir was not added to the drug combination.

Patient ACH6085, who also started HAART with relatively stable and preserved CD4⁺ T cell counts, had an undetectable LPR to TT, which could be measured immediately after HAART commenced. Significant levels of CTL activity, measured before therapy, increased early during HAART and were maintained almost unchanged during the subsequent follow-up. In this patient, as well as in the 2 described below, no p24-specific LPR was ever detected.

Patient ACH211, previously classified as a long-term survivor because of asymptomatic infection for >9 years with con-

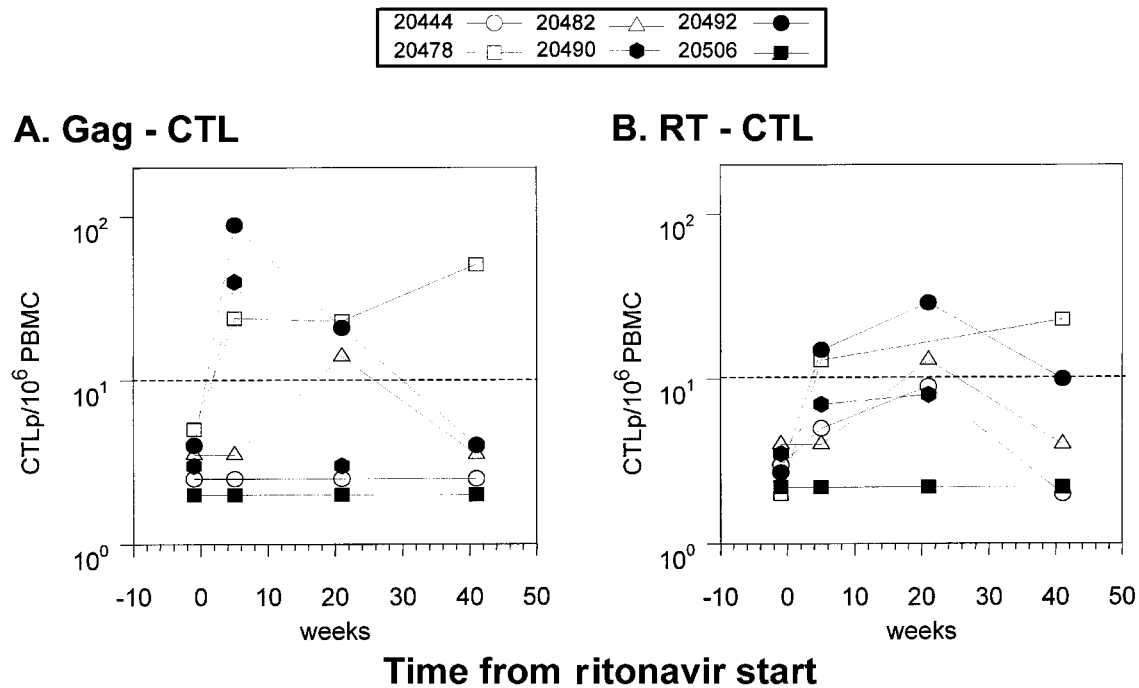


Figure 4. Frequency of circulating cytotoxic T lymphocyte (CTL) precursors specific for (A) human immunodeficiency virus type 1-Gag and (B) reverse transcriptase (RT) before and during 9 months of highly active antiretroviral therapy in 6 patients enrolled in the NUCB2019 study [4]. Horizontal dashed lines represent the assay detection limit.

served CD4⁺ T cell numbers, presented a steady decline of CD4⁺ T cells in the 3 years preceding HAART. Response to *Candida*-specific LPR and HIV-1-specific CTL activity, quite vigorous during early infection, was totally undetectable at start of therapy, when CD3 mAb reactivity was also greatly reduced. After 1 month of HAART, the response to *Candida* and CTL activity was already restored to levels comparable with those measured in samples from early infection and did not change significantly during the follow-up.

Patient ACH1095 started HAART after a prolonged period of reduced T cell responsiveness and a steady decline of CD4⁺ T cell numbers. *Candida*-specific LPRs and HIV-1-specific CTL activity were restored within the first month of therapy. This patient showed a partial therapy failure, with increasing viral load after 4 months of HAART. In association with the failure in virus control, response to *Candida* was no longer detectable in the last sample tested, and frequencies of HIV-1-specific CTL increased during the follow-up.

Discussion

Functional recovery of T cells during drug-induced suppression of HIV-1 replication has been reported with respect to responses to mitogens and microbial Ags [10–12]. We performed simultaneous measurements of lymphocyte responses to microbial and HIV-1 Ags to assess the relevance of improved

T cell function for HIV-1-specific T cell immunity. The data presented here demonstrate that lymphocyte responses to recall Ags substantially and persistently improved during HAART, reaching levels comparable with those measured in the same subjects during early or stable asymptomatic infection. This suggests that at least partial protection against common opportunistic agents is achieved. Similarly, comparable frequencies of CMV-specific CD4⁺ T cells, as detected by a sensitive assay based on early activation markers and flow cytometry, were found both in untreated HIV-1-infected patients with no history of CMV disease and in HAART-treated patients with quiescent CMV disease. In contrast, lower frequencies of CMV-specific CD4⁺ T cells were detected in patients with active CMV disease [26].

The relative decrease in responses to recall Ags in the late samples of some patients included in the present study, however, could be a sign of transient, although prolonged, effect on T lymphocyte function despite a persistent reduction of viral load. This finding deserves further characterization and could be explained alternatively by the dynamic changes in the T cell population. We recently reported that a reduction in T cell receptor (TCR) repertoire diversity occurs initially during HAART [27] in association with the phase of predominant repopulation by T cells of the memory phenotype. This phenomenon could be related to clonal expansion driven by Ags present at that time. These expansions subside once the Ags are eliminated and the

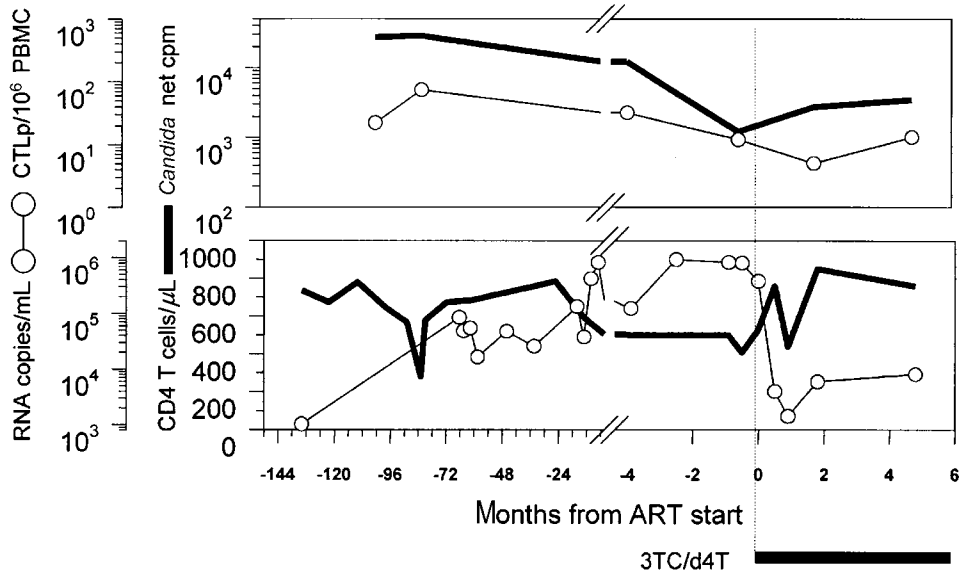
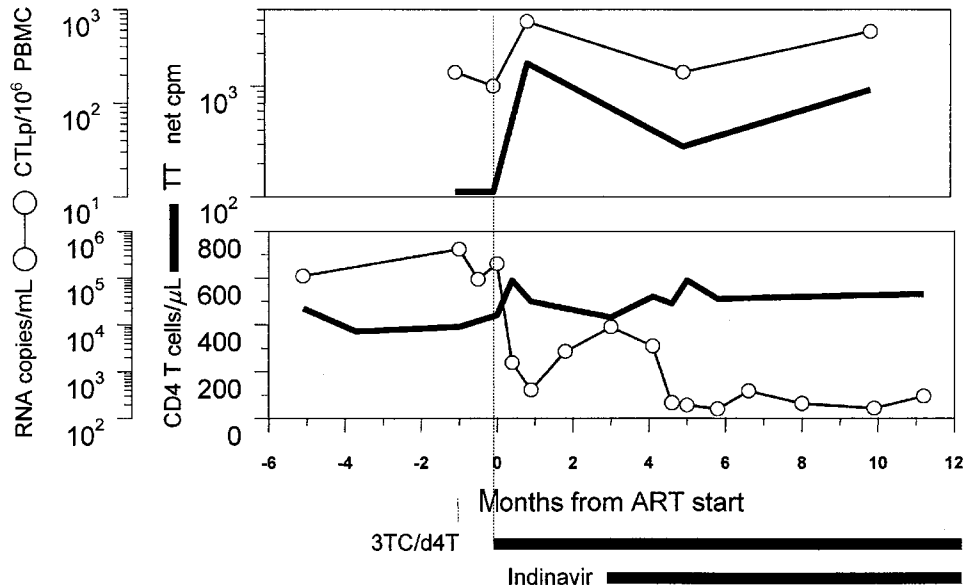
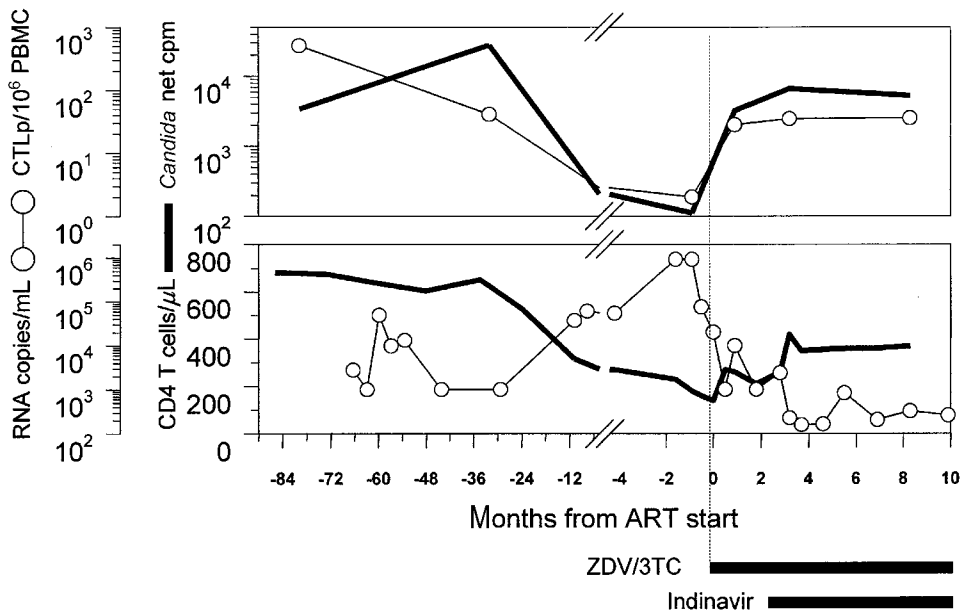
A. ACH68**B. ACH6085**

Figure 5. Impact of highly active antiretroviral therapy (ART) on T cell responses during long-standing human immunodeficiency virus type 1 (HIV-1) infection in 4 HIV-1-infected homosexual men participating in the Amsterdam Cohort. Upper panels depict the sum of Gag- and reverse transcriptase-specific cytotoxic T lymphocyte precursor (CTLp) frequencies (○) and the lymphoproliferative responses to a representative recall antigen, as specified (thick solid line). PBMC, peripheral blood mononuclear cells; TT, tetanus toxoid; 3TC, lamivudine; d4T, stavudine.

TCR diversity decreases, approaching normal distribution after several months of HAART [27, 28]. Similarly, reconstitution of pathogen-specific T cell immunity appears to induce pathological processes that are involved in the reaction to preexisting microbes and that cause clinical manifestations referred to as “immune restoration disease” [29]. In contrast, loss of control

on viral load despite no significant changes in the number of circulating CD4⁺ T cells, as observed in the follow-up of patient ACH1095, may rapidly induce a deterioration of Ag-specific responses. This mimics the early loss of responses to recall Ags without the significant CD4⁺ T cell depletion observed during the natural history of HIV-1 infection [30].

C. ACH211



D. ACH1095

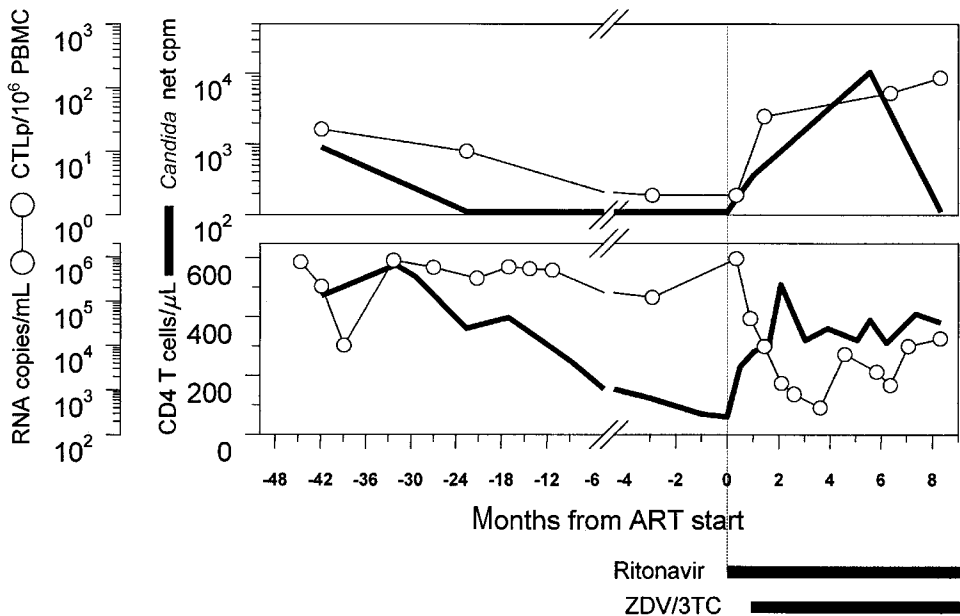


Figure 5. (continued) Lower panels depict CD4⁺ T cell numbers (thick solid line) and HIV-1 RNA plasma load (○). PBMC, peripheral blood mononuclear cells; 3TC, lamivudine; ZDV, zidovudine.

The detection of recall responses, conceivably in the absence of the relevant Ags *in vivo*, as we can reasonably assume at least for TT, suggests that immunological memory, and therefore the capacity to respond when the Ags are encountered again, is restored. This is compatible with the evidence of memory cells recirculating after having been trapped in lymphoid

tissue [12]. Indeed, Li et al. have shown that recovery of recall Ag responses was dependent upon a significant CD4⁺ memory T cell increase, together with a profound and sustained viral load reduction, in a group of patients with low baseline CD4⁺ T cell levels who were undergoing HAART [31]. However, appearance of, or increase in, recall Ag-specific responses in pa-

tients with high numbers of circulating CD4⁺ T cells who were undergoing therapy (such as patients ACH6085, ACH68, 20492, and 20444), and who therefore had smaller recirculating fractions [12], suggests that this mechanism may be only partly responsible for the regained reactivity to Ags. As a possible additional mechanism involved in the recovery of T cell function, decreased levels of proinflammatory cytokines due to viral load reduction may both mobilize lymphocytes from tissues and render them less susceptible to activation-induced apoptosis. A decrease in circulating tumor necrosis factor- α , known to induce lymphocyte apoptosis and functional suppression [32], was found during HAART in association with restored delayed-type hypersensitivity responses [33].

In general, the data now available on reconstitution of memory T cell responses indicate that no substantial deletion of Ag-specific CD4⁺ T cells occurs during HIV-1 infection [34]. This is apparently also the case when heavy exposure to the relevant pathogen occurs, as suggested by the vigorous and early responses to *Candida* in patients 20484 and 20490 and to PPD in patient 20478. This observation is in agreement with the mentioned trend to normalization of the CD4⁺ TCR repertoire after 6 months of HAART [28].

T cell responses to HIV-1 antigens follow a different pattern. Conceivably as a direct consequence of the recovery of T cell function achieved in the early phases of therapy, HIV-1-specific CTLps became detectable in some patients immediately after HAART was started and subsequently either remained stable or diminished up to disappearance after 9 months of successful therapy. In agreement with the requirement of relatively conserved T cell function to detect circulating CTLps in our assay, as extensively reported by us [22], no HIV-1-specific CTL activity was detected in the patients included in the NUCB2019 study before HAART was begun. The disappearance of HIV-1-specific CTLs during HAART has been elegantly demonstrated by direct staining with HLA/peptide tetrameric complexes, a technique that does not imply T cell responsiveness [35, 36]. In our study, a similar pattern of the disappearance of functionally detectable CTLps was seen in 2 patients, whereas 4 other patients, once CTLs became detectable during HAART, maintained stable CTLp frequencies. This discrepancy may reflect the less profound and persistent viral load reduction achieved in the latter patients, with residual viral replication occasionally detected in the assay employed. However, in 1 case of partial therapy failure associated with the emergence of drug-resistant viruses, CTLp frequencies progressively increased, together with viral load. This finding confirms the recovered lymphocyte capacity to respond to antigenic stimuli, although it poses the question of what the participation and efficiency of HIV-1-specific CTLs are in containing viral replication. Similar results have been recently reported by Dalod et al., measuring CTL activity in bulk cultures of lymphocytes stimulated with autologous activated lymphoblasts [37]. In their

patient group, the two patterns of either stable CTL detection or disappearance during therapy were related either to persistent residual viral load or to complete shutdown of viral replication, respectively.

In contrast to variably detectable HIV-1-specific CTLs, CD4⁺ T cells proliferating in response to p24 remained undetectable in patients treated with HAART late in HIV-1 infection. The finding of vigorous p24-specific LPRs in subjects undergoing HAART starting from symptomatic acute HIV-1 infection was greatly emphasized [38]. We cannot conclude at this stage that similar responses can never be achieved during treated advanced infection, but the virtual absence of antigen associated with viral load suppression argues against this possibility. The discrepancy between LPRs to recall Ags and HIV-1 p24 suggests that p24-specific CD4⁺ T memory cells either are not generated or are deleted during infection. Because recirculation from lymphoid tissue is a dominant mechanism of early T cell number increase during therapy, we can at least conclude that these cells are not compartmentalized in sites of viral replication in patients with long-standing infection. Similar data, extended also to HIV-1-Env-specific T cells, have been recently reported by Plana et al. [39], confirming the discordant behavior of LPRs to microbial Ags (CMV) and HIV-1 Ags. However, the persistent detection of p17-specific LPRs in some patients suggests that HIV-1-specific CD4⁺ T cells can be recruited during HAART, either because of regained function of previously primed and undeleted lymphocytes or by de novo priming, given the favorable conditions established during therapy. LPR to p17 was undetectable in a small group of asymptomatic HIV-1-infected patients with relatively preserved T cell function [17], but the existing knowledge on this issue is limited, and it is currently difficult to establish whether it has any relevance for the clinical outcome. The apparent paucity of HIV-1-specific CD4⁺ T cells may result in poor help for CTLs and their progressive disappearance [36]. It has been suggested that infected dendritic cells may provide activation signals for CD8⁺ T cells in the absence of CD4⁺ T cell cross-priming [40], a mechanism that could explain the vigorous expansion of HIV-1-specific CTLs during untreated HIV-1 infection [21, 22]. During HAART, infection of dendritic cells is likely to be prevented and CTL stimulation does not occur, as no significant CD4⁺ T cell help is available. In contrast, when HAART is administered before seroconversion, prevention of dendritic cell infection may allow the presentation of uninfected Ags able to prime CD4⁺ T cells, [38] which are spared from subsequent infection.

In conclusion, our results suggest that HAART during advanced HIV-1 infection induces a partial regaining of T cell competence with no evidence of repertoire regeneration, which may occur at later times. This opens the possibility of active immunizations to optimize protection against infections.

Acknowledgments

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