Decreased Expression of ζ Molecules by T Lymphocytes Is Correlated with Disease Progression in Human Immunodeficiency Virus–Infected Persons

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In human immunodeficiency virus type 1 (HIV-1) infection, functional activities of T lymphocytes are impaired. Analogous to tumor-infiltrating T lymphocytes from cancer patients, in whom poor proliferative responses are associated with fewer ζ molecules, this study compared expression of CD3ζ molecules by T lymphocytes from HIV-infected persons and healthy controls. Flow cytometry and immunoblotting revealed significantly diminished ζ expression by CD3, CD4, and CD8 T lymphocytes from AIDS patients but not from persons without AIDS. ζ-mRNA levels were also decreased in cells from AIDS patients. CD3ζ expression correlated significantly with CD4 cell counts and HIV-1 RNA levels; impaired expression of CD3ζ molecules appeared to be reversible upon virus load reduction following highly active antiretroviral treatment (HAART). Thus, reduced expression of CD3ζ molecules by T lymphocytes from HIV-infected persons correlates with disease status. Investigations into CD3ζ expression by subpopulations of peripheral T lymphocytes and by T lymphocytes in lymphoid tissues will contribute to the understanding of immune reconstitution of HIV-infected patients following HAART.

Clinically, human immunodeficiency virus (HIV) infection varies from an asymptomatic phase to full-blown AIDS, characterized by opportunistic infections related to severe defects in cellular immunity. Prior to a profound decrease in the number of peripheral CD4 T lymphocytes, T lymphocytes display impaired proliferative capacity and interleukin-2 (IL-2) production early in HIV infection. Initially, T lymphocyte responses to recall antigens and CD3 cross-linking are diminished, and then their responses to alloantigens and phytohemagglutinin (PHA) are diminished [1–5]. Incubation of T lymphocytes with HIV gene products, such as gp120 and gp41, results in altered cellular functions and in perturbations in lymphocyte signaling [6–8]. This indicates that altered signaling may underlie the impaired functional activities of T lymphocytes from HIV-infected persons.

One of the earliest events upon ligation of the T cell receptor complex (TCR) is activation of receptor-associated protein tyrosine kinases (PTKs) p56lck and p59fyn, which then phosphorlylate multiple protein substrates, including CD3ζ molecules [9, 10]. These molecules contain 3 potential tyrosine phosphorylation motifs [11], which, after phosphorylation, serve as docking sites for SH2 domain–containing signaling proteins, such as the PTK ζ-associated protein-70 (ZAP-70) [12–14]. Upon binding to ζ molecules, ZAP-70 is tyrosine phosphorylated and thereby activated [13, 14]. This TCR-associated PTK network up-regulates the activity of several signaling pathways, particularly that mediated by phospholipase Cγ1 (PLCγ1) [15–17]. Tyrosine phosphorylation of PLCγ1 results in increased PLC catalytic activity and activation of the inositol phosphatidyl pathway, yielding the release of diacylglycerol and inositol-1,4,5-trisphosphate [18]. These second messengers activate protein kinase C and induce Ca++ mobilization, requirements for the activation of transcription factors for the IL-2 gene promoter, resulting in IL-2 secretion and effector functions [19, 20].

Impairment of the CD3-mediated proliferative response of T lymphocytes from persons with early HIV infection is associated with altered early protein tyrosine phosphorylation, lower levels of p56lck [21, 22], and constitutively higher levels [21] or higher specific activity of p59fyn [22] than in T lymphocytes of healthy controls. Analogous to tumor-infiltrating T lymphocytes from cancer patients, in which poor proliferative responses are associated with lower levels of both p56lck and ζ molecules [23–25], the present study focused on whether expression of CD3ζ molecules by T lymphocytes from HIV-infected persons is lower than in healthy controls.

Materials and Methods

Antibodies. Murine IgG1 monoclonal antibodies (MAbs) TIA-2 [26] and 6B10.2 directed against intracellular epitopes of ζ mol-
ecules were obtained from Coulter (Hialeah, FL) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. The hybridoma cell line, producing the negative control murine IgG1 MAb 6D3 directed against CD14, was obtained from the American Type Culture Collection (Rockville, MD). We purchased fluorescein isothiocyanate (FITC)-conjugated F(ab)2 goat anti-mouse immunoglobulin (GAMFITC, Southern Biological Associates [SBA], Birmingham, AL), peroxidase-conjugated goat anti-mouse immunoglobulin (GAMPO, SBA), and phycoerythrin (PE)-conjugated murine IgG1 directed against human CD3 (Leu-4, anti-CD3PE), CD4 (Leu-3a, anti-CD4PE), and CD8 (Leu-2a, anti-CD8PE) (Becton Dickinson, Mountain View, CA). FITC-conjugated murine IgG1 directed against human CD69 (anti-CD69FITC) was obtained from PharMingen (San Diego).

Subjects. From March 1996 to October 1996, we enrolled 18 men and 3 women in this study (median age, 42 years; range, 27–65 years). All were patients of the Leiden University Medical Center Infectious Diseases outpatient clinic. By the 1987 Centers for Disease Control (CDC) case definition system [27], 10 patients were categorized as group IV-C1 on the basis of ≥1 previous opportunistic infection (cytomegalovirus [CMV] retinitis, Candida esophagitis, Pneumocystis carinii pneumonia, cryptococcal meningitis, Mycobacterium avium, Cryptosporidium enteritis) or as group IV-D if the subject had Kaposi’s sarcoma. Hereafter, these patients together with 1 patient with severe wasting (IV-A) will be referred to as AIDS patients. The other 10 patients were asymptomatic (CDC group II, n = 7) or mildly symptomatic (CDC group IV-C2, n = 3) and will be referred to as HIV-positive patients. Numbers of CD4 cells (mean ± SD) for the AIDS and HIV-positive subjects, respectively, were 32 ± 64 cells/μL (range, 1–208) and 270 ± 150 cells/μL (range, 86–598). All AIDS patients and 3 HIV-positive patients were on antiretroviral combination treatment consisting of zidovudine/lamivudine, zidovudine/zalcitabine, or lamivudine/zalcitabine. At that time, none was being treated with HIV protease inhibitors. One patient was on maintenance therapy for Mycobacterium tuberculosis infection. In addition to the antiretroviral combination therapy, 12 patients received prophylaxis drugs for P. carinii. In addition, 2 subjects received fluconazol prophylaxis because of recurrent oral candidiasis, 2 had maintenance treatment for CMV retinitis, and 2 received fluconazol prophylaxis and maintenance treatment for both CMV retinitis and disseminated M. avium infection. Controls were 15 healthy volunteers from the hospital staff (14 men, 1 woman; median age, 30 years; range, 21–50 years) without risk factors for HIV; CD4 T lymphocyte counts were not determined in this group.

CD4+ cells and virus load. CD4+ T lymphocytes in HIV-infected patients were measured at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam) or at the Central Clinical Haematological Laboratory, Leiden University Medical Centre (LUMC). HIV-1 RNA copies/mL plasma (detection limit ≤400 copies/mL) were determined by Amplicor HIV-1 Monitor test kit (Roche Diagnostic Systems, Branchburg, NJ) at the LUMC Department of Virology.

Cell isolation. Forty milliliters of peripheral blood was collected by venipuncture, then heparinized, and subjected to ficoll-hypaque density gradient centrifugation. Peripheral blood mononuclear cells (PBMC) collected from the interphase were washed with PBS containing 0.5 U/mL heparin and then resuspended to a concentration of 1 × 10^6–2 × 10^6 cells/mL PBS. This suspension contained ~50%–60% T lymphocytes; the remaining cells were monocytes (20%–25%), NK cells (12%–15%), and B lymphocytes (~5%). Cell viability was >95% (determined by trypan blue exclusion).

ζ molecule expression assessed by fluorescence-activated cell sorter. PBMC were fixed with 0.5% formaldehyde for 20 min at 4°C, washed in ice-cold PBS, and stored overnight at 4°C. Expression of ζ molecules by T lymphocytes was measured by an indirect immunofluorescence assay and fluorescence-activated cell sorter (FACS) analysis, as described elsewhere [26] with minor modifications. In short, PBMC were resuspended at a concentration of 5 × 10^6 cells/mL PBS containing 1% bovine serum albumin (PBS-BSA) and 20 μg/mL digitoxin (Sigma Chemical, St. Louis) and were incubated on ice for 10–15 min. After this treatment, >95% of the cells were permeable, as determined by trypan blue uptake. Next, the cells were incubated in 1% (vol/vol) normal goat serum for 5 min to block nonspecific binding sites, washed, and incubated with either MAb TIA-2, 6B10.2, or 63D3, an isotype-matched irrelevant MAb. After 30 min of incubation on ice and 3 washes, the cells were incubated with GAMFITC in PBS containing 0.05% Tween 20 for 30 min on ice. Excess GAMFITC was removed by washing. Free (F(ab)2, site of cell-bound GAMFITC were blocked by using 1% (vol/vol) normal mouse serum. Then the cells were stained for 30 min with anti-CD3PE, -CD4PE, or -CD8PE. After a wash, the cells were subjected to flow cytometry (FACScan; Becton Dickinson). The lymphocyte population was gated by forward-sideward scatter characteristics. The relative expression of ζ molecules by CD3+, CD4+, and CD8+ cells was calculated as a ratio of the median fluorescence intensity (MFI) of cells incubated with the TIA-2 or 6B10.2 MAb to that of cells incubated with the isotype-matched irrelevant antibody.

Biochemical analysis of ζ mRNA expression. After removal of the monocytes from the PBMC suspension by adherence to plastic, the nonadherent cells were collected and resuspended to a concentration of 2 × 10^7 cells/mL buffer containing 1% Nonidet P-40, 0.01 M triethanolamine-HCl at pH 7.8, 0.15 M NaCl, 5 mM EDTA, 0.02 mg/mL soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride, 0.02 mg/mL leupeptin, 20 mM iodoacetamide, and 0.1 U/mL aprotinine, and were maintained for 30 min at 4°C. Next, nuclear debris were removed by centrifugation of the lysates for 30 min at 10,000 g. The proteins in the supernatants were separated by SDS-PAGE on a 12% gel under reducing conditions and were transferred to a 0.2-μm proton nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The membrane was soaked in 5% low-fat milk to block nonspecific binding sites, and then incubated with 0.1 μg/mL MAb 6B10.2 directed against ζ molecules. MAb 6B10.2 was used in these experiments because of its suitability for immunoblotting, as stated by the manufacturer. Proteins were visualized with GAMPO and enhanced chemiluminescence (Amersham International, Amersham, UK). Band densities were measured with Image Quant software (Molecular Dynamics, Sunnyvale, CA).
Figure 1. Expression of CD3ε molecules by T lymphocytes from AIDS patients, human immunodeficiency virus (HIV)-positive asymptomatic or mildly symptomatic patients, and healthy controls. ε expression was determined in permeabilized cells by use of monoclonal antibody (MAb) TIA-2, CD3PE counterstaining, and flow cytometry and was expressed as the ratio of median fluorescence intensity of cells incubated with TIA-2 MAb to that of cells incubated with isotype-matched irrelevant MAb.

Figure 2. Correlation between expression of CD3ε molecules and plasma human immunodeficiency virus type 1 (HIV-1) RNA levels (A) and CD4+ lymphocyte counts (B). ε expression was determined in permeabilized cells by use of monoclonal antibody (MAb) TIA-2, CD3PE counterstaining, and flow cytometry and expressed as the ratio of median fluorescence intensity of cells incubated with TIA-2 MAb to that of cells incubated with isotype-matched irrelevant MAb.

Expression of CD3ε molecules by T lymphocytes. The expression of ε molecules by CD3ε lymphocytes from AIDS patients was

Analysis of CD69 expression by cultured lymphocytes. PBMC were cultured in the presence or absence of 1 μg/mL PHA (Murex Diagnostics, Dartford, UK) at a concentration of 10⁶ cells/mL Dulbecco’s MEM (Gibco BRL, Paisley, UK), supplemented with 10% heat-inactivated human serum, 3.5 mg/mL glucose, 3.7 mg/mL NaHCO₃, 2 mM L-glutamine, 100 U/mL penicillin G, and 100 μg/mL streptomycin for 20 h at 37°C. Next, the cells were harvested, washed twice in PBS-BSA, and stained with anti-CD69FITC and anti-CD3PE for 30 min. After removal of excess antibodies by washing, the cells were fixed in 1% formaldehyde and subjected to flow cytometry. The relative expression of CD69 by CD3ε cells was calculated as a ratio of the MFI of cells cultured with and without PHA.

Statistical analysis. Results are presented as individual values or as mean ± SD. The significance of differences in expression of ε molecules and CD69 expression among cells from healthy controls, HIV-positive subjects, and AIDS patients was calculated by the Mann-Whitney U test; correlations between CD3ε expression and peripheral CD4+ T cell numbers, virus load, and CD69 expression were determined by Pearson’s correlation test.

Results

Expression of ε molecules by T lymphocytes. The expression of ε molecules by CD3ε lymphocytes from AIDS patients was
Figure 3. Expression of CD3γ molecules by T lymphocytes from AIDS patients detected by gel electrophoresis and immunoblotting. Peripheral blood lymphocytes from 2 AIDS patients and 2 healthy controls were lysed in parallel by use of Nonidet P-40. Proteins in lysates (3 × 10^6 cell equivalents) were separated by 12% SDS-PAGE under reducing conditions, transferred to nitrocellulose membranes, and immunoblotted with monoclonal antibody 6B10.2. Band densities were measured with Image Quant software (Molecular Dynamics, Sunnyvale, CA). AU, arbitrary units; ND, not determined.

Figure 4. Expression of CD3γ molecules by CD4+ and CD8+ cells from AIDS patients, compared with cells from human immunodeficiency virus (HIV)-positive asymptomatic or mildly symptomatic patients and healthy controls. γ-expression was determined in permeabilized cells by use of monoclonal antibody (MAb) TIA-2 counterstained with CD4PE and CD8PE, analyzed by flow cytometry, and expressed as the ratio of median fluorescence intensity of cells incubated with TIA-2 MAb to that of cells incubated with isotype-matched irrelevant MAb.
lower amounts than in cells from the healthy donor (figure 3). Thus, both FACS and Western blot analysis showed reduced expression of $\zeta$ molecules by cells from AIDS patients.

T lymphocyte activation by specific antigens results in a decrease in the cellular content of CD3$\zeta$ molecules [31]. Decreased expression of CD3$\zeta$ molecules by T lymphocytes from AIDS patients, therefore, could result from antigenic stimulation due to previous opportunistic infections. To investigate this possibility, we assessed CD3$\zeta$ expression by T lymphocytes from HIV-negative persons with opportunistic infections frequently seen in AIDS patients. One person had a history of disseminated $M$. avium infection; the other was a kidney transplantation recipient with CMV infection. CD3$\zeta$ expression by T lymphocytes from both patients was comparable with that of cells from control donors (83%–90% of control levels). These results indicate that HIV itself, rather than the opportunistic infections resulting from HIV infection, most likely causes reduced expression of $\zeta$ molecules by T lymphocytes from AIDS patients.

Expression of $\zeta$ molecules by CD4$^+$ and CD8$^+$ T cells. Detailed analysis of the expression of $\zeta$ molecules by the various subpopulations of T lymphocytes revealed a significant decreased expression of $\zeta$ molecules by CD4$^+$ lymphocytes from AIDS patients compared with cells from HIV-positive patients and healthy donors (figure 4A). Expression of $\zeta$ molecules by CD8$^+$ cells from AIDS patients was significantly lower than that from cells from healthy controls and lower, although not significantly ($P = .06$), than that from cells from HIV-positive patients (figure 4B). Overall, expression of $\zeta$ molecules was decreased in both CD4$^+$ and CD8$^+$ cells from AIDS patients.

PCR analysis of $\zeta$ chain-specific mRNA. To learn whether the decreased expression of $\zeta$ molecules by T lymphocytes from AIDS patients is regulated at the transcriptional level, we assayed $\zeta$-specific mRNA in these cells by use of reverse transcription and PCR amplification. The results showed that decreased expression of $\zeta$ molecules by T lymphocytes from AIDS patients is associated with decreased mRNA levels for this protein (figure 5A, 5B). As a control, T lymphocytes from 2 patients expressing $\zeta$ molecules at a slightly reduced level had minor (figure 5C) or no reductions (figure 5D) in $\zeta$ mRNA levels compared with that in cells from healthy controls.

Expression of CD69 by T lymphocytes on stimulation with PHA. To investigate whether decreased expression of $\zeta$ molecules could be involved in the impairment of functional activities of T lymphocytes from HIV-infected patients as a functional readout, we determined the expression of the early activation antigen CD69 [32] upon stimulation of these cells with PHA. Only a minority of T lymphocytes from patients and healthy controls, 7.6% ± 2.2% and 8.8% ± 5.3%, respectively, expressed CD69 when cultured in the absence of a stimulus. When cultured in the presence of PHA, >50% of the cells expressed CD69: 58.4% ± 28.4% and 68.1% ± 12.4% of cells from patients and healthy controls, respectively. The intensity of PHA-induced CD69 expression by CD3 T lymphocytes from...
HIV-infected patients was significantly lower ($P = .003$) than that from cells from healthy donors (figure 6A). We found a good correlation ($r = .76, P = .007$) between CD3$\zeta$ expression and PHA-mediated CD69 expression (figure 6B), indicating that for CD69 expression, $\zeta$ expression correlates with functional activities of T lymphocytes.

**Expression of CD3$\zeta$ molecules by T lymphocytes after highly active antiretroviral therapy.** Since there was a relation between CD3$\zeta$ expression and virus load, as a pilot study, we examined whether highly active antiretroviral therapy (HAART); a combination of 3 antiviral drugs including at least 1 protease inhibitor) was accompanied by improved expression of CD3$\zeta$ molecules in 3 subjects. Shortly after initiation of HAART, HIV-1 RNA copies in plasma of all patients dropped to levels near the detection limit of the assay; this was accompanied by an increase in the expression of $\zeta$ molecules by CD3$^+$, CD4$^+$, and CD8$^+$ lymphocytes (figure 7). In 1 patient, virus loads decreased further to undetectable levels in the next month; this was associated with a further increase in the expression of $\zeta$ molecules (figure 7A). In the other 2 patients, within 1 month (figure 7B) and within 5 months (figure 7C) after the start of therapy, virus loads increased, which was associated with decreased expression of $\zeta$ molecules by their T lymphocytes. For comparison, variability in CD3$\zeta$ expression by T lymphocytes from 4 healthy controls (determined $2\times$ or $3\times$ during the same period) was $\sim10\%$. Together, these data suggest that effective treatment of AIDS patients with HAART results in enhanced expression of $\zeta$ molecules by their T lymphocytes.

**Discussion**

In this study, we found that expression of $\zeta$ molecules is down-regulated in T lymphocytes from HIV-infected persons, in relationship to disease progression. Expression of $\zeta$ molecules by T lymphocytes from AIDS patients, but not from HIV-positive persons with mild or no symptoms, was reduced by $\geqslant35\%$, as measured by flow cytometry and immunoblotting. The reduction in CD3$\zeta$ molecules was specific and not a generalized reduction in the expression of the CD3 complex, since we found no decrease in the expression of CD3$\epsilon$ molecules. We are the first to demonstrate reduced levels of mRNA for $\zeta$ molecules in cells from AIDS patients, thereby extending the results of 2 studies that reported decreased expression of $\zeta$ molecules by T lymphocytes from HIV-infected subjects [33, 34]. On the basis of these findings, we suggest that defects in or upstream of transcription of $\zeta$ account for the reduced expression of this protein. Furthermore, we found a significant correlation between expression of CD3$\zeta$ molecules and CD4$^+$ cell counts and an inverse correlation between $\zeta$ expression and plasma HIV-1 RNA levels. It is of interest that impaired expression of CD3$\zeta$ molecules appeared to be reversible in AIDS patients after HAART. Altered expression of CD3$\zeta$ molecules thus correlates with disease progression in persons infected with HIV and is thereby concurrent with therapy success in these patients.

Whether decreased expression of CD3$\zeta$ molecules can explain functional defects of T lymphocytes from AIDS patients [1–5] is not clear. We found a significant correlation between expression of CD3$\zeta$ molecules and expression of the early activation antigen CD69 by T lymphocytes upon stimulation with mitogens. Whether decreased expression of CD3$\zeta$ molecules underlies other functional defects of T lymphocytes from HIV-infected patients is beyond the focus of this study. However, others have reported that defective HIV-specific cytolytic ac-

![Figure 6. Phytohemagglutinin (PHA)-induced expression of CD69 (A) by T lymphocytes from human immunodeficiency virus (HIV)-infected patients and healthy controls and correlation with CD3$\zeta$ molecules (B). Peripheral blood mononuclear cells from patients (●) and healthy controls (○) were cultured in presence and absence of 1 μg/mL PHA for 20 h, stained with anti-CD69FITC and anti-CD3PE, and subjected to flow cytometry. Results are expressed as the ratio of median fluorescence intensity (MFI) of cells cultured with and without PHA. Expression of CD3$\zeta$ molecules was determined in freshly isolated cells by permeabilization, staining with monoclonal antibody (MAb) TIA-2, CD3PE counterstaining, and flow cytometry and expressed as the ratio of MFI of cells incubated with TIA-2 MAb to that of cells incubated with isotype-matched irrelevant MAb.](https://academic.oup.com/jid/article-abstract/180/3/649/809365)
Figure 7. Reversibility of impairment in expression of CD3ζ molecules by T lymphocytes from 3 AIDS patients after highly active antiretroviral therapy (HAART). Patient 1 (A), who had no prior antiretroviral treatment, began with stavudine, lamivudine, and indinavir; patient 2 (B) was treated with zidovudine and lamivudine and then with ritonavir; patient 3 (C), who was previously treated with zidovudine and lamivudine, received stavudine, didanosine, ritonavir, and saquinavir. Plasma human immunodeficiency virus type 1 (HIV-1) RNA levels were determined by Amplicor HIV-1 test kit (detection limit, $\leq 400$ copies/mL). CD4+ cells/μL during month 1 of HAART rose from 273 to 307, 107 to 197, and 15 to 38 for patients 1–3, respectively. Expression of ζ molecules was determined, as described in legends of figures 1 and 4. CD3ζ expression by T lymphocytes from healthy controls during same period was 7.6 ± 1.5 (n = 17).
tivity by T lymphocytes from HIV-infected patients correlated with reduced expression of CD3ζ molecules by these cells [34]. Furthermore, loss of CD3ζ molecules may result in suppression of antigen-specific proliferation of T lymphocytes [35]. Together, decreased expression of CD3ζ molecules most likely contributes at least to some functional defects in T lymphocytes from AIDS patients.

We did not find decreased expression of CD3ζ molecules by T lymphocytes from asymptomatic or mildly symptomatic HIV-infected persons, as reported elsewhere [33, 34], even when we used an antibody with a lower affinity for phosphorylated ζ molecules. The reason for this discrepancy is not clear. T lymphocytes from HIV-infected subjects at early stages of disease have altered early protein tyrosine phosphorylation and PTK levels associated with impaired proliferative responses [21, 22]. Our data suggest that such alterations and not a loss of CD3ζ molecules account for functional defects of T lymphocytes early in infection.

A second question pertains to the mechanisms underlying the reduced expression of ζ molecules by T lymphocytes from AIDS patients. Since antigenic stimulation of T lymphocytes results in enhanced TCR turnover and reduced levels of ζ molecules [31], antigenic stimulation due to previous opportunistic infections could account for the reduced expression of CD3ζ molecules in AIDS patients. However, our observation that T lymphocytes from HIV-negative persons who had been exposed to opportunistic agents, such as CMV and M. avium, did not have reduced expression of CD3ζ molecules excludes this possibility. Another possibility is that HIV-derived proteins, such as gp120, gp41, tat, and nef, which diminish functional activity of T lymphocytes and alter lymphocyte signaling [6–8, 36–40], affect expression of ζ molecules by T lymphocytes. Preliminary investigations into this possibility revealed no effect of HIV gp120 on expression of ζ molecules by T cells from healthy donors (data not shown). Alternatively, reactive oxygen intermediates produced by macrophages may induce decreased expression of ζ molecules in T lymphocytes, as has been reported elsewhere, for tumor-infiltrating T lymphocytes [35, 41]. Of interest, the level of glutathione, the main intracellular defense against oxidative stress, is decreased in plasma and leukocytes of HIV-infected subjects [42], indicating that reactive oxygen intermediates may contribute to oxidative stress [44].

HIV-1 infection is characterized by a chronic state of immune activation [45] and substantial shifts in the composition of T lymphocyte subsets, including a preferential loss of memory CD4+ T lymphocytes [3] and a relatively increased number of T lymphocytes with a Th2 profile in the circulation [46, 47]. The major goal of antiretroviral therapy, therefore, is not only to suppress viral replication but also to achieve some degree of immune reconstitution. Indeed, substantial increments in the number of memory CD4+ T lymphocytes, diminished expression of T lymphocyte activation markers (e.g., HLA-DR, CD38, and CD25), and significant improvements of antigen-specific responses by peripheral blood lymphocytes following HAART have been reported elsewhere [48, 49]. We studied too few patients for definitive conclusions in regard to up-regulation of ζ molecules by T lymphocytes after HAART. In future studies, measurement of CD3ζ expression in the various subsets of T lymphocytes may contribute to the understanding of restoration of immune competence in patients during HAART. Furthermore, since lymphatic tissues harbor large depots of viral RNA and many infected cells [50], we anticipate more severely reduced levels of ζ molecules in cells at these sites, compared with peripheral T lymphocytes. The reduction in expression of CD3ζ molecules by lymphatic T lymphocytes may be reversible with significant virus load reductions in lymphatic tissues during HAART [51]. If so, redistribution of memory T lymphocytes previously trapped in the lymph nodes upon antiretroviral therapy [52] could result in normalization of CD3ζ expression by peripheral T lymphocytes. Future experiments will focus on expression of CD3ζ molecules by T lymphocytes in lymphoid tissues from patients before and during HAART.

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