Downregulation of Increased CD95 (APO-1/Fas) Ligand in T Cells From Human Immunodeficiency Virus-Type 1–Infected Children After Antiretroviral Therapy

To the Editor:

In a recent article in *Blood*, Yang et al[1] reported that they found no evidence for abnormally elevated expression levels of CD95 ligand (L) mRNA in either fresh or cultured peripheral blood lymphocytes from 9 human immunodeficiency virus (HIV)-infected individuals with absolute T-cell counts of 200 to 500 cells/μL (CDC class 2) when compared with 6 uninfected healthy controls. These findings are in striking contrast to data previously published by us[2] showing elevated CD95L mRNA levels in freshly isolated peripheral blood T cells from symptomatic HIV-1–infected children.

Yang et al[1] used a sensitive quantitative reverse transcription-polymerase chain reaction similar to the one developed in our laboratory.[2,3] However, these investigators used unseparated peripheral blood lymphocytes as the source for total RNA instead of isolated T cells. T, B, and NK lymphocytes differ with respect to spontaneous or stimulation-induced production of CD95L mRNA.[3] In our hands, the striking difference in CD95L mRNA levels between HIV-infected children and noninfected controls could repeatedly be shown only in freshly isolated T cells.

Theoretically, the T-cell isolation procedure using immunomagnetic beads lasting up to 2 hours would allow for ongoing specific RNA synthesis to proceed. Therefore, blood samples from both patients and controls received exactly the same treatment. Thus, our data might only prove that CD95L mRNA synthesis is more easily inducible in T cells from HIV-1–infected children than in T cells from healthy controls. However, CD95L expression is increased in HIV-infected individuals also on the protein level. We performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting using a monoclonal mouse IgG antibody raised against a peptide corresponding to the extracellular part of the human CD95L molecule (Dianova, Hamburg, Germany) on cell lysates from purified T cells of a healthy control either freshly isolated or after 4 or 20 hours of stimulation with phorbol myristate acetate and ionomycin. The amount of CD95L protein detected in the lysate did not differ between unstimualted control cells and cells stimulated for 4 hours and increased significantly after 20 hours of stimulation. The lysate of unstimulated freshly isolated T cells from an HIV-1–infected patient contained at least as much CD95L protein as the control cells stimulated for 24 hours.

When following CD95L-mRNA levels in isolated T cells from 11 of our patients during 12 different courses of new antiretroviral treatment with reverse transcriptase inhibitors (zidovudine, didanosine, zalcitabine, and lamivudine, either alone or in combination), a significant reduction by 61% ± 8% (mean ± SEM; 1,441 ± 159 fg/250 ng RNA before *v* 564 ± 141 fg/250 ng RNA after 8 weeks of therapy; $P < .001$) was observed (Fig 1). At the same time point, an overall decrease of HIV viral load of 81% ± 12% was seen, whereas no consistent changes in the $CD4^+$ T-cell counts could be noted during the short observation period after the start of therapy (mean change from baseline, −9% ± 8%). The reduction in CD95L-mRNA levels was more pronounced in patients exhibiting a good response to treatment (>1.0 $\log_{10}$ copies/mL reduction in HIV plasma viral load) than in poor responders (<1.0 $\log_{10}$ copies/mL reduction in HIV plasma viral load and/or progressive immunologic deterioration during the following 6 months). One patient showed a pronounced decrease in CD95L-mRNA levels and a decrease in viral load of approximately 1.0 $\log_{10}$ copies/mL. In this patient, serial determinations of CD95L-mRNA levels in T cells and HIV viral load in plasma showed a close relationship between both parameters (Fig 2). The initial decrease of viral load and CD95L-mRNA after starting therapy with zidovudine and didanosine was followed by an increase in viral load after 12 weeks that was paralleled by an increase in CD95L-mRNA levels and a concomitant decrease in $CD4^+$ T-cell counts.

We conclude from our data that constitutive expression of CD95L is increased in freshly isolated peripheral blood T cells from HIV-1–infected children. Studies addressing CD95L mRNA expression in HIV-infected individuals should use freshly isolated lymphocyte subpopulations rather than bulk peripheral blood lymphocytes as the RNA source. Cryopreservation of mononuclear cells or any delay

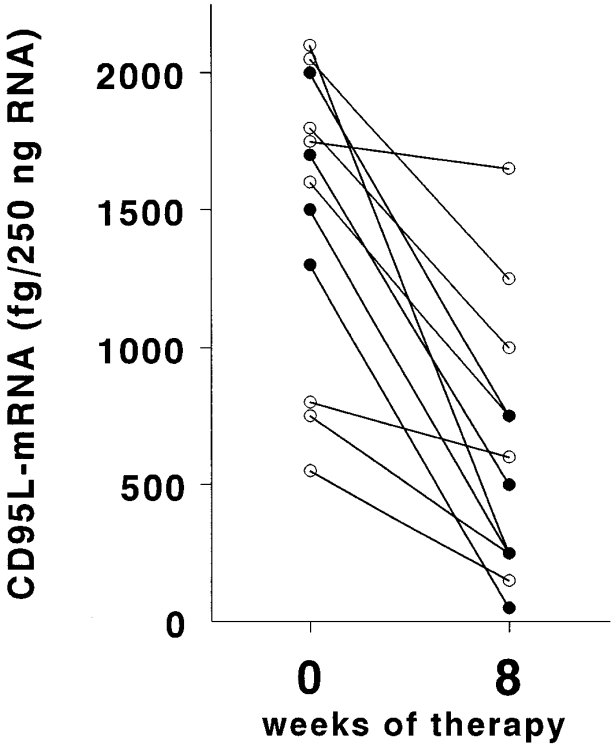

Fig 1. CD95L-mRNA levels in freshly isolated T cells from 11 HIV-1^+ children before and after 8 weeks of therapy with new or additional reverse transcriptase inhibitors (12 treatment courses). Response to treatment was classified either according to the reduction in HIV-1 plasma viral load achieved at that time point or based on clinical and immunologic observations ([●] good response, >1.0 $\log_{10}$ copies/mL reduction in viral load; [○] poor response, <1.0 $\log_{10}$ copies/mL reduction in viral load). Mean CD95L-mRNA levels after 8 weeks of therapy were significantly lower than before therapy ($P < .001$; two-tailed paired Student's *t*-test). HIV-1 plasma viremia was determined using either a commercially available polymerase chain reaction kit (Amplicor Monitor; Roche Diagnostics, Grenzach-Whylen, Germany) or a sensitive second generation branched-chain DNA (bDNA) assay (Quantiplex HIV RNA 2.0 Assay; Chiron Diagnostics, Ferning, Germany) as recommended by the manufacturer.

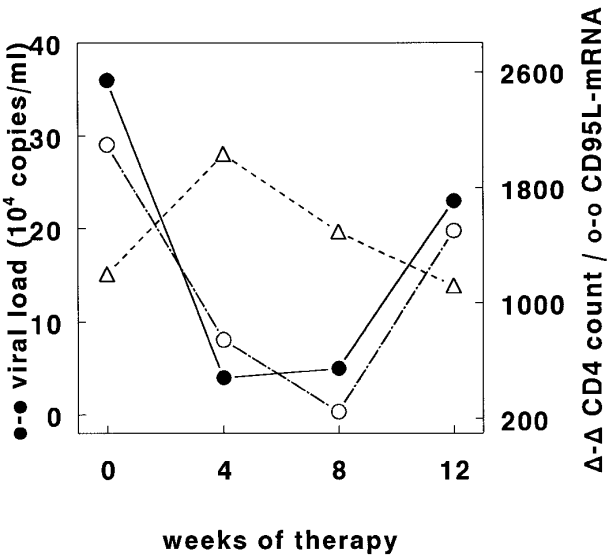

Fig 2. HIV-1 viral load in plasma ([●] 10^4 copies/mL), absolute $CD4^+$ T-cell counts ([△] cells per microliter), and CD95L-mRNA levels in freshly isolated T cells ([○] femtograms per 250 ng total RNA) during antiretroviral combination therapy with zidovudine and didanosine in an HIV-1^+ infant. Initial reduction of HIV-1 viral load and CD95L-mRNA levels were paralleled by an increase in $CD4^+$ T-cell counts. However, as early as 12 weeks after the introduction of antiretroviral treatment, HIV-1 viral load and CD95L-mRNA levels increased and $CD4^+$ T-cell counts decreased.

in processing the blood and RNA samples should be avoided because these procedures usually result in a decrease rather than an increase in measurable CD95L mRNA levels (I.H. and K.-M.D., unpublished observations).

The data presented here also indicate that increased CD95L production in freshly isolated T cells from HIV-1–infected children can be downregulated to near-normal levels by effective antiretroviral treatment. The CD95 receptor/ligand system critically regulates physiological elimination of T cells.[4] In peripheral T cells, activation-induced expression of CD95L mediates apoptosis after binding to CD95L on the surface of the T cell. Upregulation of CD95L in (uninfected) peripheral blood T cells from HIV-1^+ individuals may be either caused specifically by cellular uptake of HIV-1 Tat[5] or may reflect general stimulation of lymphomonocytic cells observed during HIV infection.[6,7] In addition to T cells, infection of human macrophages with laboratory HIV strains in vitro leads to an upregulation of CD95L expression in these cells that caused apoptotic death of uninfected T lymphocytes.[8] We previously showed inhibition of CD95-mediated apoptosis in T cells from HIV-infected children by disrupting CD95 receptor/ligand interaction using blocking anti-CD95 F(ab′) antibody fragments.[2] Given the important role of CD95L in T-cell apoptosis, we speculate that effective antiretroviral treatment (including HIV protease inhibitors) that leads to significant reduction of virus replication will also lead to decreased activation-induced death of uninfected T cells, thus restoring impaired T-cell homeostasis and function. Furthermore, monitoring CD95L mRNA expression in T cells may give important information on self-destructive immune stimulation in HIV-infected individuals and might be used as an additional surrogate marker for disease progression and treatment response.

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Response

To the Editor:

Böhler et al have pointed out a discrepancy between our respective studies[1,2] with regard to spontaneous expression of Fas ligand (FasL; or CD95L) by peripheral blood cells from HIV$^+$ individuals. We did not address their findings in our report, which was in press at the time their report was published, but are happy to have the opportunity to do so now. We used quantitative competitive polymerase chain reaction to analyze FasL levels in unfractionated peripheral