To the Editor:

In a recent article in Blood, Yang et al. 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 showing elevated CD95L mRNA levels in freshly isolated peripheral blood T cells from symptomatic HIV-1-infect ed children.

Yang et al. used a sensitive quantitative reverse transcription-polymerase chain reaction similar to the one developed in our laboratory. 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. 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 unstimulated 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 log10 copies/mL reduction in HIV plasma viral load) than in poor responders (<1.0 log10 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 log10 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...
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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REFERENCES

Response
To the Editor:
Boehler et al have pointed out a discrepancy between our respective studies 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
Downregulation of Increased CD95 (APO-1/Fas) Ligand in T Cells From Human Immunodeficiency Virus-Type 1–Infected Children After Antiretroviral Therapy

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