Case Report

Change in Viral DNA and mRNA Burdens in Peripheral Blood Mononuclear Cells in a Patient with HIV-1 after Stopping Anti-retroviral Treatment

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Case: There have been few reports on virological effect as observed through changes in viral DNA or mRNA burdens in HIV-1 infected cells during the interruption of highly active anti-retroviral therapies (HAART). We observed a patient with HIV-1 infection who was obliged to stop anti-retroviral drugs suddenly. After periodically measuring viral DNA and mRNA burdens in peripheral blood nuclear cells after stopping anti-retroviral drugs, we discussed the safety of interrupting HARRT in chronic HIV-1 infected patients.

Reselts: Although his plasma viral loads (VLs) were sustained at 3 weeks, they rapidly increased from an undetectable level to 2.2×10^6 copies/ml at 4 weeks, and his viral mRNA and DNA burdens also increased. After medication was restarted, VL, viral mRNA and DNA burdens all decreased again to the levels indicated before the interruption.

Discussion: Well-controlled patients undergoing HAART may avoid virological failure even if their drugs are stopped for a certain period. Our data suggested that short-term interruption of HAART could be applied to patients with chronic HIV-1 infection in some situations.

Key words: viral load (VL), viral DNA burdens, viral mRNA burdens, highly active anti-retroviral therapies (HAART)

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Introduction

We sometimes need to stop anti-retroviral treatment of patients with HIV-1 infection for a certain period. This can occur when the patient cannot take medicine, or structured treatment interruptions (STI) are considered[1–4]. Many investigators have analyzed the virological or immunological effects after the interruption of anti-retroviral treatment. Some investigators observed only changes in plasma viral loads (VLs) and others observed only changes in HIV-1 specific immunity during the interruption of highly active anti-retroviral treatment (HAART)[3–5]. However, there have been few reports on virological effect as observed through changes in viral DNA or mRNA burdens in HIV-1 infected cells.

We observed a patient who was suddenly obliged to stop the anti-retroviral drugs which had been administered for about 3 years. We regularly measured his CD4 positive cell counts, viral DNA and mRNA burdens in peripheral blood mononuclear cells (PBMCs) as well as his VLs. We observed how these values changed during the clinical course. We then discussed the safety of a short-term interruption of treatment in chronically HIV-1 infected patients from the changes in these parameters.

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Case Report

The patient was a 36-year-old Japanese man with hemophilia A and both HIV-1 and HCV infection. He contracted *pneumocystis carinii* pneumonia (PCP)

complicated with pneumonia by cytomegalovirus (CMV) in February 1995. In April 1997, a regimen, consisting of zidovudin, lamivudine, saquinavir was started. Although zidovudin was substituted for sanilvudin because of anemia in July 1997, he continued to take these drugs with good adherence. His CD4 positive cell count in peripheral blood was 6/μl and VL was more than 1.0×10^6 copies/ml before the medication. His VL decreased below the undetectable level 12 weeks after medication initiation and was sustained for 28 months. His CD4 positive cell count increased to 392/μl. Maintenance therapies for PCP and CMV infection had already ceased.

He entered our hospital with a sudden onset of right upper quadrant abdominal pain on January 11, 2000. He also had jaundice and high fever. Liver function tests were abnormal and inflammatory response was recognized in hematological and blood chemistry tests. Ultrasonography and percutaneous transhepatic cholangiography findings showed many stones in the gall bladder, thickening of the gall bladder wall and dilatation/obstruction of the common bile duct due to a large gallstone. He was diagnosed with choledocholithiasis complicated with acute liver damage. No pathogenic organisms causing opportunistic infection were detected. He received treatment for choledocholithiasis while fasting. His anti-retroviral drugs were interrupted during the fasting. He was also medicated with corticosteroids for the severe liver damage from 2 to 6 weeks after stopping the anti-retroviral drugs. Eight weeks later, the fasting was discontinued and the anti-retroviral drugs were restarted with the same regimen as previously.

We periodically measured his VLs, his CD4 positive cell count, his viral DNA and mRNA burdens in PBMCs from the initiation of drug-interruption (0 week) to 28 weeks later. CD4 positive cell counts were measured by flowcytometer[6]. Genomic DNA in PBMCs was extracted using the standard method and mRNA was extracted using a Quickprep Micro mRNA Purification Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) after counting the PBMCs. The quantitative polymerase chain reaction method with detection by solid phase DNA was utilized for the assay of viral mRNA and DNA burdens in PBMCs as previously described[7]. Amplicore HIV-1 Monitor Kit Ver. 1.5 (Roche Diagnostics, Branchburg, NJ, USA) assay was utilized for the measurement of VL.

Although his VLs were sustained at 3 weeks, they rapidly increased from an undetectable level to 2.2×10^6 copies/ml at 4 weeks (Fig. 1). Through genotypic analysis of the drug-resistant mutations, we found that the virions which appeared at that time were wild-type HIV-1 strains. Both his viral mRNA and DNA burdens also increased when his VL increased. After his medication was restarted, his viral mRNA and DNA burdens decreased again to their previous levels at 4 weeks. His VL also decreased to the undetectable level at 28 weeks. Although the CD4 positive cell count temporally decreased when his VL increased, the degree

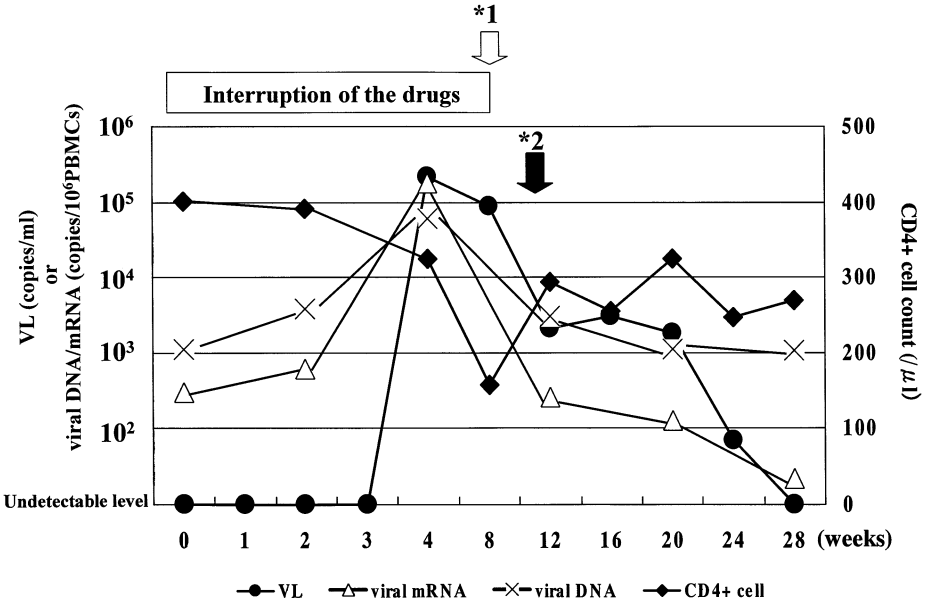

Figure 1 Clinical course of the patient.
*1: Restart of anti-retroviral drugs with the same regimen. *2: Laparoscopic surgery.

of decrease was not so pronounced and it gradually increased after restarting the medication. He had no opportunistic infections during the observation period. We could not perform genotypic analysis of the drug-resistant mutations before the interruption or after VL decrease to the undetectable level due to the small number of HIV-1 virions.

Discussion

This patient has been medicated with undetectable VLs and no symptoms of opportunistic infections for several years. Although viral burdens in PBMCs as well as VL increased when the medication was stopped for a certain period, they decreased again to the previous levels after restarting the medication. Furthermore, no drug-resistant strains appeared during the course of treatment and the initial levels of viral burdens continue to be sustained. Our data indicated no virological failure when medication was stopped for a certain period in well-controlled patients medicated for several years whose VLs remained undetectable by HAART. Some investigators may object to our interpretation because viral burdens in PBMCs do not necessarily indicate the total body viral loads in HIV-1 infection[8,9]. However, others have made quantitative analysis of latent infected cells and total body viral loads in HIV infection by measuring viral burdens in PBMCs[10–14]. In accord with their findings, we considered viral burdens in PBMCs, especially the viral DNA burdens, to be one of the surrogate markers of total body viral loads.

His CD4 positive cell count temporarily decreased during the interruption, which may have been due to the increase of CD4 positive cell destruction. However, he had mild lymphocytopenia in peripheral blood as he was medicated with corticosteroids during the anti-retroviral drug interruption. As the percentage of CD4 positive cells in lymphocytes did not significantly change (from 12.3 to 9.2), the possibility of a low CD4 positive cell count in calculation could not be denied.

We also observed another 2 patients with chronic HIV-1 infection who had 2-week interruptions in their treatment. Their VLs, viral burdens in PBMCs and CD4 positive cell counts did not change during the interruptions (data not shown). As our data covered the findings from only a few patients and during a single interruption, we could not discuss the virological effects of STI in chronic HIV-1 infection. However, the data suggest that it is possible to interrupt treatment safely for a certain period in well-controlled patients with chronic HIV-1 infection.

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