Reconstituted Immunity against Persistent Parvovirus B19 Infection in a Patient with Acquired Immunodeficiency Syndrome after Highly Active Antiretroviral Therapy

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We discovered a patient with AIDS with persistent B19 infection who had slow resolution of anemia after he commenced receiving HAART without intravenous immunoglobulin. The patient’s anemia recurred when the initial course of HAART failed, but it remitted slowly after salvage therapy was instituted. However, circulating B19 was still detectable by nested polymerase chain reaction 1 year after commencement of salvage therapy. Immunoglobulin G and immunoglobulin M antibodies against B19 were not detected by means of enzyme-linked immunosorbent assay when the anemia initially resolved, but they were detected after the patient commenced receiving salvage therapy. The absence of antibody response after the initial remission of parvovirus B19 infection suggested that cellular immunity was an important component of reconstituted immune function against B19 after the patient received HAART. The humoral response that was restored later was abnormal; it had strong reactivity to nonstructural protein NS-1 and poor generation of neutralizing antibodies against linear epitopes unique to minor capsid protein VP1.

Patients with HIV type 1 (HIV-1) infection may have chronic and severe anemia caused by persistent parvovirus B19 infection [1]. Although persistent parvovirus B19 infection could be cured by use of iv immunoglobulin (IVIG) treatment in immunodeficient patients, organ transplant recipients, and patients with leukemia [2–4], HIV-infected patients with very low CD4 counts have required maintenance IVIG therapy [5–7]. The inability of patients with AIDS to develop protective antibodies explained the failure to eradicate parvovirus B19 infection. Recently, treatment with highly active antiretroviral therapy (HAART) has been reported to result in complete remission of persistent parvovirus B19 infection in patients with AIDS [8, 9]. However, the reconstituted immunity against parvovirus B19 was not characterized. We describe the reconstitution of humoral immunity against parvovirus B19 after administration of HAART to a patient with an AIDS-associated persistent infection.

CASE REPORT

The patient, a 32-year-old Chinese man who was born in Myanmar, had sexual contact with commercial sex workers before he immigrated to Taiwan in 1990. He was found to have HIV-1 infection during a routine health checkup in 1996. One month later, in October 1996, he was admitted to our hospital because of a 3-day history of fever, watery diarrhea, headache, and vomiting. Cryptococcal meningitis was diagnosed by
culture of CSF specimens, and the patient was treated successfully with amphotericin B and fluconazole. His CD4 cell count was 16 cells/mm³, and antiretroviral therapy with zidovudine and zalcitabine was started after the patient was discharged. In May 1997, the antiretroviral therapy regimen was switched to zidovudine, lamivudine, and saquinavir (hard gel). Unfortunately, the patient was noted to have an unsteady gait and slurred speech in June 1997. CT of the head done after injection of contrast media showed multiple lesions with decreased density and abnormal enhancement in right basal ganglion and periventricular areas. Cerebral toxoplasmosis was suspected, and the patient was treated with pyrimethamine and clindamycin for 3 weeks.

Although there was a significant reduction in the patient’s HIV-1 load after he began receiving HAART (table 1), cytomegalovirus retinitis was found in August 1997; the patient was treated with iv ganciclovir. Zidovudine was replaced by zalcitabine because the patient experienced severe anemia and leukopenia. Ten weeks after the treatment with iv ganciclovir, ganciclovir was injected intravitreally, because of the concern that iv administration of the drug was causing persistent anemia. He did not receive a blood transfusion after 3 October 1997. However, severe anemia recurred in February 1998, and blood transfusion was required monthly. The patient decided to discontinue receiving antiretroviral therapy in March 1998, and agreed to receive salvage therapy with stavudine, didanosine, and indinavir in June 1998.

The possibility of persistent parvovirus B19 infection was suspected and subsequently proved by means of PCR in June 1998. The patient was not treated with IVIG because of its expense. Chronic anemia slowly resolved after institution of salvage therapy, and blood transfusions were no longer required after November 1998. Follow-up MRI of the patient’s brain, which was performed in July 1998, showed resolution of the previously noted lesions.

**Measurement of HIV-1 load and CD4 counts.** The patient’s CD4 counts were measured by means of flow cytometry (FACSCalibur; Becton-Dickinson). HIV-1 RNA levels were determined by use of reverse transcriptase PCR (Amplicor HIV-1 Monitor, version 1.5; Roche).

**Parvovirus B19 ELISA and immunoblots.** Serial serum samples were obtained from the patient and stored at −70°C. Parvovirus B19 IgM and IgG antibodies were detected by use of 2 commercially available EIAs (Immuno-Biological Labs and Biotrin), respectively. Parvovirus B19 has 1 minor and 1 major viral capsid protein, VP1 (83 kDa) and VP2 (58 kDa), respectively, that are encoded in the same reading frame, with VP1 having an additional 227 amino acids at its amino terminus [10]. For IgM and IgG ELISA, recombinant parvovirus B19 protein VP2 was coated on microtiter wells. The serum samples were also tested with parvovirus B19 immunoblots (recomBiotin Parvovirus B19 IgG/IgM; Mikrogen). The antigens blotted to nitrocellulose membrane were purified recombinant proteins that were separated according to their molecular weight by means of SDS–polyacrylamide gel electrophoresis. They included, in the order of decreasing molecular weight, the whole nonstructural protein (NS-1); the N-terminal portion of VP1, which encompassed the VP1-unique portion as well as the N-terminal half of VP2 (VP-N); the C-terminal half of VP2 (VP-C); and the VP1-unique N-terminal portion (VP-1S). All the

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<th>Table 1. Laboratory findings, according to month and year, in a patient with AIDS who had persistent parvovirus B19 infection.</th>
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<td>Finding</td>
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<td>CD4 cell count, cells/mm³</td>
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**NOTE.** B19, parvovirus B19; ND, not done.

a The patient received treatment with zidovudine, lamivudine, and saquinavir.
b The patient received treatment with stavudine, didanosine, and indinavir since June 1998.
c HIV-1 load was 0.409 × 10⁻⁰ copies/mL, as determined by means of branched DNA analysis (Chiron).
d Cutoff value for IgM, 0.2 U.

e Cutoff value for optical density for IgG, <0.245 for IgG.

f Parvovirus B19 load is expressed as the highest-fold dilution of DNA extracted from 10 μL of plasma that is detectable by use of parvovirus B19 PCR.
g B19 DNA was still detectable by use of nested PCR.
Figure 1. Measurement of plasma parvovirus B19 DNA by use of semiquantitative PCR. Serial 10-fold dilutions of DNA extracted from 10 μL plasma were tested. The expected 551-bp PCR product could be detected up to 10^9-fold dilution as early as August 1997. A 5-log_{10} decrease in parvovirus B19 load was noted on 5 December 1997, when anemia resolved. The parvovirus B19 load returned to its original level in June 1998, when salvage therapy began, and then slowly decreased to the low level when anemia resolved on 30 November 1998. Circulating parvovirus B19 DNA could only be detected by use of nested PCR after 26 January 1999 (data not shown). M, 100-bp DNA ladder.

procedures were performed according to the manufacturer’s instructions.

Measurement of plasma parvovirus B19 DNA by use of semiquantitative PCR. Detection of circulating parvovirus B19 DNA by use of PCR was performed as described elsewhere [11]. For performance of semiquantitative PCR, DNA that had been extracted from 30 μL of plasma was dissolved in 15 μL of distilled water. Serial 10-fold dilution was made by adding 5 μL of the DNA sample to 45 μL of distilled water repeatedly. In each PCR reaction, 5 μL of diluted DNA was used. Nested PCR was performed as described elsewhere [11] if PCR product was not detected in the first round. Plasma samples that tested negative for parvovirus B19 DNA were included each time that PCR was performed.

RESULTS

In August 1997, the patient was found to have ≥10^8 copies of parvovirus B19 DNA per 10 μL of plasma (figure 1), but parvovirus B19 DNA was not detected by use of nested PCR performed on 16 July 1997. Severe anemia was noted on 26 August 1997 (table 1) and was attributed to the toxicity of zidovudine and ganciclovir to bone marrow. Remission of anemia occurred on 5 December 1997 and was initially thought to be the consequence of the discontinuation of iv ganciclovir; however, it was actually associated with a 5-log_{10} decrease in parvovirus B19 load (figure 1). As a result of the failure of HAART in December 1997, anemia recurred in February 1998. The parvovirus B19 load rebounded to its original level when salvage therapy was started in June 1998. The suppression of HIV-1 replication achieved by salvage therapy resulted in gradual control of parvovirus B19 infection (figure 1). Parvovirus B19 DNA was still present in the plasma samples obtained on 26 January and 20 July 1999; however, it was detectable only by use of nested PCR (data not shown).

Use of IgG and IgM ELISA failed to detect antibodies against parvovirus B19 at the onset of infection. When anemia resolved in December 1997, the result of the IgG parvovirus B19 ELISA was still negative, but weak reactivity to VP-C and VP-N was visible on IgG immunoblot strip (figure 2). After salvage therapy, strong IgM and IgG antibodies against parvovirus B19 were detectable in both ELISA and on immunoblots from samples obtained from the patient after 28 September 1998 (table 1; figure 2). However, reactivity against the VP1-specific linear epitopes represented by VP-1S as well as the N-terminal half of VP-N was still weak in IgG immunoblots throughout the follow-up period of 10 months. In contrast, as shown in a patient with erythema infectiosum (figure 2), an immunocompetent host develops strong antibodies against VP1 shortly after the appearance of antibodies against VP2 [3]. In addition, the...
Figure 2. Serial IgM and IgG parvovirus B19 immunoblots. NS-1, nonstructural protein; VP-1S, VP1-unique portion; VP-N, contains VP-1S and the N-terminal half of VP2; VP-C, the C-terminal half of VP2. IgG antibodies directed to VP-C and VP-N were poorly developed when temporary remission of parvovirus B19 infection occurred on 5 December 1997. After salvage therapy began on 19 June 1998, strong IgM and IgG reactivity against NS-1 and VP2 were detected in immunoblots. However, the antibody response to VP1 was still defective in contrast to the fully developed anti-VP1 observed in a patient with erythema infectiosum (E.I.). C, positive result for control serum sample.

unusually strong reactivity to nonstructural protein NS-1 was found [12].

DISCUSSION

Humoral immunity is important for the control of parvovirus B19 infection [3]. Patients who were receiving immunosuppressive agents had durable remission of persistent parvovirus B19 infection and developed anti–parvovirus B19 antibodies after IVIG therapy [4, 13], whereas patients with AIDS generally had no antibody response to parvovirus B19 before and after IVIG treatment [1, 5, 7]. Antibodies with reactivity to VP1 linear epitopes and VP2 conformational epitopes had been shown to have neutralizing capability [14, 15]. Furthermore, experiments that used monoclonal antibodies generated against parvovirus B19 proteins showed that the monoclonal antibodies that interacted with linear VP1 epitopes were more potent than were the monoclonal antibodies that recognized conformational VP2 epitope in the neutralization of parvovirus B19 infection [15]. Therefore, the development of VP1-specific antibodies after the initial immune response to VP2 appears to be important for the elimination of parvovirus B19 viremia.

We discovered that the reconstituted humoral response to parvovirus B19 proteins was incomplete in our patient after he received salvage therapy; the antibody reactivity against linear epitopes of VP1 was poorly developed, which is similar to what has been reported with regard to patients with leukemia who were receiving chemotherapy [3]. The strong immune response to NS-1 in the patient is compatible with the observation that antibodies against nonstructural protein were found in patients with persistent parvovirus B19 infection [12]. However, neutralizing antibodies against VP2 conformational epitopes that may help to clear the virus from circulation were not studied. Overall, the humoral immune response against parvovirus B19 developed in the patient after salvage therapy was inefficient in the neutralization of parvovirus B19 infection, a finding that we base on the fact that clearance of parvovirus B19 viremia from 28 September 1998 through 26 January 1999 was slow despite the presence of quantitatively adequate parvovirus B19 antibodies. The slow clearance likely was not a defect in phagocytic cells because of the rapid rate of clearance of circulating parvovirus B19 that has been reported in literature [1] and noted in one of our patients with AIDS who had persistent
parvovirus B19 infection 2 weeks after commencement of IVIG therapy (M. Y. Chen, unpublished data).

By tracing the onset of parvovirus B19 infection, we found that the patient was infected and had anemia 10 months before a correct diagnosis was made. During that time, the patient was admitted to the hospital because of cytomegalovirus retinitis; the HIV-1 virus load was $1.18 \times 10^7$ copies/mL after the patient had been treated with the initial course of HAART for 3 months. The parvovirus B19 concentration decreased and anemia resolved 15 weeks after the onset of parvovirus B19 infection. It has been reported that the concentration of virus has stabilized at $\sim 10^3–10^7$ copies/mL of serum after the commencement of IVIG therapy in patients with AIDS who have persistent parvovirus B19 infection [1]. The level of circulating viral DNA during remission of anemia in our patient was consistent with that reported elsewhere. It is surprising that the transient control of parvovirus B19 infection was not accompanied by the antibody responses that were noted later during successful salvage therapy. The immune function should have been improved after suppression of viral replication [16] when the patient contracted parvovirus B19 infection. The absence of humoral immune response suggested that cellular immune response might play an important role in limiting parvovirus B19 infection.

In conclusion, HAART alone was capable of resolving anemia in a patient with AIDS who was given a delayed diagnosis of persistent parvovirus B19 infection. The IgM and IgG immune response to VP2 linear epitopes was restored after the patient began receiving HAART, but reactivity to VP1 linear epitopes remained hampered. Study of parvovirus B19 load and antibody response throughout the clinical course of persistent parvovirus B19 infection in the patient showed evidence that suggested the important role of cellular immunity in the reconstituted immune function against parvovirus B19 infection. The use of IVIG together with HAART to treat patients with AIDS who have persistent parvovirus B19 infection may impact antibody response and requires further study.

References


