Human Herpesvirus 6B Infection of the Large Intestine of Patients with Diarrhea

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Four patients had severe diarrhea after undergoing stem cell transplantation. Human herpesvirus 6B (HHV-6B) DNA was detected in large intestine tissue specimens and in peripheral blood mononuclear cells. In situ hybridization was positive for HHV-6B DNA in the nuclei of goblet cells and, sometimes, in the histiocytes in the submucous region of the large intestine, which suggests that HHV-6B may infect and reactivate in these cells.

Human herpesvirus 6 (HHV–6) belongs to the β-herpesvirus subfamily, which also includes human cytomegalovirus (HCMV) and human herpesvirus 7. HHV-6 was first isolated from the PBMCs of patients with AIDS and other lymphoproliferative disorders [1], and it is now classified into 1 of 2 variants (HHV-6A and HHV-6B) [2]. The primary infection caused by HHV-6B is exanthema subitum (ES), a common febrile illness associated with rash in infancy [3]. The clinical symptoms of ES usually are mild, but some patients exhibit such complications as diarrhea, a bulging fontanelle, encephalitis, bronchopneumonia, convulsions, and liver dysfunction [4].

Because patients with ES have diarrhea with high frequency [4], and because gastroenteritis is often found in immunocompromised patients for whom HHV-6B DNA is detectable in PBMCs [5], we thought that HHV-6B could be responsible for the gastric symptoms. We have investigated the presence and localization of HHV-6B DNA in the large intestine of patients who experienced severe diarrhea after stem cell transplantation (SCT).

Four patients for whom the detectable HHV-6B DNA load was found to be >10^3 copies per 1 μg of DNA isolated from the large intestine were assessed by PCR analysis [6, 7], antibody testing [8], and in situ hybridization (ISH). Cultures for the detection of bacteria and fungi, as well as PCR for the detection of HCMV, revealed none of these pathogens in the diarrhea of the 4 patients.

Patient 1. A 4-year-old boy who had acute lymphocytic leukemia diagnosed underwent SCT; positively selected CD34+ cells were used. Three months after transplantation, the patient exhibited neither neurological symptoms nor leukopenia (WBC count, 3970 cells/μL [82% neutrophils]), but he had a high fever and a skin rash that was not likely to be associated with graft-versus-host disease (GVHD). Moreover, he had severe, bloody diarrhea that did not lessen after administration of immunosuppressive therapy. An intestinal biopsy was performed to obtain specimens for pathological examination. A high number of HHV-6B DNA copies was detected by PCR in the large intestine (>1000 copies per 1 μg of DNA), PBMCs (>1000 copies per 1 × 10^3 cells), and plasma (10–100 copies per 10 μL), and HHV-6B was isolated from the PBMCs and plasma with high frequency. Histological examination revealed slight erosion and edema, which suggested chronic proctitis. There was no evidence of GVHD. ISH showed that much of the HHV-6B DNA had localized to the nuclei of the goblet cells and to the histiocytes of the large intestine (figure 1a). His diarrhea was so severe that he was provided ganciclovir and foscarnet alternatively; a large dose of gamma globulin was also administered. His severe diarrhea lessened after long-term treatment. The patient died of septic shock 9 months after transplantation.

Patient 2. A 1-year-old boy who had acute megakaryocytic leukemia diagnosed underwent SCT; positively selected CD34+ cells were used. One month after transplantation, he had a high fever and severe, bloody diarrhea, but he did not have any other symptoms caused by HHV-6B or GVHD, such as skin rash, neurologic complications, and neutropenia (WBC count, 1810 cells/μL [78% neutrophils]). HHV-6B DNA was detected by PCR in the large intestine (>1000 copies per 1 μg of DNA), PBMCs (100–1000 copies per 1 × 10^3 cells), and plasma (10–100 copies per 10 μL). Histological examination revealed interstitial edema, hemorrhage, and diffuse mucosal inflammation without ulceration, which suggested chronic inflammation. HHV-6B DNA localized to the nuclei of the goblet cells and to the histiocytes of the large intestine (figure 1b).
Figure 1. In situ hybridization for human herpesvirus 6B (HHV-6B) in the tissues of the large intestine. Positive signals are shown in violet. (a) Patient 1. Strongly positive signals (arrows) were observed within the nuclei of goblet cells and within histiocytes. HHV-6B was also detected in plasma. (b) Patient 2. Positive signals (arrows) were observed within the nuclei of goblet cells and within histiocytes. HHV-6B was also detected in plasma. (c) Patient 3. Positive signals (arrows) were observed only within the nuclei of goblet cells. No HHV-6B was detected in plasma. (d) Patient 3. After the patient was treated with immunosuppressants and ganciclovir, no positive signals were observed. (e) Patient 4. Strongly positive signals (arrows) were observed only within the nuclei of the goblet cells. No HHV-6B was detected in plasma.

The diarrhea lessened, and the amount of HHV-6B DNA in plasma could not be detected after treatment with ganciclovir had been received.

**Patient 3.** A 7-year-old boy who had acute lymphocytic leukemia diagnosed underwent bone marrow transplantation. Three months after transplantation, the patient exhibited neither neurological manifestations nor leukopenia (WBC count, 5000 cells/μL [83% neutrophils]), but he had a high fever and a skin rash that was likely due to GVHD. Moreover, he experienced severe, bloody diarrhea. A biopsy of the large intestine was performed. HHV-6B DNA was detected by PCR in the large intestine (100–1000 copies per 1 μg of DNA) and PBMCs (100–1000 copies per 1 × 10⁶ cells) but not in plasma. Histological examination revealed crypt degeneration, edema, and monocytic infiltration, which suggested acute GVHD. ISH revealed that HHV-6B DNA localized only to the nuclei of the goblet cells of the large intestine (figure 1c). After the patient received treatment with immunosuppressants and ganciclovir, his diarrhea lessened. However, 2 months after completion of treatment, his high fever, rash, and diarrhea became worse, although he did not experience neurologic symptoms or neutropenia (WBC count, 2700 cells/μL [95% neutrophils]). A second biopsy of the large intestine was performed. Findings of histological examination implied that the patient had GVHD. HHV-6B DNA was no longer detected in the intestinal specimen by PCR and ISH (figure 1d). After treatment with immunosuppressants only, the patient’s diarrhea lessened.

**Patient 4.** A 9-year-old girl with acute myelocytic leukemia underwent SCT with the use of cord blood. One month after transplantation, she experienced a high fever, rashlike GVHD, and bloody diarrhea without neurological symptoms or leukopenia (WBC count, 4000 cells/μL [71.7% neutrophils]). HHV-6B DNA was detected by PCR in the large intestine (100–1000 copies per 1 μg of DNA) and the PBMCs (10–100 copies per 1 × 10⁶ cells) but not in plasma. Histological examination revealed abnormalities that included mucosal degeneration and infiltration of lymphocytes, which suggested acute GVHD. The results of ISH showed that HHV-6B DNA
localized only to the nuclei of the goblet cells of the large intestine (figure 1c). After treatment with immunosuppressants and ganciclovir, the patient’s diarrhea resolved.

The titer of antibodies to HHV-6B did not increase significantly between the acute phase and the convalescent phase of diarrhea, except in patient 2 (data not shown). This finding suggests that the ability to produce antibodies did not improve as a result of immunosuppression after transplantation.

Discussion. Diarrhea is frequently observed in association with HHV-6 in cases of ES [4] and in patients with immunosuppression [5], but the pathological features of the infected intestinal tissue have been unknown [9]. In this study, we report the detection of HHV-6B DNA in specimens of the large intestine obtained from patients with diarrhea. Infection with HCMV and bacteria, which are common pathogenic agents of diarrhea in immunocompromised patients, was excluded.

Two patients (patients 1 and 2) had detectable HHV-6B DNA in plasma, and the findings of histological examination of the large intestine indicated inflammation, not GVHD. In contrast, 2 other patients (patients 3 and 4) did not have detectable HHV-6B DNA in plasma, and histological findings for the large intestine indicated GVHD. Detection of DNA in plasma indicates cell-free viremia, in which HHV-6B reactivates throughout the body. These data suggest that there are ≥2 types of diarrhea associated with HHV-6B. First, HHV-6B infection is suspected to be the causative agent of diarrhea, as in patients 1 and 2, who had reactivation of HHV-6B throughout the body. Second, HHV-6B infection is suspected to be the cause of GVHD, as in patients 3 and 4, and HHV-6B reactivation in the large intestine may be superimposed on the GVHD or stimulated by the severe diarrhea. However, additional work with many more tissue samples obtained from the large intestines of patients who had without HHV-6B in their plasma will be required before this idea can be confirmed. These results emphasize the need to have all immunocompromised patients with diarrhea undergo examinations for HHV-6B to determine whether antiviral agents will be an effective treatment for diarrhea.

GVHD and the immunosuppressive therapy used in preparation for transplantation result in severe intestinal and systemic immunodeficiency, predisposing patients to opportunistic infections, including the reactivation of human herpesviruses. Treatment of GVHD with immunosuppressants increases the risk of infection. Thus, it is easier to treat infections that are not accompanied by GVHD. HHV-6B DNA was detected in the large intestine, PBMCs, and plasma of patients 1 and 2, and antiviral therapy was an effective cure for the diarrhea. However, when 2 potential causes of diarrhea (infection and GVHD) were present, as in patients 3 and 4, diarrhea was more difficult to treat. Because HHV-6B was detected in the large intestine of both of these patients, the patients were treated with antiviral drugs in addition to immunosuppressive therapy, and their diarrhea lessened.

We demonstrated the presence of HHV-6B by ISH, but the proportion of infected cells in patient 1 was much more than that seen in the other patients. In patients 3 and 4, the proportion was much less than that in patient 1. It was the reason why HHV-6B might reactivate only in the large intestine. We need additional studies to explain the difference between patients 1 and 2, but we suspect that there were differences in the immunity of the patients. In patient 2, the titer of antibodies to HHV-6B significantly increased between the acute phase and the convalescent phase of diarrhea, which suggests that the ability to produce antibodies improved in patient 2 but not in patient 1.

In summary, although the number of patients reviewed was small, it is likely that HHV-6B infects and reactivates in goblet cells of the large intestine. Further studies that involve many more tissue samples obtained from the large intestine are needed to confirm whether HHV-6B causes diarrhea.

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References

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