Human Herpesvirus-6 Infection in Bone Marrow Transplantation

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Twenty-five pediatric patients who received bone marrow transplantation (BMT) were studied prospectively to determine the relationship between BMT and human herpesvirus-6 (HHV-6) infection by the virus isolation from peripheral blood and/or bone marrow and by determining neutralizing antibodies to HHV-6 during the 2 months following BMT. All of the 25 donors and the recipients were immune to HHV-6 at the time of BMT and the virus was not isolated from them. HHV-6 was isolated from peripheral blood and/or bone marrow mononuclear cells in ten (40%) of the 25 recipients between day 14 and day 22 of BMT, but not from any other day. Two additional recipients showed a significant increase in the antibody titer. Thus, infection with HHV-6 was confirmed in 12 (48%) of the 25 recipients. Four of the 12 developed skin rashes; three of these four had a febrile episode when the virus was isolated, whereas none of the remaining 13 developed the skin rash. These results suggest a frequent infection with HHV-6 only a few weeks after BMT and a close association between the infection with the virus and the development of skin rashes.

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Materials and Methods

Patients. Twenty-five patients underwent BMT between July 1988 and July 1990 at the Division of Hematology-Oncology, Children's Medical Center, The Japanese Red Cross Nagoya First Hospital, and the Department of Internal Medicine, Fujita Health University School of Medicine. The group was composed of nine patients with acute lymphoblastic leukemia (ALL), seven with acute nonlymphoblastic leukemia (ANLL), one with chronic myelogenous leukemia (CML), three with non-Hodgkin's lymphoma (NHL), one with Hodgkin's disease (HD), three with aplastic anemia (AA), and one with adenoleukodystrophy (ALD). They were aged 1 to 16 years with a mean age of 8. Seventeen patients were boys and 8 were girls. Details of conditioning regimen and graft-versus-host disease (GVHD) prophylaxis are shown in Table 1. In brief, patients with hematologic malignancies were conditioned with LPAM 180 mg/m² plus BU 16 mg/kg or LPAM 180 mg/m² plus 12 Gy total body irradiation. Patients with AA were conditioned with CY 200 mg/kg plus 7.5 Gy total lymphoid irradiation. A patient with ALD was conditioned with BU 16 mg/kg plus CY 200 mg/kg. Twenty-three patients received bone marrow from human lymphocyte antigen (HLA) identical siblings. One patient received bone marrow from an HLA phenotypically identical parent. Another patient received autologous bone marrow. Patients received 2.0 to 6.2 × 10⁹ (median 3.2 × 10⁹) marrow cells per kilogram body weight. GVHD prophylaxis consisted of MTX alone or plus cyclosporine. Patients received intravenous (IV) γ-globulin preparations weekly during the first 3 months as prophylaxis of cytomegalovirus infection.

Experimental design. Samples were obtained after the project was thoroughly explained to the guardians of the patients. Heparinized peripheral blood and/or bone marrow were collected from the donor and the recipient at the time of BMT and weekly from the recipient after BMT. Clinical data were collected by the authors.

Isolation and identification of HHV-6. The procedures for isolation and identification of HHV-6 were described elsewhere. Briefly, peripheral blood MNCs and plasma were separated by Ficoll-Hypaque gradient centrifugation using heparinized blood samples. Cells and plasma from patients were cocultured (1 × 10⁹/mL) with MNCs obtained from cord blood in RPMI 1640 culture medium supplemented with 20% heat-inactivated fetal bovine serum, 0.1 U/mL recombinant human interleukin-2, and 5 μg/mL phytohemagglutinin (PHA)-P. The ratio of cells from the patient to those from cord blood was approximately 1:1. The cultures were maintained at 37°C in a CO₂ incubator and observed daily under a microscope; the medium was changed twice weekly. Viral isolates were identified primarily by morphologic changes of cultured cells, which had the characteristics of pleomorphic, balloon-like large cells. The cells were then mounted on a slide, air dried, and fixed with cold acetone and methanol (1:1) mixture. Well-characterized antibody to HHV-6 from a patient with exanthem subitum, which had no antibody activity to five other human herpesviruses, was used for the virus identification by an indirect immunofluorescence (IF) assay. The cells were incubated with 1:10 diluted antibody to HHV-6 for 1 hour at 37°C. After washing three times with phosphate-buffered saline (PBS), fluorescein-conjugated goat antibody to human IgG, one drop diluted to 1:40, was added to the fixed cells. The slide was incubated for 1 hour at 37°C, washed with PBS, and examined with a fluorescence microscope. When the cells...
Virus isolation was confirmed when thin section electron microscopic examination of the cells revealed that viral particles morphologically similar to herpes group viruses were present.

Neutralization test. Details of the method for neutralization test (NT) were described elsewhere. Briefly, serial twofold serum dilutions prepared on disposable plastic trays containing 96 wells were mixed with an equal volume (100 μL) of HHV-6 preparation strain) containing 10⁵ tissue culture infective doses (TCID₅₀ per 0.1 mL of the virus. The cell-free virus was prepared from supernatant tissue culture fluid after centrifugation of the virus-infected cord blood MNC cultures. RPMI 1640 medium supplemented with 30% heat-inactivated fetal bovine serum, 0.1 U/mL of recombinant human interleukin-2, 5 μg/mL of phytohemagglutinin-p, and suitable antibiotics were used for the culture of MNCs and the virus and diluent of sera. After 1 hour of incubation at 37°C, 2 x 10⁶ cord blood MNCs were added to each well and incubation was continued for 7 days. The antibody titer was determined as the reciprocal of highest dilution serum that completely prevented large cell dysfunction and no diarrhea in the 12 recipients with the evidence of HHV-6 infection. They were diagnosed clinically as having acute GVHD. However, histologic findings of their skin biopsy specimen obtained during the episode of rashes supported the diagnosis of acute GVHD in one recipient (number 4, Table 2). The patient developed chronic GVHD thereafter. There was no skin rash in the

RESULTS

Results from neutralizing antibody titers to HHV-6 and isolating the virus from peripheral blood and/or bone marrow samples in 25 donor and recipient pairs are summarized in Table 2. All of the 25 donors were immune to HHV-6 with antibody titers ranging from 4 to 128 and no virus was isolated from their samples. All 25 recipients had NT antibodies ranging from 8 to 128 at the time of BMT and no virus was isolated from their blood samples at that time. Eleven strains of HHV-6 were isolated from peripheral blood or bone marrow MNCs (but not from plasma) in ten (40%) (numbers 1 to 10, Table 2) of 25 recipients; five on day 14 of BMT, four on day 15, one on day 21, and one on day 22. No virus could be isolated before or after that time in the ten recipients. Two additional recipients (numbers 11 and 12, Table 2), showed a 16-fold increase in antibody titer during the 2-month observation period after BMT, although the virus was not isolated from their blood samples. Thus, HHV-6 infection was confirmed in 12 (48%) of 25 recipients during the 2-month period following BMT. In the remaining 13 recipients (numbers 13 to 25, Table 2), there was no significant increase in antibody titer nor the viremia during the same observation period.

Among 12 recipients with the evidence of HHV-6 infection, four (numbers 1 to 4, Table 2) developed skin rashes between day 6 and day 40 of BMT. Three (numbers 1, 2, and 4, Table 2) of the four had a febrile episode between day 6 and day 21 of BMT. There was no hepatocellular dysfunction and no diarrhea in the 12 recipients with HHV-6 infection. They were diagnosed clinically as having acute GVHD. However, histologic findings of their skin biopsy specimen obtained during the episode of rashes supported the diagnosis of acute GVHD in one recipient (number 4, Table 2) of the four. The patient developed chronic GVHD thereafter. There was no skin rash in the

exhibited strong nuclear and cytoplasmic staining, virus isolation was considered positive. The cells shown to have positive reactivity by indirect IF staining were then studied by electron microscopy. Virus isolation was confirmed when thin section electron microscopic examination of the cells revealed that viral particles morphologically similar to herpes group viruses were present.†
remaining 13 recipients who had no evidence of HHV-6 infection.

**DISCUSSION**

In the present study we found that during the 2 months following BMT 48% of marrow graft recipients developed HHV-6 infection. This was confirmed by morphologic changes of the cultured cells, specific IF staining with the antibody to HHV-6, and virion structure by electron microscopy. Recently, Frenkel et al.\(^\text{10}\) reported the isolation of a new human herpes virus, human herpesvirus-7 (HHV-7), which shares some genomic homology with HHV-6. Since HHV-6 and HHV-7 exhibit similar cytopathic effects and virion structure, HHV-7 may be included in the 11 virus strains of the present study. However, at present, there is not enough information about genomic and antigenic properties of the virus.

It is likely that the HHV-6 infection that occurred after BMT is due to activation of the virus in the body of the recipients, since all of the donors and recipients were immune to HHV-6 and no virus was isolated from them at the time of BMT. However, we don’t know whether 11 strains of HHV-6 isolated in the present study were derived from the donor or the recipient or from other sources. HHV-6 viremia is often found in the acute stage of exanthem subitum,\(^\text{7}\) but it is hard to isolate the virus from blood after recovery or from healthy individuals with antibody.\(^\text{7}\) The virus probably remains latent in the body after primary infection as do other human herpesviruses. If the virus was derived from the donor, it must have been latently infecting the donor’s marrow cells or blood cells and was activated to replicate after transfer to a recipient. Although a large proportion of healthy adults with the antibody has been reported to have HHV-6 gene sequences in their peripheral blood MNCs,\(^\text{11,12}\) it is not known whether marrow cells contain HHV-6 DNA. Alternatively, the virus may have been transferred by contaminated MNCs from other blood donors, since all of the patients received platelet and/or red blood cell transfusion several times around the time of transplantation. It is generally believed that the primary infection with HHV-6 acquired early in life confers permanent immunity. However, although there was no outbreak of exanthem subitum in the ward during the observation period, reinfec tion with HHV-6 would be considered in these profoundly immunosuppressed patients since most adults with the antibody to HHV-6 excrete the virus into saliva.\(^\text{13,14}\) If the virus was derived from the recipient’s own body, it must have been harbored somewhere in the recipient’s body. It has been suggested that several tissues, such as lymph nodes,\(^\text{15}\) liver,\(^\text{16}\) kidney,\(^\text{17}\) and salivary glands\(^\text{18}\) contain HHV-6. The virus may have been reactivated from these tissues by factors such as a profound immune dysfunction or an allogeneic reaction after BMT. In order to confirm the origin of the virus isolated from recipients after BMT, gene sequences of the virus would be required to compare with those of HHV-6 isolated from same individuals before BMT, or from the donors, or from other sources. Recently, we isolated two HHV-6 strains from the blood of a child with ALL before and after BMT. Genomic analyses of both strains indicated reactivation of the virus harboring in his own body (unpublished data, April 1990).

It is of interest that the viremia was detected between day 14 and day 22 of BMT, which is almost the same time as that of reactivation of herpes simplex virus infection but earlier than those of cytomegalovirus and varicella-zoster virus infections.\(^\text{19-21}\) However, it is difficult to explain the reason why human herpesvirus infection is temporally related to various stages of the posttransplantation period.

In the present study, HHV-6 infection was confirmed by the virus isolation from blood and/or bone marrow or significant increase in antibody titer, or both. Among 10 recipients with viremia, only five showed fourfold increases in NT antibody titer, suggesting decreased antibody production during the 2 months following BMT. During the period of profound immune dysfunction, it is important to isolate the virus, as proved in the present study, or to detect the virus antigen\(^\text{22}\) or the virus DNA sequences\(^\text{17,26}\) in order to confirm the virus infection.

Another interesting finding in the present study is a correlation between the HHV-6 infection and the development of skin rash resembling acute GVHD. Although one patient had histologic evidence for acute GVHD, the skin rash appeared in four (33%) of 12 recipients with HHV-6
infection and the fever developed in three of the four simultaneously. None of the 13 recipients without the virus infection developed the skin rash, suggesting a close association between the virus infection and the skin manifestation. This finding raises the question of whether the skin rash was due to the virus infection. Recently Okuno et al. suggested that the active infection of HHV-6 in renal transplants might be due to immunosuppressive treatments for kidney rejection. However, they did not see a development of skin rashes in their patients. Temporal relationship between appearance of the rash and the viremia observed in the present study suggests the causal relationship, but does not draw a conclusion for the relationship because the development of the skin rash preceded the occurrence of viremia in one patient, was almost simultaneous in two patients, and the viremia preceded the development of the skin rash in another patient. In primary HHV-6 infection in infancy, exanthem subitum, HHV-6 viremia is found frequently in a febrile stage of the disease and followed by maculopapular skin rash. Active infection with the virus may have produced skin rash in the three recipients. However, detection of HHV-6 antigen or the virus gene sequences in the skin samples would be required to confirm this point.

REFERENCES

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T Yoshikawa, S Suga, Y Asano, T Nakashima, T Yazaki, R Sobue, M Hirano, M Fukuda, S Kojima, and T Matsuyama