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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was considered positive. The cells shown to have positive reactivity were mixed with an equal volume (100 µL) of HHV-6 preparation. RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum, 0.1 U/mL of recombinant human interleukin-2, 5 FgimL of phytohemagglutinin-p, and suitable antibiotics were used for the culture of MNCs and the virus infective dose_50_ (NT) were described elsewhere. Briefly, serial twofold serum dilutions prepared on disposable plastic trays containing 96 wells and diluent of sera. After 1 hour of incubation at 37°C, the antibody titer was determined as the reciprocal of highest dilution that completely prevented large cell dysfunction.

RESULTS

Results from neutralizing antibody titers to HHV-6 and isolating the virus from peripheral blood and 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
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\(^{19}\) 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 virus isolation from blood and/or bone marrow or skin rash appearance in four (33%) of 12 recipients with HHV-6 infection, although one patient had histologic evidence for acute GVHD. Although 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 a large proportion of healthy adults with the antibody has been reported to have HHV-6 gene sequences in their peripheral blood MNCs,\(^{17,19}\) 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, reactivation 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.\(^{19,22}\) 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,\(^{17}\) liver,\(^{21}\) kidney,\(^{22}\) and salivary glands\(^{24}\) 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.\(^{27}\) 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\(^{17}\) or the virus DNA sequences\(^{17,26}\) in order to confirm the virus infection.
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 transplant recipients 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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