Fatal Reactivation of Postnatal Cytomegalovirus Infection with Rapid Emergence of Ganciclovir Resistance in an Infant after Allogeneic Stem Cell Transplantation

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Human cytomegalovirus (HCMV) can cause serious problems after hematopoietic stem cell transplantation. The death of a pediatric transplant recipient after reactivation of a postnatal HCMV infection with bilateral retinitis and pneumonitis is described. Sequencing of the HCMV UL97 region revealed a compartment-specific mutation (H520Q) in urine conferring ganciclovir resistance.

CASE REPORT

A 3-month-old infant had been diagnosed with juvenile myelomonocytic leukemia (JMML), a rare myeloproliferative disorder of childhood, and was admitted 2 months later for hematopoietic stem cell transplantation (HSCT). An HLA-compatible (HLA-A, -B, -C, -DRB1, and -DQB1-matched, one mismatch in HLA-DPB1), unrelated, human cytomegalovirus (HCMV)-seronegative donor was identified, and the infant was scheduled for an allogeneic HSCT at the age of 6 months. According to the European Working Group on Myelodysplastic Syndrome protocol, the conditioning regimen included busulfan, cyclophosphamide, and melphalan. Anti-thymocyte globulin was administered as prophylaxis for graft-versus-host disease (GVHD) prior to transplantation. Post-transplantation, cyclosporine was used for GVHD prophylaxis, and only mild acute GVHD confined to the skin (maximum, 25%) had occurred. Hematopoietic reconstitution was initially protracted. Analysis of blood DNA short terminal repeats revealed 100% donor chimerism. At the time of pretransplantation evaluation, the HCMV-specific immunoglobulin G (IgG) assay was weakly positive whereas HCMV-specific IgM and HCMV DNA assays were negative. The infant had been breast-fed, but breast milk had not been tested for HCMV DNA by PCR. A retrospective PCR analysis from blood of the Guthrie card obtained during the first days of life of the newborn demonstrated HCMV DNA negativity. Therefore, HCMV-specific IgG antibodies were considered to be of maternal origin, for the mother was HCMV seropositive.

Due to severe thrombocytopenia, the infant received a leukocyte-depleted platelet transfusion from an HCMV-seropos-

itive donor at the age of 5 months. One week later, HCMV IgM was detected with increasing sample-to-cutoff ratios from 2.8 to 6.4 over the next 3 weeks. However, during this time, HCMV DNA PCR from leukocytes and urine was negative, and HSCT was performed. Acyclovir prophylaxis was routinely started 1 day after transplantation.

On day 10 posttransplantation systemic active HCMV infection was first detected by PCR from blood. Seven days later anti-HCMV therapy was started with intravenous ganciclovir (GCV) at a dose of 2×5 mg/kg of body weight per day (Fig. 1). Due to fluctuating HCMV loads and pp65-positive cells in the antigenemia assay as well as increasing myelosuppression, therapy was briefly switched to foscarnet (PFA), followed by alternate medication of GCV and PFA. However, response to therapy with GCV remained poor, which was reflected in continuing fluctuating levels of HCMV antigenemia and the development of HCMV retinitis. On day +62, the infant developed fever with increasing viral loads of HCMV and Epstein-Barr virus. A combined therapy of GCV and PFA reduced Epstein-Barr virus levels under the detection limit; however, HCMV levels remained unaltered. Immunophenotyping was performed on days 93, 104, 135, 156, and 181 post-HSCT and confirmed a lack of reconstitution of immunity with severe CD4 lymphopenia (maximum, 102 CD4⁺ cells/ μ l [data not shown]). A fulminant HCMV retinitis including massive bleeding and necrosis was diagnosed on day +113. Despite cidofovir (CDV) administration, amaurosis was diagnosed on day +132. UL97 genotyping from blood and urine samples obtained on day +113 revealed a UL97 mutation at codon 520 (H520Q) known to confer GCV resistance. However, this UL97 mutant virus strain was detectable only in urine. In a retrospective analysis, we found this GCV-resistant strain in several other urine samples but not in the very early and late phase after transplantation (Table 1). Although antiviral therapy was subsequently changed, HCMV retinitis progressed to amaurosis. The infant finally died of HCMV pneumonitis on day 198 after HSCT, after having developed disseminated HCMV disease.

Posttransplantation, HCMV monitoring was performed on a weekly basis and included blood and urine. Additionally,

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FIG. 1. Longitudinal analysis of HCMV DNA PCR, pp65 antigenemia, and antiviral therapy.

spinal fluid or nasal washes were screened for HCMV. DNA extraction and qualitative HCMV PCR using an in-house assay (GeneAmp PCR System 2400; Applied Biosystems, Weiterstadt, Germany) were performed as described earlier (15). Quantitative HCMV PCR was performed using the Cobas Amplicor CMV Monitor (Roche Diagnostics, Alameda, Calif.). The

detection of pp65 antigenemia was performed with an immunofluorescence test (CINA kit; Argene Biosoft, Fürth, Germany) using 2×10^5 blood cells. According to the manufacturer's instructions, a positive result of the pp65 antigen assay is defined by two or more positive cells per 2×10^5 leukocytes. HCMV IgM was determined by CMV-IgM-enzyme-labeled-antigen test PKS Medac (Medac, Hamburg, Germany), and HCMV IgG was determined by enzyme-linked immunosorbent assay (ETI Cytok G; DiaSorin Diagnostics, Dietzenbach, Germany). Direct amplification and UL97 genotyping were performed as described previously (12). Briefly, DNA was extracted directly from patient specimens, and sequences between nucleotides 1234 and 1961 of the UL97 coding region were amplified by PCR on the LightCycler (Roche Diagnostics, Mannheim, Germany) using the SybrGreen amplification mix. For sequencing the ABImed cycle sequencing kit (Applied Biosystems, Darmstadt, Germany) was used according to the manufacturer's instructions. UL97 fragments were sequenced in duplicate and from both directions using the primers UL97-1274, 5'-AGC TGG CGT GCA TCG ACA G-3', and UL97-1935, 5'-GCG ACA CGA GGA CAT CTT GG-3'. Sequences were determined and aligned with published se-

TABLE 1. Results of UL97 genotyping^a

Day post-HSCT	Specimen	HCMV UL97 genotyping direct from specimens
38	Plasma	WT
38	Urine	WT
80	Urine	Mut 520, 30–40%
96	Nasal wash	WT
109	Urine	Mut 520, 40–50%
113	Blood	WT
113	Urine	Mut 520, 30–40%
114	Spinal fluid	WT
150	Urine	No result
150	Plasma	WT (Mut <10%?)
172	Urine	WT

^a On day 113 after HSCT, HCMV with 100% mutation at position 520 in UL97 could be isolated from urine. WT, wild type; Mut, mutation.

quence data to detect previously described drug resistance mutations (7).

HCMV infections and reactivations are a serious threat to patients undergoing HSCT despite available preemptive antiviral therapy (6). Current drugs for antiviral treatment include GCV, PFA, and CDV. GCV, at present the drug of first choice, is monophosphorylated by the virally encoded UL97 protein (13), whereas CDV and PFA do not require activation by the UL97 protein. Several mutations in the coding region of UL97 have been described, inducing resistance of the respective cytomegalovirus strains to GCV (7).

This report describes a rare case of HCMV retinitis in an infant receiving a hematopoietic stem cell transplant for JMML. JMML was diagnosed based on the fulfillment of criteria such as monocytosis, hepatosplenomegaly, lymphadenopathy, no *bcr/abl* rearrangement, granulocyte-macrophage colony-stimulating factor hypersensitivity of bone marrow myeloid colonies, and myeloid precursors on a bone marrow smear. Although viral infections including HCMV may mimic JMML, which might lead to false conclusions and treatment (14), our patient had no evidence of active HCMV infection at the time of evaluation pretransplantation. A low-level anti-HCMV IgG but no anti-HCMV IgM response was detectable. HCMV IgG positivity of the mother suggested that IgG was of maternal origin. Therefore, a seronegative donor had been chosen for transplantation, thereby eliminating the possibility of transferring HCMV-specific T cells. Furthermore, a perinatal infection was largely excluded by a negative HCMV PCR result of the Guthrie card. However, the definite onset of primary HCMV infection remains unclear.

Our retrospective analyses suggest a postnatal infection acquired probably by breast-feeding shortly before transplantation. The primary infection was obviously asymptomatic. We assume that the conditioning regimen prior to transplantation provoked an HCMV reactivation associated with rapid emergence of a GCV-resistant virus at an early stage post-HSCT (day 80) after only 63 days of cumulative GCV therapy (Table 1). Whereas UL97 genotyping from blood and other body fluids such as cerebrospinal fluid did not detect a mutant virus population, genotyping from urine showed, besides wild-type virus, 30 to 50% GCV-resistant virus containing a mutation at codon 520 (H520Q). The mutation H520Q is located in a functionally critical domain of UL97 and was responsible for the lowest level of residual GCV phosphorylation (5.2% compared to wild type) (1). On day 150, analysis of HCMV sequence from plasma revealed a mixture of approximately 90% wild-type virus and 10% H520Q mutant virus. This compartment-specific, GCV-resistant UL97 mutant strain was only transiently detectable in urine samples. The virus isolated from urine has also been phenotypically tested in an in vitro drug sensitivity assay performed as focus reduction (12). The 50% inhibitory doses were 72.8 μ M for GCV, 271 μ M for PFA, and 5.1 μ M for CDV, respectively. Concerning our laboratory definitions the virus was considered phenotypically resistant to GCV but sensitive to PFA and CDV. Therefore, sequencing of the viral polymerase (UL54) was not considered. However, the results of biological assays should be interpreted with caution,

particularly since mixed virus populations (like here in urine) are involved and discordant results can be observed in genotypic and phenotypic assays. Resistant virus was absent from a urine sample obtained on day +172, shortly before the infant's death.

In our patient, retinitis and subsequent amaurosis occurred early posttransplantation; however, this complication has been reported by other groups to develop at later stages in the transplant setting. Crippa et al. reported the occurrence of retinitis and/or blindness due to HCMV as a late complication post-HSCT with a median time of diagnosis at day 251 (range, 106 to 356 days) (4). In contrast to our patient, retinitis responded well to antiviral treatment with GCV, PFA, or CDV, without occurrence of relapse in that study. Also, patients with low lymphocyte counts, previous HCMV reactivation, and chronic GVHD were identified to be at high risk for developing HCMV retinitis. A higher frequency of HCMV retinitis has been suggested by the increased use of unrelated donor transplants and therefore a delayed immune reconstitution (11).

It has been pointed out that viral clearance is inefficient without an adequate immune response and that lymphopenia, especially in children, is associated with an increased risk of developing GCV resistance early in the transplant period (5, 6). The constantly low lymphocyte counts of our patient were primarily induced by the conditioning regimen prior to HSCT and maintained by the necessary immunosuppression. Although we reduced and eventually stopped immunosuppression because of ongoing HCMV activity, CD4 T-cell counts did not rise to higher levels and remained below 200/ μ l. This lymphopenia was paralleled by recurrent episodes of myelosuppression that most likely occurred because of antiviral therapy after good initial engraftment.

The high level of viral replication (due to primary infection), a prolonged antiviral therapy, and administration of immunosuppressive drugs such as cyclosporine and corticosteroids as well as the conditioning regimen required for HSCT (and therefore an incompetent immune response with low CD4 and CD8 T-cell counts) are all factors that favor the emergence of resistant viruses during a course of therapy (2).

GCV-resistant HCMV strains are isolated relatively frequently in the blood or urine of immunodeficient virus-infected patients after antiviral therapy (5, 8). Notably, HCMV resistance has been linked with mutations in the HCMV UL97 protein in up to 90% of isolates examined (3). In a well-designed prospective study, Jabs et al. (10) followed patients to assess shedding of resistant HCMV in blood or urine, and they found that 11% of patients shed virus by 6 months of GCV therapy and that these patients have a ninefold-greater risk of developing contralateral retinitis. These data suggest that approximately 50% of cases in which contralateral retinitis developed by 6 months are associated with genotypic GCV-resistant HCMV in the blood or urine. However, Imai et al. (9) reported that only approximately 20% of cases, for which the median duration of GCV exposure was 5.5 months, were the result of drug-resistant virus. Whether the simultaneous detection of two different HCMV genotypes in separate body compartments is of clinical relevance remains to be established.

This case demonstrates the importance of rapidly evaluating the possibility of emerging GCV resistance in children in the early posttransplant phase. Even with preemptive therapy

(based on PCR monitoring for HCMV), which has reduced lethal HCMV-related complications in the past, there is clearly need for improved treatment options such as adoptive immunotherapy with CD4 and CD8 HCMV-specific T cells. This methodology has made impressive advances and will be instrumental in reconstitution of antiviral immunity and protection against viruses and tumors.

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