Drug-resistant human cytomegalovirus infection in children after allogeneic stem cell transplantation may have different clinical outcomes

Tobias Eckle, Lothar Prix, Gerhard Jahn, Thomas Klingebiel, Rupert Handgretinger, Barbara Selle, and Klaus Hamprecht

Three seropositive pediatric recipients of allogeneic stem cell transplantation out of a group of 42 patients receiving T-cell–depleted, unrelated transplants and 37 patients receiving T-cell–depleted, haploidentical transplants were monitored longitudinally for human cytomegalovirus (HCMV) infection and the emergence of antiviral drug resistance. Early in the posttransplant course, all 3 patients developed HCMV mutations conferring drug resistance to ganciclovir. One child additionally developed multidrug resistance to foscarnet and cidofovir, with mutations in the viral phosphotransferase gene (UL97) and the DNA-polymerase gene (UL54) being found. These data show that resistant HCMV infection does not necessarily correlate with a severe clinical outcome. The early detection of genotypic resistance up to 129 days before the emergence of phenotypic resistance and the dissociation of resistance patterns among different body sites emphasize the importance of genotypic analyses of different DNA specimens for an efficient antiviral therapy. T-cell–depleted children having transplantation might be at an increased risk for the development of drug resistance. (Blood. 2000;96:3286-3289)

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Introduction

Despite the availability of effective antiviral drugs, human cytomegalovirus (HCMV) is still a serious infectious complication after allogeneic bone marrow transplantation (BMT).1,2 However, the limited number of reports concerning drug-resistant HCMV infection might suggest that this problem occurs very infrequently in this clinical setting.2

In contrast, ganciclovir (GCV) resistance during antiviral treatment of HCMV retinitis in adults with acquired immunodeficiency syndrome (AIDS) has been shown to appear frequently after more than 3 months of therapy,3,4 and resistance to foscarnet (PFA)5 and cidofovir (CDV)6 has been described after even longer durations of therapy.

In the BMT setting, no reports exist about CDV resistance and only very few about GCV and PFA resistance.2 Nevertheless, there are indications that children with combined immune deficiencies after T-cell–depleted BMT are at a high risk of developing early GCV resistance.7

Of 79 children who underwent allogeneic peripheral blood stem cell transplantation (PBSC) at our institution, 3 subjects are presented who had a striking course of HCMV infection and development of resistance to various antiviral drugs. Because culture-based resistance screening is not a practical method for routine use,8,9 therapy was monitored by molecular analysis of sequential specimens from various body sites for typical mutations conferring GCV resistance.

Study design

Transplantation procedure

The 3 patients belong to a sequence of pediatric patients of whom 42 (having transplantation between October 1995 and March 2000) received positively selected, highly purified CD34+ peripheral blood stem cells from unrelated donors and 37 (having transplantation between August 1995 and March 2000) received these cells from familiarly mismatched donors. The ClinMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) CD34+ selection device was used for positive selection.10 The mode of transplantation is detailed in Table 1. Because of the low T-cell number, no prophylaxis for graft-versus-host disease was necessary (Table 1). After transplantation, antiviral therapy was administered with acyclovir until day +100, Pneumocystis carinii prophylaxis with trimethoprim-sulfamethoxazole until day +200, and antimycotic and antibiotic prophylaxis with fluconazole or itraconazole and colistin until day +100.

Monitoring of HCMV infection and development of resistance

All patients, except seronegative recipients of seronegative transplants, were screened weekly for HCMV infection by HCMV polymerase chain reaction (PCR) from plasma and leukocytes until day +100.11 Antiviral therapy with GCV was initiated (initiation, 5 mg/kg twice a day; maintenance, 5 mg/kg per day) when 2 consecutive PCR results from leukocytes were positive. GCV-resistant infection was alternatively treated with PFA (3 × 10 / 3 × 50 mg/kg per day) or CDV (5 mg/kg per week).

Especially in the 3 patients presented here, in the case of a high viral load during antiviral therapy and because of clinical suspicion, genotypic8 and phenotypic12 resistance screening was performed from all specimens available. Semi quantitative limiting-dilution nested PCR from native plasma was performed, as described,13 whenever HCMV plasma DNAemia was detected.

Sequence analyses of codons 439 to 696 of the UL97 gene and codons 365 to 1084 of the UL54 gene (viral polymerase) were performed by using BigDye-Terminator chemistry on an ABI 310 machine (PE Applied Biosystems, Weiterstadt, Germany).

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situation deteriorated and he died at day 167.

Remarkably, an additional resistance mutation for GCV was found in the blood in Table 1. Thereafter, the patient’s pulmonary function was monitored closely. Despite the administration of antiviral therapy, the patient’s lung function continued to decline, and he died on day 167.

In the 3 pediatric patients, mutations conferring GCV resistance emerged early, after 25, 53, and 30 days of GCV therapy after PBSCT. This is a testament to the difficulty of establishing effective antiviral therapy in the presence of multidrug resistance in an AIDS patient.

Case 2: fatal clinical outcome in a patient with multidrug resistance and HCMV encephalitis. This patient had transplantation with a disseminated HCMV infection during the conditioning procedure. Upon observation of GCV resistance, an emerging multidrug resistance was detected, and a new antiviral therapy was initiated. However, the HCMV-associated encephalitis worsened, and the patient died on day +287.

Table 1. Clinical characteristics of recipients of highly purified peripheral blood stem cells

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Underlying disease</th>
<th>Donor HLA-A, -B, -DR match</th>
<th>CD34+ cell infusion × 106 cells/kg</th>
<th>CD34+ cells × 107 cells/kg</th>
<th>Donor leukocyte infusion after PBSCT</th>
<th>Disease after PBSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>Innate T-cell defect, autoimmune hemolytic anemia†</td>
<td>Mother; 3/6 antigens matched</td>
<td>50</td>
<td>18.7</td>
<td>No infusion</td>
<td>Chronic restrictive lung disease</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>AML M4 CR2; del(11)(q23); initial hyperleukocytosis/CNS infiltration <strong>AAML—BFM93</strong> regimen</td>
<td>Unrelated; 6/6 antigens matched</td>
<td>20.5</td>
<td>14.1†</td>
<td>Day +112 after PBSCT</td>
<td>Gut and skin</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>AML M2 CR1; t(8;21); initial infiltration of CNS, orbitae, pleura, lymph nodes <strong>AAML—BFM93</strong> regimen</td>
<td>Mother; 5/6 antigens matched</td>
<td>13.4</td>
<td>13.6</td>
<td>Days +72 and +100 after PBSCT</td>
<td>Autoimmune hemolytic anemia</td>
</tr>
</tbody>
</table>

PBSCT indicates peripheral blood stem cell transplantation; IV, intravenous; IG, immunoglobulin; ALG, antilymphocyte globulin; AML, acute myeloblastic leukemia; CR2, second complete remission; CNS, central nervous system; ATG, antithymocyte globulin; GvHD, graft-versus-host disease; CMV, cytomegalovirus; CRI, first complete remission.

Results and discussion

In the 3 pediatric patients, mutations conferring GCV resistance emerged early, after 25, 53, and 30 days of GCV therapy after PBSCT. Because patient 1 received GCV before PBSCT, he had had GCV therapy for 93 days when genotypic resistance was first detected.

The sudden emergence of mutations in blood specimens was observed within 12, 29, and 9 days after the last negative genotypic test result. After genotypic resistance in the 3 children had been detected, viral isolates were obtained 10, 124, and 169 days after PBSCT.

Case 1: fatal clinical outcome in a patient with chronic restrictive lung disease. The patient, as described elsewhere, was treated with GCV before PBSCT because of pneumonitis following recurrent HCMV infections. After transplantation, the development of resistance coincided with a 10-fold increase in the viral DNA titer in plasma; an increase is often suggested as a marker for resistance development. An immediate switch in therapy from GCV to PFA after the detection of genotypic resistance resulted in a prompt decrease in the viral load. However, HCMV could not be eliminated from the blood. Remarkably, an additional resistance mutation for GCV was found uniquely in a urine isolate (Table 2). Thereafter, the patient’s pulmonary situation deteriorated and he died at day +170.

Case 3: patient with an asymptomatic HCMV infection and GCV resistance. Initial HCMV leukoDNAemia manifest on day +65 was treated with GCV. When genotypic resistance was detected, the patient was negative for HCMV in the plasma by PCR. Therapy was switched to CDV for 5 weeks until day +150. When HCMV plasmaDNAemia disappeared, therapy was stopped. Since day +169, the mutation conferring resistance was no longer detectable in sporadically HCMV PCR-positive leukocyte specimens. The first viral isolate from this
Our data strengthen the consideration that children having T-cell–depleted BMT or PBSCT may have an increased risk of resistance development. In contrast to the patients described by Wolf et al, our patients did not suffer from severe immunologic disorders. Additionally, our patients received highly purified stem cells, whereas their patients underwent T-cell–depleted BMT. Therefore, neither the mode of transplantation nor the general immune situation may solely explain the early emergence of drug resistance.

Rigorous T-cell depletion can increase the risk for opportunistic infections. As a consequence, the immune recovery, especially the recovery of specific immune cells, may play an important role in defending against these infections. In the case of an HCMV infection, the lack of a specific immune response could allow high viral replication rates during antiviral therapy. Thus, studies in pediatric recipients of T-cell–depleted transplants are needed to determine the relation between antiviral drug resistance in the context of HCMV-specific cellular immune reconstitution and immune cell function.

### Table 2. Longitudinal HCMV monitoring and the relation between antiviral therapy and development of mutations in UL97 and UL54

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Specimens (days after PBSCT)</th>
<th>PCR</th>
<th>Viral load*</th>
<th>Therapy (cumulative days)</th>
<th>IC50 (μmol/L)†</th>
<th>UL97 mutations</th>
<th>UL54 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serum – 67</td>
<td>GCV 0, PFA 0</td>
<td>GCV 29, PFA 0</td>
<td>Negative</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Serum – 18</td>
<td>–</td>
<td>GCV 68, PFA 0</td>
<td>–</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Serum + 26</td>
<td>+</td>
<td>GCV 75, PFA 30</td>
<td>+</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte + 32</td>
<td>+</td>
<td>GCV 81, PFA 30</td>
<td>1.4</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Urine + 149</td>
<td>+</td>
<td>GCV 103, PFA 125</td>
<td>9.8</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Plasma + 44</td>
<td>+</td>
<td>GCV 93, PFA 30</td>
<td>–</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Urine + 54</td>
<td>+</td>
<td>GCV 103, PFA 30</td>
<td>13</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td>2</td>
<td>Serum – 14</td>
<td>+</td>
<td>GCV 0, PFA 0</td>
<td>–</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte – 1</td>
<td>+</td>
<td>GCV 0, PFA 0</td>
<td>+</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte + 6</td>
<td>+</td>
<td>GCV 0, PFA 0</td>
<td>+</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td>3</td>
<td>Plasma – 3</td>
<td>+</td>
<td>GCV 0, PFA 0</td>
<td>+</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Serum – 30</td>
<td>+</td>
<td>GCV 0, PFA 0</td>
<td>+</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte – 65</td>
<td>+</td>
<td>GCV 0, PFA 0</td>
<td>+</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte + 129</td>
<td>+</td>
<td>GCV 48, PFA 14</td>
<td>8</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte + 169</td>
<td>+</td>
<td>GCV 48, PFA 35</td>
<td>8</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>CSF + 263</td>
<td>+</td>
<td>GCV 179, PFA 67</td>
<td>13</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte – 106</td>
<td>+</td>
<td>GCV 41</td>
<td>–</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>Leukocyte + 224</td>
<td>+</td>
<td>GCV 48, PFA 35</td>
<td>8</td>
<td>GCV, PFA, CDV</td>
<td>D515E; L516M; I521T†</td>
<td>Wild type</td>
</tr>
</tbody>
</table>

HCMV indicates human cytomegalovirus; PBSCT, peripheral blood stem cell transplantation; PCR, polymerase chain reaction; IC50, 50% inhibitory concentration; GCV, ganciclovir; PFA, foscarnet; CDV, cidofovir; CSF, cerebrospinal fluid.

*Limiting-dilution nested PCR from native plasma/serum; + indicates undiluted (corresponding to 103 genome equivalents/mL), ++ indicates dilution of 10; +++ indicates dilution of 100.
†Phenotypic resistance testing of viral isolates: cutoff values for resistance: GCV, IC50 > 6 μmol/L; CDV, IC50 > 4 μmol/L; PFA, IC50 > 400 μmol/L.
‡Mutated codon is located adjacent to known codons involved in drug resistance.

Review of the literature

Until now, only one case of both genotypic and phenotypic antiviral drug resistance in the adult BMT setting has been documented. In contrast, Wolf et al showed the early development of resistance in 4 of 6 children after T-cell–depleted BMT. Our data strengthen the consideration that children having T-cell–depleted BMT or PBSCT may have an increased risk of resistance development. In contrast to the patients described by Wolf et al, our patients did not suffer from severe immunologic disorders. Additionally, our patients received highly purified stem cells, whereas their patients underwent T-cell–depleted BMT. Therefore, neither the mode of transplantation nor the general immune situation may solely explain the early emergence of drug resistance.
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