Development of drug-resistant herpes simplex virus infection after haploidentical hematopoietic progenitor cell transplantation

Amelia A. Langston, Istvan Redei, Angela M. Caliendo, Jyoti Somani, Don Hutcherson, Sagar Lonial, Silvana Bucur, Judy Cherry, Andrew Allen, and Edmund K. Waller

An unusually high incidence of acyclovir- and foscarnet-resistant herpes simplex virus (HSV) infection was noted after lymphocyte-depleted blood hematopoietic progenitor cell (HPC) transplantation from HLA-haploidentical family donors. Fourteen adults with hematologic malignancies underwent blood HPC transplantation from haploidentical family donors. Pheresis products were stringently depleted of T and B cells by immunomagnetic adsorption, and patients received no immunosuppression after transplantation. HSV reactivation occurred in all 7 evaluable HSV-1– or HSV-2–seropositive patients, at a median of 40 days after transplantation. Susceptibility testing of clinically resistant viral isolates demonstrated acyclovir resistance in all 5 cases tested. Second-line therapy produced only partial responses, and in vitro evidence of foscarnet resistance developed rapidly in all 3 patients treated with foscarnet. Healing of lesions coincided with T-cell recovery. The prolonged immunodeficiency associated with stringent lymphocyte depletion of the graft appears to strongly predispose to emergence of drug-resistant HSV. Furthermore, immune reconstitution is necessary for eradication of infection. (Blood. 2002;99:1085-1088)

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Study design

Patient population and transplantation

Between September 1999 and October 2000, 14 adults with hematologic malignancies underwent blood HPC transplantation from HLA-haploidentical family donors at Emory University Hospital, according to an institutional review board–approved investigational protocol. Pretransplantation conditioning therapy began on day −10 and consisted of fractionated total body irradiation, thiopeta, fludarabine, and antithymocyte globulin (ATG; 13 patients), or busulfan, cyclophosphamide, fludarabine, and ATG (1 patient). Donors underwent leukopheresis for 2 to 5 days after subcutaneous administration of granulocyte colony-stimulating factor (G-CSF; 10 μg/kg per day). Donor pheresis products were stringently depleted of T and B cells by a combination of CD34+ selection and T- and B-cell–negative selection using the Isolex 300i system (version 2.5; Baxter HCC, Roundlake, IL), and immediately administered to the recipient through a central venous catheter. No posttransplantation immunosuppression was given for graft-versus-host disease prophylaxis. Patients with positive pretransplantation serologies for HSV-1, HSV-2, or varicella zoster virus (VZV) received prophylactic acyclovir (5 mg/kg intravenously every 8 hours) from day −1 until establishment of neutrophil engraftment. Patients also received prophylactic ciprofloxacin, penicillin, and liposomal amphotericin B during the neutropenic period. Preemptive ganciclovir was given for cytomegalovirus (CMV) reactivation,2 as monitored by weekly plasma polymerase chain reaction for CMV DNA (Roche Diagnostics, Indianapolis, IN). Quantitation of blood T-cell, B-cell, and natural killer (NK) cell subsets was performed by standard flow cytometry at set intervals after transplantation.

HSV reactivation, cultures, and susceptibility testing

Reactivation of HSV was defined as development of one or more clinical lesions, with isolation of HSV by standard culture methods. Isolates were not typed. Initial treatment of HSV lesions consisted of famciclovir or intravenous ACV, except in 3 patients who were treated with foscarnet because initial lesions developed while receiving ACV or ganciclovir. In cases of clinical failure on ACV or ganciclovir, a new culture was obtained and the isolate was sent for drug susceptibility testing by modified plaque reduction assay (Children’s Hospital of Pennsylvania Laboratories, Philadelphia). Patient follow-up is reported as of August 1, 2001.

Results and discussion

Characteristics of the 14 patients treated according to protocol are shown in Table 1; data are also presented for patients with and...
without HSV reactivation. Two patients (both HSV seropositive) died on posttransplantation days 6 and 8, leaving 12 patients fully evaluable for evidence of HSV reactivation. All of the 12 patients who survived beyond posttransplantation day 8 achieved granulocyte engraftment (absolute neutrophil count [ANC] > 500/µL for at least 2 consecutive days), at a median of 11 days after transplantation (range, 9-27 days). Median values for subsets of CD4+ T cells, CD8+ T cells, and CD56+ NK cells at intervals after transplantation are shown in Table 1.

Table 2 provides clinical and virologic details of HSV disease among study patients. Evidence of HSV reactivation was seen in all 7 evaluable HSV-1 or HSV-2 seropositive patients, versus none of the 5 patients seronegative for HSV. As shown in Table 1, there were no other significant differences between patients with and without HSV infection. HSV reactivation occurred at a median of 11 days after transplantation (range, 12-136 days), at which time the median blood CD4 cell count was 3.5/µL (range, 0.44-µL). Sites of reactivation included oral mucosa (3 patients), esophagus (2 patients), and anogenital area (3 patients); one patient had lesions at 2 sites (anogenital and esophagus). In 5 patients, lesions developed while the patient was receiving either intravenous ACV for primary HSV prophylaxis (2 patients) or ganciclovir for treatment of CMV reactivation (3 patients). No patient was receiving immunosuppressive medications at the time of HSV reactivation.

Susceptibility testing was conducted on isolates from 5 patients, either because of development of lesions while on ACV or ganciclovir, or failure to respond to initial therapy; all 5 isolates were ACV resistant by in vitro testing. ACV-resistant lesions failed to respond completely to alternative therapy, including higher doses of ACV, foscarnet, or cidovir. In vitro resistance to foscarnet developed in all 3 patients who were treated with foscarnet. Eventual healing of lesions correlated with immune recovery; one patient has developed recurrent ACV-resistant HSV lesions in association with a drop in the CD4 cell count (Table 2). The 2 patients with anogenital HSV posed local management challenges because ulcers were large (> 6 cm diameter), but no one developed life-threatening HSV disease or secondary complications despite the long duration of active lesions in surviving patients. Interestingly, in this group of patients all episodes of CMV and VZV reactivation responded promptly to ganciclovir or ACV therapy, respectively (data not shown).

Acyclovir-resistant HSV infections occur almost exclusively in immunocompromised patients and are most commonly seen in individuals infected with human immunodeficiency virus.14,15 Several lines of evidence highlight the importance of cell-mediated immunity (mediated by T cells and NK cells) in providing protection from HSV reactivation as well as healing of established lesions.16-19 In the patient population described here, significant NK cell recovery occurred early after transplantation, but did not prevent viral reactivation or facilitate clearance. Healing of HSV lesions correlated with recovery of CD4+ and CD8+ T cells, which was delayed. These data are consistent with in vitro and animal studies implicating CD4+ and CD8+ effector T cells as essential for clearance of HSV and healing of lesions.16,18

Our experience illustrates the importance of drug-resistant HSV in alternative donor transplantation, particularly when stringent lymphocyte depletion is used. The HSV prophylaxis regimen used was not adequate for prevention of HSV reactivation in this clinical setting, although using the same antiviral prophylaxis, we have seen ACV-resistant HSV infection in only 2 (3.4%) of 59 patients receiving transplants from matched unrelated donors at our institution since January 1997 (versus 5 [36%] of 14 patients in the current series, P = .002). Administration of higher doses of prophylactic ACV for longer periods before and after transplantation may reduce the incidence of HSV reactivation and development of drug resistance in these patients; however, it may not completely eliminate the problem given that most reactivation episodes in this series occurred during antiviral therapy. Combination or sequential antiviral prophylaxis may be more effective than single-agent therapy, although this remains to be tested.

On the basis of these data, we have modified the HSV prophylaxis for this protocol: HSV-seropositive patients receive ACV 10 mg/kg intravenously every 8 hours from day −4 through engraftment, followed by valacyclovir 1000 mg orally twice a day until at least 6 months after transplantation and achievement of a CD4 count more than 200/µL. To date, 6 seropositive patients have received the more aggressive prophylaxis; 2 patients have had HSV reactivation, with only one case of ACV-resistant HSV infection.

Acknowledgments

We wish to thank staffs of Emory University Hospital Ward 7E, the Emory Clinic Ambulatory Infusion Clinic, and the Emory Bone Marrow and Stem Cell Transplant Center for clinical care of patients. We also thank Vickie Bartlett for transplant coordination, Grier Banks and the staff of the Emory University Hospital Clinical Microbiology Lab for expert technical assistance, and Sylvia Ennis for assistance in preparation of the manuscript. Isolex cell selection devices were generously provided by Nexell Therapeutics (Irvine, CA) and Baxter HCC.
### Table 2. Characteristics of HSV reaction episodes

<table>
<thead>
<tr>
<th>Age/ gender</th>
<th>Diagnosis</th>
<th>Pretransplantation HSV serology</th>
<th>Posttransplantation day HSV documented</th>
<th>CD4 count at HSV reactivation (no./mm²)</th>
<th>Clinical course of HSV</th>
<th>HSV sensitivity data</th>
<th>CD4 count at HSV resolution (no./mm²)</th>
<th>Survival (d) or cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>40/M</td>
<td>ALL-CR2</td>
<td>+/−</td>
<td>+12</td>
<td>4</td>
<td>Oral ulcers developed on IV ACV (prophylaxis), and stabilized on high-dose famciclovir. Lesions resolved −6 mo after transplantation, in association with immune reconstitution.</td>
<td>Day − 97: ACV resistant</td>
<td>Day − 138: ACV resistant, foscarnet sensitive</td>
<td>156 Alive d − 692</td>
</tr>
<tr>
<td>32/M</td>
<td>NHL (refractory)</td>
<td>+/+</td>
<td>+57</td>
<td>8</td>
<td>Buttocks ulcer developed while on no antivirals. Lesion stabilized on IV ACV and then famciclovir. Lesions resolved −6 mo after transplantation.</td>
<td>Day + 131: ACV resistant, foscarnet sensitive</td>
<td>24 306, relapse</td>
<td></td>
</tr>
<tr>
<td>30/M</td>
<td>CML-CP2</td>
<td>+/+</td>
<td>+61</td>
<td>0</td>
<td>Buttocks and esophageal ulcers developed on ganciclovir and stabilized on foscarnet. Lesions resolved −6 mo after transplantation in association with immune reconstitution.</td>
<td>Day + 118: ACV resistant, foscarnet resistant (after 6 wk foscarnet therapy)</td>
<td>242 Alive day + 538</td>
<td></td>
</tr>
<tr>
<td>39/F</td>
<td>AML-CR1</td>
<td>+/+</td>
<td>+22</td>
<td>0</td>
<td>Buttocks and genital ulcers developed on IV ACV and progressed on foscarnet. Partial response to cidofovir. Lesions resolved −9 mo after transplantation, in association with immune reconstitution. Lesions recurred on day +378, in association with drop in CD4 to 22/mm³. HSV still active on high-dose foscarnet.</td>
<td>Day + 47: ACV resistant, foscarnet sensitive</td>
<td>Day + 113: ACV resistant, foscarnet resistant (after 10 wk foscarnet therapy)</td>
<td>208 Alive day + 448</td>
</tr>
<tr>
<td>21/M</td>
<td>ALL (refractory)</td>
<td>+/−</td>
<td>+48</td>
<td>44</td>
<td>Esophageal ulcer developed on ganciclovir, and stabilized on foscarnet. Patient died with active ulcer (still culture +).</td>
<td>Day + 69: ACV resistant, foscarnet resistant (after 3 wk foscarnet therapy)</td>
<td>Not tested NA 73, aspergillosis</td>
<td></td>
</tr>
<tr>
<td>47/M</td>
<td>AML (refractory)</td>
<td>+/+</td>
<td>+40</td>
<td>Not tested</td>
<td>Oral ulcers developed on ganciclovir. Patient died with active lesions 2 d after diagnosis of HSV.</td>
<td>Not tested NA 42, aspergillosis, multorgan failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46/M</td>
<td>AML-CR1</td>
<td>+/+</td>
<td>+136</td>
<td>3</td>
<td>Oral ulcers developed while on no antivirals. Patient treated with high-dose foscarnet. Died with active lesions 8 d after diagnosis of HSV.</td>
<td>Not tested NA 144, relapse</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALL indicates acute lymphocytic leukemia; CR2, complete response; CP2, chronic phase 2; AML, acute myelogenous leukemia; IV, intravenous; for other abbreviations, see Table 1.

### References

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