Chronic Vulvar Ulceration in an Immunocompetent Woman Due to Acyclovir-Resistant, Thymidine Kinase–Deficient Herpes Simplex Virus

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A 34-year-old healthy woman presented with a 15-month history of persistent, nonhealing vulvar ulcerations due to herpes simplex virus (HSV) type 2. Extensive dermatologic workup and serial skin biopsies failed to reveal an underlying vulvar dermatosis or autoimmune bullous disorder. Virologic studies revealed resistance to acyclovir in vitro due to deficiency in thymidine kinase activity. Serum antibody to human immunodeficiency virus was negative on two occasions, separated by 1 year. Immunologic evaluation showed normal HSV-specific proliferative and CD8 cytotoxic T lymphocyte responses as well as normal NK cell function. Vulvar lesions failed to heal in association with trials of topical trifluridine and oral valacyclovir but resolved completely with the application of 1% foscarnet cream. No recurrence of HSV has been observed in 24 months of follow-up to date.

Clinically significant acyclovir-resistant herpes simplex virus (HSV) infections were relatively rare until the onset of the AIDS epidemic in the mid-1980s [1, 2]. Acyclovir-resistant HSV isolates have been identified in immunocompetent patients, although drug resistance has not generally correlated with clinical disease [3]. In fact, animal models have shown attenuated virulence of certain acyclovir-resistant HSV isolates, with reduced mucocutaneous pathogenicity and decreased recurrence of latent disease [4, 5].

Acyclovir is a selective and specific inhibitor of HSV replication because its antiviral activity depends on two virally encoded enzymes, thymidine kinase (TK) and DNA polymerase. Once acyclovir has diffused into the cell, the viral TK converts it to acyclovir monophosphate, and cellular enzymes add two more phosphates to generate the active antiviral metabolite, acyclovir triphosphate. Acyclovir triphosphate has two mechanisms of action: It can bind to and inactivate the viral DNA polymerase directly, or it can be incorporated into and terminate new viral DNA synthesis [6, 7]. Resistance to acyclovir may result from mutations in the viral genes encoding TK or DNA polymerase [8–12]. Changes in the TK gene have led to viruses that are either deficient, altered, or low-activity phenotypes. Altered DNA polymerase mutants have been identified in clinical isolates of HSV recovered from immunocompromised patients but are rare [13–15].

Clinically significant acyclovir-resistant HSV disease has previously been limited to severely immunocompromised patients, especially AIDS patients [16]. In 1993, however, Kost et al. [17] described an immunocompetent, human immunodeficiency virus (HIV)–negative man with a history of unsafe intercourse with HIV-positive men and frequently recurring genital herpes that was resistant to treatment with high-dose oral acyclovir. Virologic characterization of this patient’s isolate revealed acyclovir-resistant HSV-2 with a TK-altered phenotype.

We report the first case of clinically significant acyclovir-resistant disease caused by a TK-deficient HSV isolate from an otherwise healthy HIV-negative woman. Serial HSV isolates were recovered from persistent nonhealing vulvar erosions. Trials of high-dose oral acyclovir, topical trifluridine, and oral valacyclovir were unsuccessful in controlling disease. However, application of topical foscarnet (1% cream) produced dramatic clinical response, with complete reepithelialization of HSV erosions, repeatedly negative viral cultures, and remission of disease.

Case Report

In July 1993, a healthy, 34-year-old white woman presented to the Stanford Dermatology Clinic with continuous, painful, and...
pruritic vulvar erosions and an adherent, mucoid vulvovaginal discharge for the preceding 15 months. Prior viral cultures from the vulvar area through June 1993 were reportedly positive for HSV-2, and a skin biopsy in April 1992 showed intense inflammation and multinucleated cells indicative of HSV infection.

Physical examination revealed well-demarcated, tender, erythematous erosions on the labia minora and clitoral hood. A gray-white pseudomembranous exudate was present over several erosions, and scant clear vaginal discharge was noted. The oropharynx showed no erosions, aphthous ulcers, or evidence of lichen planus. Skin examination was otherwise unremarkable.

The patient recalled the first symptomatic outbreak of genital herpes in July 1990, following an episode of unprotected sexual intercourse. Initial outbreaks occurred every 4–5 weeks thereafter and healed in response to oral acyclovir (e.g., 200 mg five times daily). The patient reported no lesions when not receiving treatment from August 1991 to December 1991. However, in early 1992, she noted abrupt worsening of her genital herpes, with prolonged healing of vulvar erosions and new lesions occurring on a weekly basis. Over the next 12–15 months, the patient was treated with intermittent doses of acyclovir ranging from 200 mg five times daily to 600 mg five times daily, with continued slow healing of vulvar lesions and frequent recurrences both while receiving and while not receiving acyclovir therapy. Although individual lesions eventually healed over several weeks, the patient developed recurrent vulvar erosions at different sites on a continual basis beginning in April 1992. A diagram of the patient’s clinical course of therapy is shown in figure 1.

Serum antibodies to HIV were absent on two occasions, in 1992 and 1993. Further immunologic workup was done in July 1993 to rule out occult immunodeficiency. A quantitative immunoglobulin assay revealed normal IgG, IgA, and IgM levels. The percentage of CD4 and CD8 lymphocyte subsets (CD4:CD8 ratio, and CD20) were normal, as were absolute lymphocyte counts. A fluorescent anti-nuclear antibody titer was positive (≥1:640) in a speckled pattern. Further tests for collagen vascular disease showed negative antibodies to double-stranded DNA, extractable nuclear antigens, and to SS-A (Ro) and SS-B (La), effectively excluding systemic lupus erythematosus, mixed connective tissue disease, Sjogren’s syndrome, or subacute cutaneous lupus erythematosus.

An HSV-2 vulvar isolate (15923) collected in June 1993, before any antiinflammatory agents were introduced or antiviral medications restarted, was found to be resistant to acyclovir in vitro (ID₅₀ 17.5 μg/mL) (table 1). Repeat vulvar biopsy in July 1993 showed ulceration with a lichenoid lymphocytic infiltrate and overlying lichen simplex chronicus. No specific viral changes were noted in the biopsy specimen, and direct immunofluorescence testing was negative for autoimmune bullous diseases, such as pemphigus vulgaris, cicatricial or bullous pemphigoid, or bullous lupus erythematosus. The biopsy specimen suggested a possible diagnosis of lichen planus, although the lack of oral mucosal findings did not support a diagnosis of erosive vulvar lichen planus.

Beginning in July 1993, the patient received oral acyclovir (800 mg five times daily), with topical application of mupirocin ointment to vulvar erosions thrice daily. The patient noted moderate improvement in vulvar pain and discharge while receiving the above regimen, and vulvar pruritus decreased with oral antihistamines.

Because of the possibility of underlying lichen planus, low-potency topical steroids (desonide 0.05%) were added to the regimen in August 1993, with significant reduction in vulvar pain and burning. Over the ensuing 3 months, mid- to high-potency topical steroids (mometasone furoate, 0.1%, and clobetasol propionate, 0.05%, ointments) were prescribed for possible erosive vulvar lichen planus. A trial of oral prednisone was started in early December 1993, and within 2 weeks, the patient’s vulvar erosions were nearly reepithelialized while receiving systemic steroids and high-dose oral acyclovir (800 mg five times daily). However, in January 1994, her symptoms worsened despite continuation of this regimen and after a gradual prednisone taper. Viral cultures done every 2 weeks were positive for HSV-2, vulvar erosions showed poor healing, and the thick, pseudomembranous vulvovaginal discharge reappeared. In vitro resistance to acyclovir was again documented in 2 vulvar HSV-2 isolates from January 1994 (14849 and 14848), obtained before cessation of low-dose systemic steroids and oral acyclovir (table 1). Repeat vulvar biopsy showed a similar lichenoid infiltrate and ulceration without specific herpesvirus findings.

Because of lack of effect, oral acyclovir was discontinued in early February 1994, and the prednisone taper continued. An HSV isolate collected in mid-February while the patient was receiving oral prednisone alone at 20 mg every other day (14847) again showed acyclovir resistance. A trial of trifluorothymidine (TFT; 1% ophthalmic solution) was initiated in late February 1994 and continued after oral prednisone was stopped in March 1994. TFT mixed with bacitracin zinc-polymyxin B sulfate and Aquaphor (Beiersdorf, Norwalk, CT) ointments was applied thrice daily for 2 weeks without healing of vulvar erosions. Serial alterations in therapy over the total 8-week course included the addition of topical steroid therapy, application of TFT 1% solution alone, and the use of topical TFT mixed with mupirocin ointment, all without clinical or virologic effect. Topical TFT therapy was discontinued in April 1994.

In May 1994, repeat vulvar biopsy while the patient was receiving no topical or systemic therapy showed histologic changes characteristic of herpetic infection, including ulceration, adjacent multinucleated giant cell formation, and intranuclear viral inclusion bodies within epithelial cells. Direct immunofluorescence testing on the biopsy tissue was again negative for autoimmune bullous disease.

In September 1994, a trial of oral valacyclovir was initiated (1 g four times daily for 6 weeks, followed by 2 g four times daily for 8 weeks), without significant clinical improvement or conversion of viral cultures to negative. Peak steady-state plasma levels of acyclovir were found to be high while the patient was receiving both the lower and higher doses of valacyclovir (9.6 and 18.5 μg/mL, respectively). Valacyclovir was discontinued in January 1995. Repeat antiviral susceptibility testing confirmed acyclovir resistance and susceptibility to foscanet, both before and after valacyclovir cessation (isolates 15583 and 15582, respectively; table 1).

Therapy with topical foscanet cream (1%) was initiated in June 1995. The cream was applied with a gloved hand five times daily, with dramatic reduction in chronic pain and burning within the first 24 h, and marked reepithelialization of all erosions evident within 5 days. Complete reepithelialization was noted at 2 weeks. Viral cultures were negative for HSV-2 at 2 weeks and 4 weeks,
Figure 1. Clinical course of immunocompetent woman with acyclovir-resistant genital herpes. Reported onset of genital HSV-2 infection was July 1990, followed by onset of chronic vulvar erosions in April 1992 (outbreaks reported by patient denoted by short vertical lines). HSV-2 recurrences and persistent vulvar erosions were documented by office visits to Stanford Dermatology beginning in July 1993 (longer vertical lines); +, − indicate viral cultures positive and negative, respectively, for HSV-2. No further HSV-2 infection was detected clinically or virologically after July 1995. Assigned numbers are shown for HSV-2 isolates evaluated in detail (susceptibility and neurovirulence testing). Initiation and dose escalation of acyclovir and valacyclovir and courses of topical and systemic corticosteroids, topical trifluorothymidine, and topical foscarnet cream are drawn in relation to time line.

and no clinical recurrence was evident at weeks 6 and 10 of the foscarnet trial. According to the patient, a small vulvar erosion recurred after 9 weeks (September 1995) but resolved completely after four applications of foscarnet cream over a 24-h period. The patient has remained free of vulvar pain and erosions since that time.

Materials and Methods

An Investigational New Drug (IND) application was approved by the US Food and Drug Administration for the use of valacyclovir (IND 46,008) and foscarnet cream (IND 47,844). Oral valacyclovir pills were supplied by Glaxo Wellcome (Research Triangle Park, NC), and foscarnet cream by Astra Pharmaceuticals (Westborough, MA).

In vitro susceptibility testing and phenotypic analysis. Antiviral susceptibility testing to acyclovir and foscarnet was done by use of the plaque reduction assay in Vero cells, as previously described [18]. TK phenotype was characterized by plaque autoradiography as described by Martin et al. [19]. Virus plaques in either Vero cells (ATCC CCL 81) or 143B cells (ATCC CRL 8303, a TK-deficient human osteosarcoma cell line) were labeled with $^{[32]}$Iodothymidine (specific activity, 2200 Ci/mmol; NEN Research Products, Boston) or $^{[14]}$Cthymidine (56 Ci/mmol; NEN), respectively. The proficiency of these isolates to phosphorylate and incorporate the labeled nucleosides was compared with that of controls following a 1-week exposure of the fixed monolayers to Kodak X-Omat film, and the isolates were classified as TK-positive, TK-deficient, TK-altered, or TK–low activity phenotypes [20]. Three of the original isolates (14849, 14848, and 15582) were mixed populations containing 2%–10% TK-positive virus and may indicate the limits of our assay methods; however, the 2 chosen for further animal study (14847 and 14848) were 98%–100% TK-deficient populations. Virus isolates were plaque-purified to homogeneity through three rounds of plaque purification, and typing was verified by type-specific polymerase chain reaction (PCR) primers. Plasma acyclovir levels were measured by scintillation proximity RIA [21]. The TK phenotypes of these isolates were further characterized by the ability of the viral TK to phosphorylate thymidine and were determined on crude extracts of TK-deficient 143B cells as described elsewhere [20]. After subtracting out the cells’ background activity, acyclovir-resistant isolates that had <1% wild type activity were classified as TK-deficient, isolates between 1% and 15% were TK–low activity types, and isolates that had 15%–100% activity were classified as TK-altered. TK-altered phenotypes are distinguished from TK-positive phenotypes by their inability to phosphorylate acyclovir in the EIAs [8].

Immunologic assays. Type-specific immunoblot analysis of HSV was done as previously described [22]. NK cell measures used peripheral blood mononuclear cells (PBMC) as effector cells and $^{[51]}$Cr-labeled K562 target cells at six effector-to-target ratios.
Table 1. Virologic characterization of serial HSV patient isolates.

<table>
<thead>
<tr>
<th>Date (lot no.) of specimen</th>
<th>Acyclovir ID50* (µg/mL)</th>
<th>Foscarnet ID50* (µg/mL)</th>
<th>TK phenotype†</th>
<th>TK activity‡</th>
<th>Genotype§</th>
<th>pfu/LD50 ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/28/93 (15923)</td>
<td>17.5</td>
<td>20.0</td>
<td>100% TK⁻</td>
<td>None detected</td>
<td>+G</td>
<td>Not tested</td>
</tr>
<tr>
<td>1/3/94 (14849)</td>
<td>4.8</td>
<td>12.5</td>
<td>90%–100% TK⁻</td>
<td>None detected</td>
<td>+G</td>
<td>2.1 × 10⁻³</td>
</tr>
<tr>
<td>1/20/94 (14848)</td>
<td>16.0</td>
<td>18.5</td>
<td>98%–100% TK⁻</td>
<td>None detected</td>
<td>−C</td>
<td>&gt;5.4 × 10⁻³</td>
</tr>
<tr>
<td>2/14/94 (14847)</td>
<td>13.0</td>
<td>23.0</td>
<td>100% TK⁻</td>
<td>None detected</td>
<td>−C</td>
<td>6.2 × 10⁻¹</td>
</tr>
<tr>
<td>12/21/94 (15583)</td>
<td>12.7</td>
<td>16.4</td>
<td>100% TK⁻</td>
<td>None detected</td>
<td>+G</td>
<td>2.0 × 10⁻²</td>
</tr>
<tr>
<td>1/18/95 (15582)</td>
<td>15.9</td>
<td>30.1</td>
<td>90% TK⁻</td>
<td>None detected</td>
<td>−C</td>
<td>1.1 × 10⁻¹</td>
</tr>
<tr>
<td>8705, TK-positive³</td>
<td>1.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8706, TK-altered³</td>
<td>13.9</td>
<td>16.4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8711, TK-deficient³</td>
<td>58.1</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MS, TK-positive**</td>
<td>—</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR6608, TK-negative**</td>
<td>—</td>
<td>2.6 × 10⁴</td>
<td></td>
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</tr>
</tbody>
</table>

NOTE. Susceptibility testing was done by plaque reduction assay. TK, thymidine kinase. Dates are given as month/day/year.

* ID50 > 100 µg/mL defines resistance to acyclovir; ID50 > 100 µg/mL defines resistance to foscarnet.

† Phenotypic characterization was done by plaque autoradiography. These phenotypes are from original isolates; all other assays, enzyme analysis, genotyping, and animal work were done with plaque-purified homogeneous TK-deficient populations.

‡ Level of thymidine kinase (TK) activity is result of at least two determinations and expressed as percentage of activity in control strain.

§ Genotype —C denotes single-base deletion within C homopolymer (5 cytosines instead of 6), which yields truncated protein of 263 aa. Genotype +G (G homopolymer) has 8 guanines vs. 7 (as in wild type) and yields truncated TK protein of ~229 aa.

¶ Plaque-forming units of virus required to kill 50% of mice inoculated intracerebrally.

[12] ** Control viruses of known virulence.

for 4 h; results are expressed as lytic units/10⁶ PBMC [23, 24]. Quadruplicate proliferation assays used 10⁶ PBMC/well and 0.4 µg/mL phytohemagglutinin (Murex Diagnostics, Norcross, GA) or UV-inactivated cell-associated HSV strain 333 or wild type varicella zoster virus prepared as described [23, 25]. After 5 days, wells were pulsed with 1 µCi of [³H]thymidine and 18 h later were harvested and analyzed by liquid scintillation; results are expressed as Δ counts per minute (cpm) (mean cpm mitogen or antigen – mean cpm for mock-infected fibroblasts). Limiting dilution assays for HSV-2 proliferative and CD8 cytotoxic T lymphocyte precursor (pCTL) responses to HSV-2 antigen were done as described [26, 27] and analyzed by x² minimization for estimation of precursor frequency [28], with results rounded to the nearest 100.

Virulence assay. Intracerebral inoculation of young mice provides a very sensitive system for determining virulence of HSV isolates. In general, isolates that have lost their TK activity do not cause mortality, whereas TK-positive isolates will kill mice with as few as 1–10 pfu. Five of the HSV isolates were inoculated into the right cerebral hemisphere with 0.03 mL of four dilutions containing 1–10,000 pfu. A group of 10 BALB/c mice (3 weeks old) was inoculated with each concentration and checked for mortality for 21 days. At this time, LD50 values were calculated by use of a computer program (Dose Effect Analysis; Elsevier Biosoft, Cambridge, UK), and plaque-forming unit/LD50 ratios were determined [29, 30]. Titer of the virus pools were determined by plaque assay in primary rabbit kidney cells. All animals were anesthetized with a mixture of ketamine-rompune before virus inoculation.

DNA purification and PCR. Vero cells were infected with each of the 6 clinical isolates and the 2 isolates recovered from infected mouse brain homogenates (14847 and 14848). HSV-specific whole cell and viral DNA was isolated and purified with the QIAamp Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. The TK of these isolates was PCR-amplified with designed primers based on the DNA sequence of the HSV-2 strain 333 TK gene (GenBank accession no. M29943). Tandem PCR with the use of an upstream biotinylated primer in one reaction and a downstream biotinylated primer in the other (Oligos Etc., Wilsonville, OR) allowed for strand-specific separation using streptavidin-coated magnetic beads following the manufacturer’s instructions (Dynal, Oslo). The PCR consisted of 300 ng of DNA, 1 × Thermopol buffer (10 mM Tris-HCl, pH 8.8, at 25°C, 10 mM [NH₄]₂SO₄, 2 mM MgSO₄, 0.1% Triton X-100), 0.5 mM MgSO₄, 250 µM dNTPs, including the use of 7-deaza-2’-deoxy-GTP in a 3:1 ratio to dGTP for sequencing high-G-C-content DNA [31], 0.5 µM upstream and downstream primers, and 0.5 U of Vent DNA polymerase (New England BioLabs, Beverly, MA). PCR products were analyzed by 1% agarose gel electrophoresis, and a band ~1.56 kb in length, the viral TK, with 90% of the TK promoter, was purified with the Wizard PCR Preps DNA Purification System (Promega, Madison, WI).

Sequencing analysis. Sequencing was done at the Glaxo Wellcome Automated Fluorescent DNA Sequencing Facility. The HSV-TK solid-phase single-stranded DNA templates were submitted with 3.2 pmol of sequence primer and DMSO, for a final concentration of 5% in the sequence reaction. Twelve oligonucleotide primers, designed on the DNA sequence of HSV-2 strain 333 TK gene, were used to sequence both strands. The fluorescent sequencing chemistry used was Dye Terminator Cycle Sequencing with AmpliTaq DNA Polymerase, FS (Applied Biosystems, Foster City, CA), and manufacturer’s protocols were followed. Sequencing was done on a DNA sequencer (model 373; Applied Biosystems) using ABI Sequence Analysis 2.1 software. Sequence editing and analysis were done with Sequencher software (Gene Codes, Ann Arbor, MI).
Results

Immunologic studies. HSV type-specific immunoblot of patient sera with HSV-1 and HSV-2 antigens showed a normal pattern of reactivity, with multiple HSV proteins indicative of HSV-2 but not HSV-1 infection. To assess cellular immunity, assays of HSV-specific proliferative and CD8 pCTL responses and NK cell function were performed. Proliferative response by PBMC for HSV-2 antigen was 34,748 cpm. This is comparable to values from 10 HIV-seronegative HSV-2-seropositive female patients with symptomatic genital HSV-2 (median $\Delta$ cpm, 23,526; range, 14,678–39,656). Lymphoproliferative responses to phytohemagglutinin ($\Delta$ cpm, 61,236) and varicella-zoster virus antigen ($\Delta$ cpm, 21,500) were also intact. Limiting dilution assay for proliferative responses gave an estimated precursor frequency of 1/1800 (95% confidence range, 1/1400–1/2200). The precursor frequencies for the same ($n = 10$) control group (median, 1/3346; range, 1/961–1/11,904) and previously healthy HSV-2-seropositive controls were similar [26]. The frequency of HSV-specific CD8 pCTL was 1/9000 (95% confidence range, 1/7000–1/12,000). NK cell assay using K562 target cells showed that PBMC contained 20.7 lytic units/10$^7$ PBMC; the percentage of specific release was 24.3 at an effector-to-target ratio of 100:1. Values for NK assay from 4 healthy persons were 19, 63, 87, and 130 lytic units/10$^7$ PBMC (Koelle DM, unpublished data).

Virologic studies. Phenotypic characterization of multiple HSV isolates by plaque autoradiography showed that all isolates tested were predominantly TK-deficient. These isolates were plaque-purified to homogeneity, and their TK activity was undetectable by both TK assay and plaque autoradiographic methods (table 1).

Five serial patient isolates were inoculated intracerebrally into mice to determine if their virulence had been attenuated. Two HSV isolates of known virulence were included as controls. The positive control was HSV-2, MS strain, and the negative control was an HSV-1 SR 6008 in which the TK region had been deleted. The number of plaque-forming units required to kill 50% of mice inoculated with each of the isolates is shown in table 1. Four of the five viruses tested that were TK-deficient had retained the capacity to kill mice. In particular, virus isolate 14847 was similar to the TK-positive control in that 10–50 pfu were lethal. Virus isolate 14848 failed to kill any mice, even when 5 × 10$^3$ pfu were inoculated. Because of this unexpected finding, a second series of samples from the same isolates were inoculated into mice with virtually identical results. The results shown in table 1 are the average of both experiments. To determine the phenotype and genotype of these viruses (both before and after replication in the mouse), brains were harvested from mice inoculated with 14847 and 14848. Tissue homogenates were prepared and retested for TK phenotype and sequenced. Both virus isolates recovered from mouse brain were still TK-deficient and had the same sequence as the virus that was injected into the mice. Furthermore, isolate 14847 was nearly as virulent as the TK-positive control, whereas isolate 14848 was avirulent.

The TK from the 6 original isolates recovered from the patient was sequenced by PCR and showed two genotypes (table 1). A single-base deletion within a homopolymer of six cytosines (C homopolymer) was found in 3 of the isolates and occurred in isolates 14847 and 14848 on repeated testing, resulting in a truncated protein of ~263 aa (wild type = 376 aa). The other 3 isolates showed insertion mutations within the guanine (G) homopolymer (eight guanines instead of seven), yielding a truncated TK protein of ~229 aa (wild type = 376 aa) [32]. Despite the differences in virulence, the 2 isolates recovered from the mice inoculated intracerebrally (14847 and 14848) showed identical genotypes to each other (as well as to the original isolates tested), with the same single-base deletion in the C homopolymer. However, although the DNA sequence for both of these isolates was identical for the TK and 90% of the TK promoter, the possibility of mutations in other loci of these isolates cannot be excluded.

Discussion

This is the first reported case of persistent, nonhealing vulvar lesions in an immunocompetent patient due to an acyclovir-resistant, TK-deficient HSV-2 infection. Despite therapeutic trials with long-term high-dose acyclovir, oral valacyclovir, and topical triflurourymidomide, vulvar erosions did not heal until topical foscarnet cream was applied. In addition, the use of foscarnet cream has resulted in a durable remission of disease. Chronic or recurrent vulvar erosions may be due to a variety of diseases, including HSV infection. A noninfectious primary vulvar dermatosis was initially considered in our patient, possibly acting as a nusus for frequently recurring HSV-2 infection. Potential skin disorders included erosive lichen planus, pemphigus vulgaris, cicatrical or bullous pemphigoid localized to the vulva, bullous lupus erythematosus (rarely reported to be associated with mucocutaneous disease), and Behcet’s syndrome. However, although the DNA sequence for both of these isolates was identical for the TK and 90% of the TK promoter, the possibility of mutations in other loci of these isolates cannot be excluded.
in immunocompetent patients has been demonstrated in vitro without significant clinical correlation [3]. Prolonged, self-limited infections may occur in the setting of acyclovir resistance, but severe disease with nonhealing lesions is uncommon in healthy patients [2, 3, 17, 36].

The patient described herein is unique in that a TK-deficient HSV infection has apparently emerged after initial improvement during acyclovir treatment, producing morbidity in an immunocompetent heterosexual person. The acyclovir-resistant HSV-2 isolates recovered from this patient were extremely difficult to characterize in the phenotypic assays and may demonstrate the limits these assays have in detecting low levels of TK activity [37]. While the isolates tested were plaque-purified three times to homogenous populations before the enzyme analysis and animal studies were done, the influence of undetectable low levels of TK-positive virus cannot be completely excluded in this patient.

Animal models are extremely efficient at detecting low levels of virulence [5, 38], as demonstrated by our studies in which 4 of 5 isolates were virulent, although at a reduced level compared with virulence of the TK-positive MS strain. Further evidence supporting the fact that these virus pools were homogenous populations of TK-deficient HSV is that the virus isolated from the brains of mice that died from infection had the same phenotype and genotype as the original infecting virus. This is in contrast to an isolate recently described by Sasadeusz and Sacks [37], who inoculated a TK-deficient virus into mice and recovered a TK-positive virus from animals that died, suggesting either selection of a TK-positive virus from a heterogeneous mixture or complementation between the TK-deficient isolate and wild type virus. However, it is important to reiterate that of the 5 TK-deficient isolates from our patient that were tested in mice, only 1 failed to kill the animals.

Phenotyping and sequencing isolates before and after mouse inoculation demonstrated TK deficiency and identical TK deletion mutations. The C and G homopolymer loci, in which the mutations in our isolates were localized, have previously been shown to be sites of high mutational activity and appear to be responsible for 40%–50% of acyclovir-resistant HSV isolates [32]. Although the entire coding region of the TK protein in our patient’s isolates was sequenced, including 90% of the promoter region and 41% of the 3’ untranslated region, we cannot exclude the possibility that some other mutational changes responsible for neurovirulence may have occurred in regions that were not sequenced.

We intensively evaluated the immune status of our patient. No evidence of HIV infection or collagen vascular disease was present. She appeared to have a normal immune response to HSV-2. The profile of antibody reactivity observed with HSV-2 immunoblot assay using patient sera was typical for long-standing HSV-2 infection [22]. Results of bulk lymphoproliferation and limiting dilution proliferation assays were within the range of values obtained for immunocompetent patients with recurrent genital HSV-2. Although rare patients with low NK activity and severe herpesvirus infections have been reported [39], our patient appeared to have intact PBMC NK activity. The frequency of HSV-specific CD8 pCTL was within the range observed for 10 immunocompetent patients with a history of genital herpes [28]. The data suggest that neither an HSV-specific nor a generalized immunodeficiency state contributed to the development of persistent acyclovir-resistant HSV infection.

Multiple therapeutic modalities were used in this patient to evoke healing. TFT is a nucleoside analogue whose activity is not dependent on phosphorylation by the virus-specific TK [40], and both in vitro susceptibility of acyclovir-resistant HSV strains [41] and clinical success with its topical application for treatment of AIDS patients with acyclovir-resistant mucocutaneous HSV infection have been described [42–44]. However, this modality was unsuccessful in our patient, who showed persistent culture-positive HSV-2 infection throughout TFT therapy. Valacyclovir is a valine ester prodrug of acyclovir that yields plasma levels of acyclovir similar to those seen with intravenous acyclovir [45]. Despite prior reports of success with use of continuous-infusion acyclovir for treatment of acyclovir-resistant infection in patients with AIDS [46–48], as well as documentation of high serum levels of acyclovir with both low and high doses of the drug, valacyclovir was unsuccessful in evoking healing of vulvar erosions in our patient.

Like TFT, foscarnet (trisodium phosphonoformate) does not require viral TK phosphorylation for its antiviral activity [50]. Foscarnet is a potent inhibitor of HSV DNA polymerase and has been shown to effectively treat acyclovir-resistant infection in immunocompromised persons when administered parenterally [51–53]. However, its systemic use is limited by potentially severe toxicity, including renal impairment, granulocytopenia, and metabolic disturbances (particularly in phosphate, calcium, and magnesium levels).

While foscarnet cream has shown no significant benefit over oral acyclovir in healthy, immunocompetent patients with recurrent genital herpes [54–56], it may have a singular role in certain subsets of patients, such as those with acyclovir-resistant disease. A recently completed pilot study of foscarnet cream in AIDS patients with acyclovir-resistant HSV-2 infection [57] showed marked reduction in pain in 50% of patients and complete healing of HSV lesions in 45% of 20 patients. Our patient showed a dramatic and lasting response to topical foscarnet cream since it was initiated in June 1995, experiencing rapid symptomatic relief and complete resolution of vulvar burning, itch, and pain within 1 day of foscarnet application, and becoming free of herpetic lesions for the first time since July 1993 or earlier.

Our patient is the first healthy and otherwise immunocompetent person in whom acyclovir-resistant, TK-deficient HSV caused chronic and recurrent, nonhealing vulvar erosions for a period of >3 years. Implications regarding similar infections in otherwise immunocompetent persons will require observation over time.
Acknowledgments

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References


