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Pathogenesis of acyclovir-resistant herpes simplex type 2 isolates in animal models of genital herpes: models for antiviral evaluations

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Abstract

Our understanding of the pathogenesis of acyclovir (ACV)-resistant herpes simplex virus (HSV) is limited, especially with regard to reactivation and recurrent disease. To further explore the pathogenesis of ACV-resistant HSV-2 viruses, we used the guinea pig model of genital HSV-2 infection to evaluate several ACV-resistant isolates of both thymidine kinase (Tk)-altered and Tk-deficient phenotypes obtained from HIV-infected patients. Two plaque-purified workpools from each isolate were initially evaluated. Each produced acute disease and at least one clinical recurrence. The two strains that produced the most severe primary disease and most recurrences, one Tk-deficient virus and one Tk-altered virus, were further evaluated and shown to produce acute and recurrent genital disease similar to that seen with wild-type viruses. Furthermore, the reactivated virus producing recurrent lesions could be a pure population with minimal Tk activity. Finally, we showed that topical foscarnet treatment could alter disease and vaginal virus replication following vaginal inoculation with these two ACV-resistant strains. Using the guinea pig model of genital HSV-2 infection, we found that recurrent disease following infection with markedly Tk-deficient viruses was more common than expected, especially in select isolates. Furthermore, this model should be useful in evaluating potential new therapies for ACV-resistant HSV strains.

Keywords: Pathogenesis; Acyclovir resistant; Genital herpes; Animal models

1. Introduction

Genital herpes simplex virus infections are increasing in frequency (Fleming et al., 1997). Disease due to acyclovir (ACV)-resistant virus, however, remains rare in immunocompetent patients, although disease and even serious disease due to resistant viruses have been reported (Kost et al., 1993; Nyquist et al., 1994; Mouly et al., 1995; Swetter et al., 1998). Serious disease due to ACV-resistant virus continues to be a problem in immunocompromised patients (Erlich et al., 1989a; Marks et al., 1989; Ljungman et al., 1990),

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limiting its usefulness in this population and requiring treatment with drugs with a poorer safety profile compared with ACV (Erlich et al., 1989b; Snoeck et al., 1994; LoPresti et al., 1998).

Resistance to ACV occurs either from mutation in the viral DNA polymerase or, more commonly, the viral thymidine kinase (Tk) gene (reviewed in Coen, 1994; Kimberlin et al., 1995). Tk mutations lead to strains that are either deficient in Tk activity and thus cannot phosphorylate ACV effectively or those that have an altered Tk. Tk-altered mutants are unable to phosphorylate ACV, but can phosphorylate thymidine. Most ACV-resistant strains are Tk deficient (Hill et al., 1991; Collins and Ellis, 1993). The deficient strains have frequently been divided into Tk negative and Tk deficient but, because the distinction is difficult and relies on the sensitivity of the assays used (Kimberlin et al., 1995), the term Tk deficient will be used here. The pathogenic potential of Tk-altered strains may be expected to be more similar to Tk + strains than Tk-deficient strains because their ability to phosphorylate thymidine is unaltered (Kimberlin et al., 1995).

The pathogenesis of ACV-resistant mutants is not clear. In mouse models, true Tk-negative mutants appear to be minimally virulent, especially in their ability to reactivate from latency (Tenser and Edris, 1987; Efstathiou et al., 1989; Jacobson et al., 1993; Coen, 1994). Most of the studies evaluating reactivation of Tk-deficient mutants have been carried out in mice infected with herpes simplex virus type 1 (HSV-1) using in vitro reactivation of explanted ganglion. In contrast, most of the cases of reactivation with ACV-resistant HSV strains in immunocompetent humans involve HSV-2 (Kost et al., 1993; Mouly et al., 1995; Swetter et al., 1998). In humans, reactivation of HSV-2 with extremely low levels of Tk activity has been reported (Safrin et al., 1991; Sasadeusz and Sacks, 1996). Furthermore, there is less information in animal models regarding the requirement for Tk activity to establish latency or reactivate with HSV-2 than HSV-1 strains, and not all mammalian species are equally permissive for latency following infection with Tk-deficient HSV strains (Meignier et al., 1988). Stroop et al. (1994) have shown that a Tk-deficient HSV-2 strain can establish latency following intranasal or ocular infection in rabbits and can reactivate in a small proportion of animals.

In a recent report, Horsburgh et al. (1998) proposed that the discrepancy between mouse models and clinical experience might be due to low but undetected amounts of Tk or from a subpopulation of Tk competent viruses. Using the guinea pig model of genital herpes, Stanberry et al. (1985b, 1986) previously reported that a Tk-deficient laboratory HSV-2 strain could establish latency and reactivate. To further explore the pathogenesis of ACV-resistant HSV-2 viruses, we used the guinea pig model of genital HSV-2 infection to evaluate several ACV-resistant isolates of both Tk-altered and Tk-deficient phenotypes obtained from HIV-infected patients. This model offers an advantage to other models in that HSV-2 spontaneously produces recurrent genital lesions in vaginally infected animals, thus allowing us to investigate for the first time the pathogenesis of recurrent genital HSV-2 disease with these viruses (Stanberry et al., 1982; Bernstein et al., 1986). Furthermore, by using plaque-purified human isolates, we were able to evaluate homogenous Tk-deficient pools. Identification of ACV-resistant isolates that produced acute and recurrent disease also allowed us to establish a model for evaluation of new antivirals, which we validated using foscarnet.

2. Methods

2.1. Viruses

The antiviral-resistant HSV-2 isolates used in these studies are shown in Table 1. All of the viruses were originally isolated from HIV-infected patients. Seven were kindly provided by Dr Sharon Safrin (University of California, San Francisco, CA) and one virus by The Burroughs Wellcome Company, currently Glaxo Wellcome (Safrin et al., 1991, 1994; Hill et al., 1991). The ACV-resistant isolates had ACV ID_{50} values of 10.5–64 μg/ml, and included Tk-deficient viruses with Tk activity of 2.4–3.6% compared with HSV-2 wild-type strain 333 (Safrin et al., 1991),

and a Tk-altered virus that retained the ability to phosphorylate thymidine but did not phosphorylate ACV (Hill et al., 1991). A foscarnet-resistant isolate and an isolate resistant to foscarnet and ACV were also kindly provided by Dr Safrin (Safrin et al., 1994). Both isolates were obtained from the same patient about 1 year apart. Isolates with ID_{50} values >3.0 μg/ml were considered ACV resistant (Hill et al., 1991).

Workpools were prepared in primary rabbit kidney cells. Isolates were then plaque purified twice using Vero cells. For plaque selection, six-well plates of Vero cells were inoculated with 7-fold dilutions of virus grown for 48 h in the presence of 5 μg/ml ACV in a methylcellulose overlay. Four plaques were randomly selected in wells with less than six plaques after each passage, reinoculated onto Vero cells in the presence of ACV and harvested when a 3–4+ cytopathic effect (CPE) was seen. For Foscarnet resistant isolates, the virus was plaque purified in the presence of 100 μg/ml foscarnet. This procedure was then repeated so final workpools were from double plaque-purified isolates. Following the second plaque purification, small workpools were prepared on Vero cells in the presence of the antivirals as already described. When cultures reached 3+ CPE, they were harvested and stored frozen (-80°C) until used undiluted in guinea pig studies, at which time they were titrated. The HSV-2 strain MS (Tk+), originally obtained from the American type culture collection (VR-540), was prepared in low passage rabbit kidney (RK) cells (Stanberry et al., 1982; Bernstein et al., 1986) and used as a control in the guinea pig model. HSV-2 strain 186 (Tk+) was also prepared in low-passage RK cells (Milligan and Bernstein, 1995, 1997) and used as a control in the mouse vaginal model.

2.2. Animal models

2.2.1. Guinea pig model

Hartley guinea pigs weighing 275–300 g (Charles River Breeding Laboratory, Wilmington, MA) were intravaginally inoculated with the various HSV-2 isolates. Following rupture of the vaginal closure membrane with a calcium alginate swab, 0.1 ml suspensions of two plaque-picked virus isolates from each patient were instilled using a plastic catheter as previously described (Stanberry et al., 1982; Bernstein et al., 1986). Guinea pigs were examined daily and the severity of the primary genital skin disease quantified on a scale of one to four (Bernstein et al., 1986; Stanberry et al., 1982). The severity of primary disease is expressed as a total lesion score, which is the sum of scores for the primary disease (day 1–14). Vaginal viral swabs were collected where indicated using a calcium alginate swab (Stanberry et al., 1982; Bernstein et al., 1986). Isolates were stored frozen at -70°C until assay for the presence of virus using RK cells (Stanberry et al., 1982; Bernstein et al., 1986). Following recovery

Table 1
Antiviral-resistant HSV-2 isolates[a]

Isolate	Acyclovir ID_{50} (μg/ml)	Foscarnet ID_{50} (μg/ml)	Tk (%)
890430 (Safrin et al., 1991)	24.0	ND	3.6
890480 (Safrin et al., 1991)	64.0	ND	3.2
890540 (Safrin et al., 1991)	10.5	ND	2.8
890560 (Safrin et al., 1991)	16.0	ND	2.0
890610 (Safrin et al., 1991)	13.0	ND	2.4
12247 (Hill et al., 1991)	26.7	ND	150.0
900395 (Safrin et al., 1994)	1.0	119	ND
920056 (Safrin et al., 1994)	43	110	ND
MS (Bernstein et al., 1986)	0.1	ND	ND

[a] The sensitivity to ACV and/or foscarnet and Tk activity are from the cited references. ACV resistance is defined as ACV $ID_{50}>3.0$ μg/ml. ND, Not determined.

from the acute disease (day 14), animals were followed daily for the development of new recurrent lesions through day 42. Animals were then sacrificed and the lumbosacral dorsal root ganglion explanted onto rabbit kidney cell monolayers and observed for reactivation (Stanberry et al., 1982; Bernstein et al., 1986).

In the experiment evaluating foscarnet activity, Hartley guinea pigs were intravaginally inoculated with 5×10^5 pfu of each virus as already described. Animals were then randomized to receive 3% foscarnet cream (Foscarnet powder was kindly supplied by Astra Pharmaceuticals and was formulated by Dr W. Ritschel (University of Cincinnati) as a 3% cream) or a placebo cream. Animals received 0.2 ml drug or placebo cream applied intravaginally, beginning 4 h after virus inoculation and continued three times daily for 10 days. Vaginal swabs were obtained on day 2 to quantitate virus replication. Animals were examined daily from days 1 to 14 for evaluation of primary disease and from days 15 to 42 for recurrent genital disease as already described.

2.2.2. Mouse vaginal model

Female Swiss Webster mice weighing 18–21 g (Harlan, Indianapolis, IN) were given 0.1 ml of a suspension containing 3 mg medroxyprogesterone acetate (Upjohn Pharmacia, Kalamazoo, MI) by subcutaneous injection 7 and 1 day prior to challenge to increase susceptibility to vaginal HSV infection as previously reported (Milligan and Bernstein, 1997). Animals were then anesthetized and the vagina swabbed with a calcium alginate swab prior to intravaginal inoculation with 15 μl of a suspension containing 1×10^6 pfu of HSV-2 strain 186 (Milligan and Bernstein, 1995). Animals were then followed daily for 21 days.

2.2.3. Assays for Tk function

The Tk phenotype was characterized by plaque autoradiography using methods similar to those previously reported (Martin et al., 1985). Briefly, virus plaques in either Vero cells or 143B cells (a Tk-deficient human osteosarcoma cell line) were labeled with ^{125}iododeoxycytidine or ^{14}C-thymidine, respectively. The efficacy of these isolates to phosphorylate and incorporate the labeled material was then evaluated after exposure to film and the isolates classified as Tk +, Tk deficient or Tk altered (Martin et al., 1985).

The method used to evaluate the ability of the viral Tk to phosphorylate thymidine was similar to that reported previously (Martin et al., 1985). Briefly, ^{14}C-TdR was used as the substrate to measure the phosphorylation of lysate from virus-infected 143B cells. The phosphorylation was then compared with wild-type control HSV-2.

2.3. Statistics

Incidence data were compared by Fisher's exact test. Comparisons of the severity of disease were carried out using the Student's *t*-test. All comparisons are two-tailed.

3. Results

3.1. ACV-resistant strains and genital disease

In the initial experiment, undiluted workpools from two plaque-purified isolates for each of six ACV-resistant, one ACV- and foscarnet-resistant, and one foscarnet-resistant human HSV-2 isolate were screened for their ability to cause acute and recurrent genital disease in three to six guinea pigs each. The sensitivity to ACV, foscarnet and the Tk activity of these isolates is shown in Table 1. As seen in Table 2, all isolates produced some primary disease and all but the dual resistant virus produced some recurrent genital lesions, although primary disease was, in general, mild and recurrences infrequent.

The isolate with the Tk-altered phenotype, 12247, produced acute disease in seven of eight animals, and this disease was the most severe of the Tk-mutated isolates evaluated. Recurrences were also the most frequent in this group. This was not unexpected as the virulence of Tk-altered strains would be expected to be similar to Tk + strains because their ability to phosphorylate thymidine is unaffected. The Tk-deficient strains, however, also produced acute disease in most of the animals inoculated and, somewhat surprisingly, also produced recurrences in the majority of

Table 2
Acyclovir-resistant HSV-2 isolates: primary and recurrent genital skin disease

Isolate	Antiviral resistance	Inoculum (log_{10} pfu)	Primary disease		Recurrent disease	
			Incidence[a]	Severity[b]	Incidence[c]	Lesion days[d]
12247A (Tk altered)	ACV	5.7	3/4	5.6 ± 1.9	3/3	8.0 ± 3.8
12247B	ACV	5.7	4/4	10.1 ± 0.4	2/2	2.0 ± 1.0
890430C (Tk deficient)	ACV	4.4	2/3	0.7 ± 0.3	2/3	1.0 ± 0.6
890430D	ACV	4.7	4/4	3.4 ± 1.5	2/4	1.0 ± 0.6
890480C (Tk deficient)	ACV	4.4	3/4	4.0 ± 1.7	4/4	7.0 ± 2.3
890480D	ACV	4.7	4/4	5.3 ± 1.5	4/4	7.3 ± 0.6
890540B(Tk deficient)	ACV	4.6	2/4	0.9 ± 0.5	1/4	0.3 ± 0.3
890540C	ACV	5.6	1/3	0.7 ± 0.7	1/3	2.0 ± 2.0
890560A (Tk deficient)	ACV	3.5	3/3	4.8 ± 0.4	3/3	3.3 ± 0.3
890560C	ACV	3.5	3/4	2.4 ± 0.9	2/4	1.0 ± 0.6
890610C (Tk deficient)	ACV	3.8	3/4	3.1 ± 1.4	3/4	3.8 ± 1.3
890610D	ACV	4.0	4/4	5.9 ± 1.4	3/4	3.3 ± 1.7
900395C (polymerase mutant)	Foscarnet	3.7	5/6	8.9 ± 1.8	ND[e]	–
900395D	Foscarnet	5.7	6/6	10.8 ± 0.6	ND[e]	–
920056B (polymerase, mutant, Tk unknown)	Both	3.7	4/6	1.5 ± 0.5	0/6	–
920056C	Both	3.5	3/6	1.3 ± 0.6	0/6	–

[a] Number of animals with symptomatic primary genital skin disease/number inoculated.
[b] As measured by the area under the lesion score day curve (mean ± S.E.).
[c] Number of animals with recurrent lesions/number available for evaluation of recurrent disease.
[d] Number of days with recurrent lesions between days 15 and 42 post-inoculation (mean ± S.E.).
[e] The severity of primary disease precluded evaluation of recurrences.

animals. Both workpools of 890480, in fact, produced primary and recurrent disease that were similar in severity to Tk+ strains (Stanberry et al., 1982, 1985a; Bernstein et al., 1986; Fowler et al., 1992). The foscarnet resistance isolate, a polymerase mutant (Safrin et al., 1994), also produced severe primary and recurrent disease, while the dual resistant isolate that contains a polymerase mutation as well as a Tk mutation produced the mildest disease and no recurrences.

In the next experiment, we evaluated the Tk-deficient strain, 890480D, that produced the most recurrences and the Tk-altered strain 12247B, at an inoculum of 1×10^6 pfu, similar to that we have used previously for the Tk+ strains MS (Stanberry et al., 1982; Bernstein et al., 1986; Da Costa et al., 1997) and 333 (Harrison et al., 1994). As seen in Table 3, both viruses replicated to a high titer in the guinea pig vagina, peaking at day 2. Primary disease was seen in almost all of the animals inoculated and, somewhat surprisingly, recurrent disease was seen in most of the animals. The severity of primary disease and the frequency of recurrence were similar to that seen following inoculation with the Tk+ MS strain of HSV-2. However, both the number of animals developing recurrent disease and the number of days with recurrent lesions was fewer, indicating that these viruses may indeed be somewhat impaired in their ability to reactivate and/or produce recurrent disease, although the effects were less than anticipated, especially for the Tk-deficient strain.

Because recurrences are thought to be rare with Tk-deficient viruses, we evaluated the Tk activity of virus isolates obtained from recurrent lesions and those obtained following reactivation of explanted dorsal root ganglion, as well as the workpool used for inoculation. HSV was recovered from three of 13 recurrences for isolate 12247B and three of ten for isolate 890480D. As seen in Table 4, it appears that the workpools used were homogeneous. The 12247B workpool is a Tk-al-

tered virus with 16% Tk activity compared with a wild-type control strain. Strain 890480 is a Tk-deficient strain with Tk activity below the level of detection. All the isolates from recurrences or those reactivated from latency in animals inoculated with the Tk-altered virus were Tk+ with activity similar to the inocula and the same altered Tk phenotype. Isolates from animals infected with the Tk-deficient strain were often Tk deficient with extremely low levels of Tk activity (0.1–0.3%). This demonstrates that viruses with extremely low-level Tk activity can reactivate and produce genital lesions in this model. Virus from at least one recurrence was Tk+, however, indicating that the inoculum either contained low levels of a Tk+ strain or that the virus had reverted.

3.2. Evaluation in a lethal mouse genital model

We further characterized these isolates in a lethal mouse genital model. As seen in Table 5, only 10% of mice inoculated with the Tk-deficient strain 890480 died, while, similar to the Tk+ strain 186, 80–100% died after inoculation with either the Tk-altered strain 12247 or the foscarnet-resistant strain 900395.

Table 3
Acyclovir-resistant HSV-2 isolates: viral replication, primary and recurrent genital skin disease

Isolate	Inoculum (log_{10} pfu)	Vaginal virus titer[a]			Primary disease		Recurrent disease	
		Day 1	Day 2	Day 3	Incidence[b]	Severity[c]	Incidence[d]	Severity[e]
12247B (Tk altered)	6.0	5.1	6.6	5.8	6/6	7.3+2.0	2/5	1.8+1.4
890480D (Tk deficient)	6.0	4.8	6.8	4.4	5/6	5.4+1.8	5/6	3.0+1.0
MS (Tk+)	6.0	6.0	6.0	ND	12/12	7.6+0.8	12/12	5.8+0.9

[a] Mean virus titer in vaginal swab samples collected on days 1, 2 and 3 post-inoculation.
[b] Number of animals with symptomatic primary genital skin disease/number inoculated.
[c] As measured by the area under the lesion score day curve (mean ± S.E.).
[d] Number of animals with recurrent lesions/number available for analysis of recurrent disease.
[e] Recurrent lesion days between days 15 and 42 post-inocultaion (mean ± S.E.).

Table 4
Characterization of viral thymidine kinase in isolates from animals infected with ACV-resistant strains

Virus	Source	Plaque Autoradiography		% Activity	ACV ID_{50} (μg/ml)[a]
		[^{125}I]ldc	[^{14}C]TdR		
12247 (Tk altered)	Workpool	TK alt/low	TK positive	16	95.4
	Recurrence	TD alt/low	TK positive	24	24.4
	Recurrence	TK alt/low	TK positive	16	45.1
	Recurrence	TK alt/low	TK positive	22	50.6
	Explant	TK alt/low	TK positive	13	25.6
	Explant	TK alt/low	TK positive	15	62.8
890480 (Tk deficient)	Workpool	TK deficient	TK negative	0	31.8
	Recurrence	TK positive	TK positive	33	6.3
	Recurrence	TK deficient	TK negative	0.2	42.3
	Recurrence	Tk deficient	Tk negative	11	32.6
	Explant	TK alt/low	TK positive	0.1	51.4
	Explant	TK deficient	TK negative	0.3	34.7
	Explant	TK deficient	TK positive	7	1.3

[a] Tested by dye uptake assay using Vero cells (Hill et al., 1991).

Table 5
Mortality using acyclovir-resistant HSV-2 strains in a mouse vaginal model

Virus	Inoculum (log_{10} pfu)	Mortality
12247 (Tk altered)	5.7	8/10
890480 (Tk deficient)	5.8	1/10
900395 (polymerase mutant)	6.0	10/10
186 (wild type, Tk+)	6.0	9/10

3.3. Effect of foscarnet on infection with ACV-resistant virus

In the previous experiment, we established that ACV-resistant strains replicated well in the vagina, and produced acute disease and recurrences at a frequency that made evaluation of antiviral drugs practical. We therefore used the same two strains, 890480 (Tk deficient) and 12247 (Tk altered), to define the effect of 3% foscarnet cream. As seen in Fig. 1 and Table 6, foscarnet treatment significantly reduced vaginal virus titers, the severity of the primary disease but not recurrent disease when begun 4 h after HSV-2 inoculation.

4. Discussion

Our understanding of the pathogenesis of antiviral-resistant HSV strains is far from complete. The most common mechanism of resistance to ACV is a mutation in the viral Tk that results in the absence or marked decrease in activity. There appears to be a relationship between Tk activity and the pathogenic potential of strains at least for HSV-1 in the mouse (Coen, 1994). In the mouse models, true Tk-deficient HSV-1 mutants display only low levels of neurovirulence, establish latency but with lower efficiency and, most strikingly, are severely impaired in their ability to reactivate from latency in the murine ganglion (Tenser and Edris 1987; Coen et al., 1989; Efstathiou et al., 1989; Jacobson et al., 1993). Tk-altered and polymerase mutants are the least attenuated and are therefore more pathogenic (Coen, 1994).

The majority of clinical ACV-resistant isolates retain some Tk activity and, despite the findings in mice with HSV-1, reactivation of ACV-resistant HSV-2 strains occurs in both immunocompetent (Kost et al., 1993; Mouly et al., 1995; Swetter et al., 1998) and immunocompromised

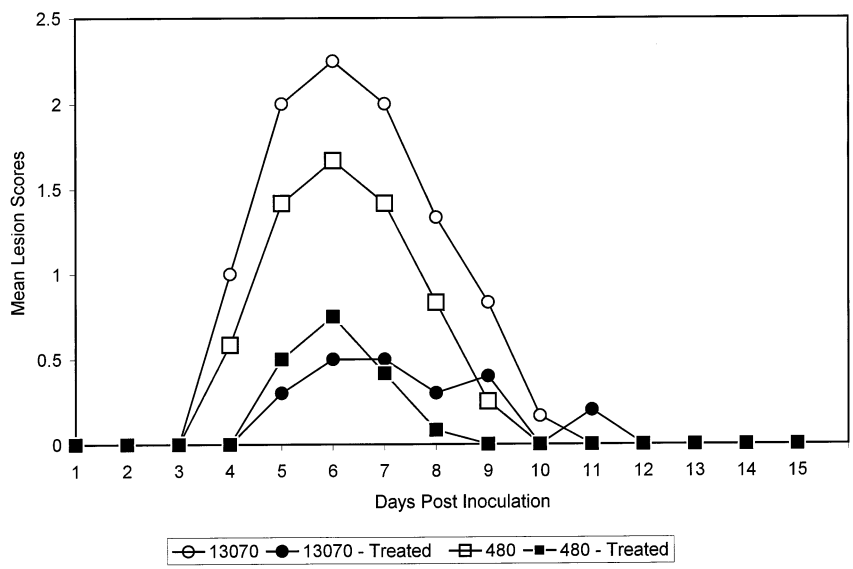

Fig. 1. Effect of topical foscarnet on acute genital HSV-2 disease in guinea pigs. Following intravaginal inoculation with two ACV-resistant HSV-2 isolates, animals received topical 3% foscarnet cream or placebo cream three times daily for 10 days.

Table 6
Effect of topical foscarnet treatment on acute and recurrent genital herpes following inoculation with two ACV-resistant HSV-2 isolates

Isolate	Treatment	Vaginal virus titer[a] (log_{10} pfu)	Primary disease		Recurrent disease	
			Incidence[b]	Severity[c]	Incidence[d]	Severity[e]
Tk altered						
12247	None	7.4 ± 0.7	6/6	9.6 ± 0.7	3/4	4.8 ± 2.1
12247	Foscarnet	3.7 ± 1.0[f]	3/5	3.7 ± 2.7[g]	4/5	4.6 ± 2.1
Tk deficient						
890480	None	8.3 ± 0.6	5/6	7.4 ± 1.2	5/6	2.8 ± 1.8
890480	Foscarnet	4.8 ± 0.5[h]	4/6	2.6 ± 0.5[f]	2/6	3.0 ± 1.0

[a] Mean virus titer in vaginal swab samples collected on day 2 post-inoculation.
[b] Number of animals with genital skin disease/number infected.
[c] As measured by the total lesion score. Calculated by using only symptomatic animals.
[d] Number of animals with recurrent lesions/number available for analysis of recurrent disease.
[e] Number of days with recurrent lesion on days 15–42 post-inoculation (mean ± S.E.).
[f] $P < 0.01$ compared with untreated control.
[g] $P < 0.02$ compared with untreated control.
[h] $P < 0.03$ compared with untreated control.

patients (Erlich et al., 1989a,b; Marks et al., 1989; Ljungman et al., 1990; Safrin et al., 1991; Snoeck et al., 1994; LoPresti et al., 1998). This suggests that either the mouse models are poorly predictive or that the ACV-resistant viruses with the Tk deficits isolated from patients express enough functional Tk for reactivation. Tk function could also be supplied in a mixed infection containing some Tk competent viruses as has been previously reported (Christophers and Sutton, 1987; Sacks et al., 1989; Sasadeusz et al., 1997). In mouse models, such mixed infection are considered more pathogenic than pure populations of Tk-deficient mutants (Gordon et al., 1983; Darby et al., 1984; Sears et al., 1985; Hwang et al., 1994).

Because little is known about spontaneous reactivation, especially with ACV-resistant HSV-2, we used the guinea pig model of genital HSV-2, the only small animal model with spontaneous reactivation, to examine the pathogenesis of several ACV-resistant isolates. Furthermore, we minimized the chance of inoculation with mixed populations by plaque purifying each isolate twice under selected pressure of ACV.

In the experiments reported in this paper, we were able to show that even viruses which express very low levels of Tk were able to produce acute disease in most animals and, most surprisingly, all were capable of reactivating and producing recurrent genital lesions, albeit mainly at reduced levels compared with Tk+ strains (Stanberry et al., 1985a; Fowler et al., 1992; Landry et al., 1992; Harrison et al., 1994). At least in one case, however, the Tk-deficient strain 890480 produced acute disease and reactivated to produce recurrent lesions at a frequency similar to Tk+ HSV-2 strains. Furthermore, we were able to show that the reactivated virus could be a pure population that produced minimal, if any, Tk. Thus, it appears that viruses with minimal Tk activity can establish latency and reactivate in the guinea pig model of genital HSV-2. It should be pointed out, however, that we also recovered Tk+ isolates from a recurrent lesion following inoculation with this Tk-deficient strain and, thus, either our inoculum did contain a small population of Tk+ virus despite our efforts or the virus reverted. These results are similar to those of Sasadeusz and Sacks (1996), who reported that an HSV-2 Tk-deficient virus recovered from an HIV-infected patient could be reactivated from the ganglia of an infected mouse as a homogeneous Tk-deficient population, but it could also either revert to or acquire neurovirulence from a mixed population

despite plaque purification and inoculation with an apparently pure Tk-deficient population. Furthermore, it supports their conclusion that the level of Tk activity necessary for spontaneous reactivation is much lower than previously appreciated. Importantly, it also extends these observations to show that virus can be reactivated spontaneously in vivo and can produce recurrent lesions that contain only Tk-deficient virus. These observations are consistent with that observed in HIV patients (Safrin et al., 1991; Sasadeusz and Sacks, 1996) as well as the immunocompetent patients reported by Swetter et al. (1998). What remains to be determined is the molecular basis to explain why selected Tk-deficient HSV-2 strains are able to reactivate to cause recurrent disease.

We have further shown that inoculation with a Tk-altered strain of HSV-2 produced acute and recurrent disease in the guinea pig that was comparable, although recurrences were reduced, with that following inoculation with Tk + strains in this model (Stanberry et al., 1985a; Fowler et al., 1992; Landry et al., 1992). This observation is consistent with the immunocompetent patient reported by Kost et al. (1993) who developed recurrent genital HSV-2 lesions due to a Tk-altered HSV-2 strain. They concluded that this variant was the most likely to appear in the general population, and our data would support this. Thus, it may be that the only reason these viruses have not become a major problem in the general population is their rarity, even in the immunocompromised patient.

The evaluation in the lethal mouse model of HSV-2 infection is also of interest. The Tk-deficient strain was lethal in only 10% of mice inoculated intravaginally compared with a mortality of 80–100% with the Tk-altered or Tk + but foscarnet-resistant strain. This probably reflects the decreased neurovirulence of the Tk-deficient virus as death is most commonly due to encephalitis in this model, and emphasizes the disparity between neurovirulence and the ability to become latent and reactivate.

In this paper, we have also described the availability of animal models to evaluate the antiviral activity of new compounds against ACV-resistant viruses. The lethal mouse model could serve as a screening model for Tk-altered or polymerase mutants. Furthermore, we have demonstrated the utility of the guinea pig model for evaluations against both Tk-deficient and Tk-altered HSV-2 viruses, the most common ACV-resistant mutants. Thus, we showed that topical foscarnet therapy could reduce acute disease and vaginal viral replication. Treatment, however, did not reduce the frequency of subsequent recurrences. This is similar to the results with other antivirals in this model (Bernstein et al., 1986). Because the main concern with HSV infection is the ability to become latent and produce recurrent disease, this model can evaluate the effects of drugs on recurrent disease. Thus, the guinea pig model presented can evaluate the effect of antiviral therapy on vaginal viral replication, acute genital disease and spontaneous genital recurrences.

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