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In Vitro and In Vivo Characterization of Herpes Simplex Virus Clinical Isolates Recovered from Patients Infected with Human Immunodeficiency Virus

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A total of 100 herpes simplex viruses isolated from lesions not responding to acyclovir (ACV) therapy were recovered from 51 patients infected with human immunodeficiency virus. In vitro analysis of these isolates included testing their susceptibility to ACV and determining their thymidine kinase (TK) phenotypes. Of the 100 isolates evaluated, 23 were ACV sensitive and 77 were ACV resistant. Seventy-four of these ACV-resistant isolates were of the TK-deficient or low-TK-producer phenotype and three were of the TK-altered phenotype. The TKs of isolates that represented each of the different autoradiographic phenotypes were further characterized by enzyme kinetics. The ability of selected isolates to cause disease in vivo was evaluated by using several mouse virulence models. Cutaneous virulence in normal and immunocompromised mice was evaluated, and neurovirulence in normal mice was determined. Latent infections were assayed by the cocultivation of trigeminal ganglia recovered from mice that had survived acute infection. These reactivated viruses were evaluated in vitro and compared with the original infecting isolate. The mechanisms of resistance and pathogenicity of these herpes simplex virus isolates recovered from patients positive for human immunodeficiency virus are similar to those reported for isolates recovered from normal and immunocompromised patients without AIDS.

The recovery of acyclovir (ACV)-resistant herpes simplex virus (HSV) clinical isolates from patients with normal immunity has not been associated with the progression of clinical disease (2, 15, 30). Normal patients that shed isolates that are ACV resistant in vitro have responded well to continued ACV therapy (31, 46, 47), and the number of normal patients shedding resistant virus is below 1% of all patients treated (11, 21), including those on suppressive oral therapy for as long as 1 year (26a).

However, ACV-resistant HSV has been recovered more frequently from immunocompromised patients such as patients undergoing organ transplantation (4, 32, 50), cancer patients (35), patients with congenital abnormalities (3, 13, 45), and patients positive for human immunodeficiency virus (HIV) (7, 22). Infection with ACV-resistant viruses can result in locally progressive mucocutaneous lesions (20, 22, 33, 39). Although a number of reports have addressed the problem of ACV-resistant HSV in the HIV-positive population, only a small number of patients and their isolates have been described previously (7, 21, 22, 43).

Concern that ACV-resistant viruses recovered from this patient group may possess novel mechanisms of resistance or may be more pathogenic than viruses recovered from normal or immunocompromised patients without AIDS led us to evaluate the in vitro and in vivo characteristics of HSV clinical isolates recovered from patients positive for HIV. These isolates were sent to us by the attending physicians, who presumed them to be ACV resistant because of the patients' poor clinical response to ACV therapy. Unfortunately, since these isolates were recovered from patients not enrolled in controlled clinical protocols, questions regarding the prevalence of ACV-resistant virus in this population cannot be addressed. We report here the evaluation of 100 HSV clinical isolates recovered from 51 patients positive for HIV.

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MATERIALS AND METHODS

Cells. Vero cells (African green monkey kidney cells; ATCC CCL 81) were grown in Eagles Minimal Essential Media (EMEM) supplemented with 5% heat-inactivated calf serum, 10 mM HEPES (*N*-[2-hydroxyethyl]-piperazine-*N*′-2-ethanesulfonic acid), 0.075% sodium bicarbonate, 100 units of penicillin G per ml, 100 μg of streptomycin per ml, 0.25 μg of amphotericin B (Fungizone) per ml, and 2 mM L-glutamine (Flow Laboratories, Inc.).

A thymidine kinase (TK)-deficient cell line (143B, a human osteosarcoma cell line; ATCC CRL 8303) was also grown in supplemented EMEM. The 143B cells were routinely checked for reversion to the TK-positive (TK^+) phenotype by autoradiography after labeling with [^{14}C]thymidine and were regularly passaged in media containing 50 μg of bromodeoxyuridine per ml to eliminate cells of the TK^+ phenotype (5).

Cells were grown and assays were performed at 37°C in a 5% CO_2 incubator.

HSV clinical isolates. The HSV clinical isolates we characterized were recovered from patients positive for HIV and whose lesions were not responding to ACV therapy. These isolates were sent to us by the attending physicians for laboratory evaluation. The initial isolation and identification were done in the clinical laboratory, and a small aliquot of the supernatant from infected cells was sent to us. The isolate was regrown in Vero cells to generate an adequate virus stock, which was then aliquoted and stored at −70°C. Most of these isolates are homogeneous with respect to their TK phenotypes as shown by [^{125}I]iododeoxycytidine ([^{125}I]IdC)

and [^{14}C]thymidine ([^{14}C]TdR) plaque autoradiography. The following isolates were plaque purified three times: Virology Laboratory numbers 10091, 11680, 11893, 12247, 10092, and 11785. The in vitro susceptibilities of the cloned viruses were similar to that of the original isolate.

In vitro assays. (i) ACV susceptibility. Virus isolates described in this study were tested for their in vitro susceptibility to ACV by the dye uptake assay. This is a semiautomated, colorimeteric method that measures a quantitative reduction in viral cytopathic effect on the basis of the amount of neutral red dye retained by viable cells (37). These susceptibility values are expressed as 50% effective doses (ED_{50}s), which are the ACV concentrations that inhibit viral cytopathic effect by 50%. The in vitro susceptibilities of selected isolates to a variety of TK-activated drugs and drugs that act directly on the viral DNA polymerase were also determined.

(ii) Plaque autoradiography. Virus isolates were analyzed for their TK phenotypes by the method of Martin et al. (34) by using plaque autoradiography with the labeled nucleosides [^{125}I]idC (specific activity, 2,200 Ci/mmol; New England Nuclear Corp.) and [^{14}C]TdR (specific activity, 56 Ci/mmol; ICN Pharmaceuticals Inc.). The [^{14}C]TdR plaque autoradiography was performed by using a modification of the method described by Tenser et al. (49). Briefly, confluent monolayers of 143B cells in 60-mm^2 plates were inoculated with between 100 and 200 PFU of test virus. After 1 h of incubation at 37°C, the inocula were removed and a 7-ml overlay of supplemented EMEM containing 0.6% agar was added to the plates. After 72 to 96 h of incubation at 37°C in a 5% CO_2 incubator, 1 ml of supplemented EMEM containing 1 μCi of [^{14}C]TdR was added on top of the agar. Following 6 h of incubation at 37°C, the label was removed and 3 ml of crystal violet stain (1% crystal violet, 10% formalin, 5% acetic acid, 60% methanol, 25% water) was added and allowed to stain overnight at room temperature. The stain and agar overlay were gently removed, and the plates were washed with water and then air dried. The rims of the plates were removed, and the monolayers were placed next to Kodak X-Omat X-ray film. Following a 1-week exposure, the film was developed.

(iii) Enzyme kinetics. Monolayers of TK-deficient 143B cells in 100-mm^2 plates were infected with virus at a multiplicity of infection of 2 PFU per cell. After 10 to 12 h of incubation at 37°C in a 5% CO_2 incubator, the monolayers were washed twice with phosphate-buffered saline (PBS), scraped into 1 ml of PBS, pelleted at 1,500 rpm in a refrigerated microcentrifuge, and resuspended in 150 μl of swelling buffer (10 mM Tris-HCl [pH 7.5], 10 mM KCl, 1 mM $MgCl_2$). Following three cycles of freezing and thawing, the cell extracts were pelleted at 11,000 × g in a refrigerated microcentrifuge. The supernatants were then transferred to fresh tubes, and the protein concentration was determined by using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, Calif.) according to the manufacturer's instructions.

The ability of the viral TK to phosphorylate [^{14}C]TdR and [^{3}H]ACV (specific activity, 25 Ci/mmol; New England Nuclear Corp.) was determined by enzyme reactions run at 37°C. The reaction mixture with [^{14}C]TdR as a substrate consisted of 15 μl of cell extract, 10 μl of water, and 25 μl of [^{14}C]TdR reaction soup (200 μM [^{14}C]TdR, 100 mM Tris-HCl (pH 7.5), 10 mM ATP, 10 mM $MgCl_2$). The reaction mixture with [^{3}H]ACV as a substrate consisted of 25 μl of cell extract and 25 μl of [^{3}H]ACV reaction soup, which was the same as above except that 200 μl of [^{3}H]ACV and 100 μM cold ACV were substituted for the TdR. At various time points (1.5, 6, 12, and 15 min), 9 μl of reaction mixture was spotted on DEAE-ion exchange chromatography paper (Whatman DE81). After air drying, the paper was washed twice for 5 min with 1 μM ammonium formate and once with 95% EtOH for 5 min and air dried again. Scintillation cocktail (Beckman Readysafe) was added, and then the vials were counted. The counts per minute were plotted versus time. This generated a slope that was used to calculate enzyme activity, which is given in picomoles per minute per milligram of protein in the cell extract. The TK phenotypes were determined by an isolate's ability to phosphorylate thymidine. This value was then compared with that for wild-type control virus (29). After the cell TK activity is subtracted as background, isolates that have <1% TK activity are classified as TK deficient (TK^D), isolates that have 1 to 15% TK activity are classified as low-TK producers, and isolates that have 15 to 100% TK activity are classified as TK altered (TK^A). Isolates with TK^A phenotypes are distinguished from those with TK^+ phenotypes by their inability to phosphorylate ACV in the enzyme assays (14).

Cutaneous virulence. Cutaneous virulence in immunocompetent hairless HRS/J (Jackson Laboratory) and immunocompromised CD-1 *nu/nu* athymic mice (Charles River) was determined. Groups of 10 mice, under light ether anesthesia, were inoculated on the snout by scarification with a 25-gauge needle, and the site was then rubbed for 10 s with a cotton-tipped applicator soaked in PBS-diluted virus or PBS alone. Samples of the virus dilution used to infect the mice were titrated on confluent monolayers of Vero cells to verify the virus dose used. Mean daily lesion scores were recorded by using the following system: 0 = no lesions, 1 = fewer than five discrete lesions, 2 = five or more lesions, 3 = confluent lesions, 4 = ulcerations or necrosis. The minimum amount of virus needed to infect 50% of the mice (MID_{50}) was calculated by a modified Karber method (45). PFU/MID_{50} ratios are expressed logarithmically.

Neurovirulence. Groups of 10 BALB/c mice (Charles River), under light ether anesthesia, were inoculated in the right cerebral hemisphere with 0.1 ml of one of five 10-fold serial dilutions of virus in PBS or with PBS alone. Samples of the virus dilution used to infect the mice were titrated on confluent monolayers of Vero cells to verify the virus dose used. Mice were observed for 30 days, and death was used as the end point. The amount of virus needed to kill 50% of the animals (50% lethal dose [LD_{50}]) was calculated by using a modified Karber method (45). PFU/LD_{50} ratios are expressed logarithmically.

Latency. HRS/J mice that survived acute infection were sacrificed 30 days after inoculation, and their trigeminal ganglia were harvested. Explanted ganglia were incubated for 5 days at 37°C in supplemented EMEM and then homogenized with a glass homogenizer (Bellco Glass, Inc.). The homogenate was then inoculated on confluent monolayers of Vero cells and checked daily for evidence of viral cytopathic effect. Latency was scored as positive or negative for recoverable virus from explanted ganglia.

The reactivated viruses were evaluated in vitro for susceptibility to ACV, and the viral TKs were analyzed by enzyme kinetics in order to compare them with that of the original infecting isolate.

RESULTS

In vitro drug sensitivities. The in vitro ACV susceptibilities of 100 HSV clinical isolates recovered from 51 patients

TABLE 1. In vitro ACV susceptibilities and TK characterization of selected HSV clinical isolates from HIV-positive patients

Patient no. or control	VL	TK phenotype	ACV ED_{50}[a] (μg/ml)	Plaque autoradiography results[b] for:		Substrate phosphorylation (% of wild-type KOS)	
				[^{125}I]IdC	[^{14}C]TdR	[^{14}C]TdR	[^{3}H]ACV
9	10091	Positive	0.2	+	+	46	20
1	11680	Altered	5.2	A	+	51	9
7	11893	Altered	9.0	A	+	64	<1
4	12247	Altered	26.9	A	+	150	<1
11	11334	Low producer	21.3	D	ND	14	<1
12	11571	Low producer	32.0	D	D	16	1.1
2	11572	Low producer	100.0	D	D	9	6
8	11575	Low producer	90.0	D	ND	18	5
1	11785	Low producer	8.4	D	D	19	2
9	10092	Deficient	22.6	D	D	6	<1
6	11359	Deficient	35.9	D	ND	3	<1
3	11360	Deficient	6.7	D	D	2	<1
3	11361	Deficient	66.4	D	D	4	<1
10	11363	Deficient	13.3	D	ND	5	<1
5	11461	Deficient	100.0	D	D	3	<1
BWS[c]		Positive	0.6	+	+	74	93
BWR[c]		Deficient	17.6	D	D	2	3
143B						6	1

[a] ACV resistance is defined as ACV ED_{50} > 3.0 μg/ml.
[b] +, TK positive; A, TK altered; D, TK-deficient; ND, not done.
[c] TK^+ and TK^D viruses recovered from child with severe combined immunodeficiency (45). BWS and BWR are viruses; 143B is a cell line.

positive for HIV were determined by the dye uptake assay with Vero cells. These isolates were recovered from lesions refractory to ACV therapy and therefore were presumed to be ACV resistant. Isolates for which ED_{50}s of ACV were less than or equal to 3.0 μg/ml were considered ACV sensitive, and isolates for which ED_{50}s of ACV > 3.0 μg/ml were considered ACV resistant.

The median ED_{50} of ACV for 23 ACV-sensitive isolates recovered from 13 patients was 0.6 μg/ml (range, 0.1 to 2.5 μg/ml). All 23 ACV-sensitive isolates are of the TK^+ phenotype as indicated by their ability to phosphorylate [^{125}I]IdC in the plaque autoradiographic assay. Forty-four patients shed 77 ACV-resistant isolates with a median ED_{50} of 34.3 μg/ml (range, 4.2 to 100 μg/ml).

Of the 77 ACV-resistant isolates, 74 had TK^D phenotypes and 3 had TK^A phenotypes. Six patients shed both ACV-sensitive and ACV-resistant viruses, and two shed ACV-sensitive virus early in therapy and ACV-resistant virus after multiple courses of ACV treatment. The temporal relationship of isolates from the other four patients is unknown.

The in vitro sensitivities of the isolates listed in Table 1 to a variety of TK-activated drugs (ganciclovir and thymidine arabinofuranoside) and drugs that act directly on the viral DNA polymerase (Vidarabine, phosphonoacetic acid, and Foscarnet) were also determined. These isolates remained sensitive to the polymerase inhibitors, and ED_{50}s for them were similar to those for wild-type control viruses. However, ED_{50}s were more than threefold less than those of the polymerase mutant control viruses, phosphonoacetic acid-resistant PAA^r (27), and a clinical isolate shown to have a polymerase defect (12). These drug profiles of these isolates suggest that they are unlikely to have a defect in their polymerase gene function.

The in vitro ED_{50}s for ACV-sensitive and ACV-resistant isolates recovered from patients positive for HIV are similar to those reported for isolates recovered from other patient groups (1, 11, 38).

In vitro analysis. Table 1 shows the in vitro results for representative isolates from this HIV-positive patient group. Included in this table are the ACV ED_{50}s, the plaque autoradiographic labeling patterns, and the comparisons of TK activities. In addition, Table 1 shows results for our control viruses, BWS and BWR. These are a pair of ACV-sensitive and ACV-resistant isolates recovered from a child with severe combined immunodeficiency syndrome (45). The enzyme activities are expressed as a percentage of the rate of substrate phosphorylation catalyzed by extracts of cells infected with KOS, an HSV type 1 wild-type laboratory strain. The isolates' TK phenotypes were determined by in vitro ED_{50}s, their parallel plaque autoradiographic patterns, and enzyme kinetic analysis. This scheme is similar to that described by Larder and Darby (29).

These results show that the TK^+ isolate, VL 10091, can phosphorylate both substrates: 46% of wild-type activity for thymidine and 20% of wild-type activity for ACV. This percentage of wild-type activity is low and may indicate an isolate that makes a reduced amount of wild-type enzyme; however, this level of activity is sufficient to phosphorylate enough ACV for this isolate to be sensitive to ACV in vitro and positive in the plaque autoradiographic assay. The plaque-purified TK^A isolates, VLs 11680, 11893, and 12247, were confirmed to have an enzyme with an altered substrate specificity as shown by their ability to phosphorylate thymidine (51, 64, and 150% of wild-type TK activity, respectively) but not ACV (9% for VL 11680 and <1% of wild-type activity for VLs 11893 and 12247). VLs 11334, 11571, 11572, 11575, and 11785 are low TK producers, phosphorylating TK above background but less than 20% of wild-type TK activity. Isolates that were found to be ACV resistant and TK^D in earlier assays were unable to phosphorylate either substrate; their activities were similar to that of the 143B cell control.

Cutaneous virulence. The PFU/MID_{50} ratios, expressed logarithmically, are depicted in Table 2.. There is little difference between the cutaneous virulence ratios for the HRS/J and athymic nude mice. The ratios are similar for the same isolates as well as for isolates with different TK

TABLE 2. In vivo results with selected HSV clinical isolates from HIV-positive patients

Patient no. or control	VL	TK phenotype	Cutaneous virulence[a] for:		Neurovirulence[b] for for BALB/c mice	Latency[c]
			HRS/J mice	Athymic mice		
9	10091	Positive	4.9	4.6	1.5	Negative
1	11680	Altered	4.7	4.9	1.6	Negative
7	11893	Altered	5.6	5.8	2.7	ND
4	12247	Altered	6.3	5.9	1.9	Positive
11	11334	Low producer	4.7	5.8	4.8	Negative
12	11571	Low producer	5.7	5.0	2.6	Negative
2	11572	Low producer	4.7	4.8	2.4	Negative
8	11575	Low producer	4.8	5.6	2.0	Positive
1	11785	Low producer	>6.5	>6.5	1.6	Positive
9	10092	Deficient	5.8	5.4	3.0	Negative
6	11359	Deficient	4.9	5.3	3.6	Negative
3	11360	Deficient	5.4	5.3	2.1	Negative
3	11361	Deficient	5.2	4.8	3.3	Negative
10	11363	Deficient	4.6	5.0	4.3	Negative
5	11461	Deficient	6.0	5.4	3.7	ND
BWS[d]		Positive	4.7	3.8	1.0	Positive
BWR[d]		Deficient	6.9	3.8	4.3	Negative

[a] Numbers are logarithmic and are PFU/MID_{50} ratios.
[b] Values shown are logarithmic and PFU/LD_{50} ratios.
[c] Recovered virus from explanted trigeminal ganglia. ND, not done.
[d] TK^+ and TK^D viruses recovered from a child with severe combined immunodeficiency (45).

phenotypes. This suggests that the ability of these isolates to produce skin lesions is roughly equivalent in these models.

Neurovirulence. The ability of representative isolates to cause lethal infection in BALB/c mice after intracerebral inoculation is also shown in Table 2. As previously reported, differences in pathogenicity of TK^+, TK^A, and TK^D viruses are easier to distinguish with neurovirulence models than with cutaneous virulence models (17, 45). This difference is illustrated by isolates from patient no. 9. The early isolate (VL 10091) was ACV sensitive and TK^+, while a later isolate (VL 10092) was ACV resistant and TK^D. The cutaneous virulence PFU/MID_{50} ratios for hairless HRS/J and athymic mice were 4.9 and 4.6, respectively, for VL 10091 and 5.8 and 5.4, respectively, for VL 10092. The neurovirulence PFU/LD_{50} ratios for these same isolates demonstrate a significant difference in pathogenicity; the ratio for VL 10091 was 1.5 and for VL 10092 was 3.0. This decrease in pathogenicity for the TK^D viruses agrees with published reports (24, 45). The PFU/MID_{50} ratios are similar to the values obtained with control viruses of the same TK phenotypes.

Latency. While viruses of all three TK phenotypes can establish latency, TK^D strains are unable to reactivate from neurons and cause recurrent disease (9, 24, 48). The results of reactivation studies with the trigeminal ganglia from HRS/J mice that survived snout inoculation are shown in Table 2. In our hands, experiments with highly virulent viruses were complicated by acute deaths that resulted from the initial infection. We were unable to test ganglia from these mice, and we found that mice that received less virus (i.e., a higher dilution) were often uninfected. This is the type of problem encountered with the TK^+ isolate VL 10091 and the TK^A isolate VL 11680, and both are listed as negative for the reactivation of latent virus. While TK^D viruses cannot reactivate (9, 48), low TK producers with diminished amounts of wild-type TK activity can reactivate (8, 25). These results are supported by our latency experiments.

The three viruses that were able to establish latent infections (VLs 11575, 11785, and 12247) had wild-type TK activities of 18, 19, and 150%, respectively. All were ACV resistant and are either low TK producers (VLs 11575 and 11785) or TK^A (VL 12247). Analyses of the viruses recovered from explanted ganglia are shown in Table 3. The progenies of the cloned isolates (VLs 11785 and 12247) were similar to the infecting virus. Both VLs 12639 and 12640 are ACV resistant and able to phosphorylate TK but not ACV. The progeny of the uncloned isolate VL 11575 was ACV sensitive and able to phosphorylate both TdR and ACV as efficiently as wild-type virus. This results emphasize the importance of doing in vitro evaluations of the reactivated virus rather than assuming that ACV-resistant virus will reactivate if ACV-resistant virus is the original infecting

TABLE 3. In vitro results with HSV isolates recovered from latently infected ganglia

Infecting virus (VL)	TK phenotype	Recovered Viruses (VL)[a]	ACV ED_{50}[b] (μg/ml)	Substrate phosphorylation (% of wild-type KOS)		TK phenotype of recovered virus
				[^{14}C]TdR	[^{3}H]ACV	
11575	Low producer	12148	2.0	332	147	Positive
11785	Low producer	12639	4.3	58	2	Altered
12247	Altered	12640	26.7	37	<1	Altered

[a] Recovered virus from explanted trigeminal ganglia.
[b] ACV resistance is defined as ACV $ED_{50} > 3.0$ μg/ml.
[c] Phosphorylation catalyzed by cells infected with KOS.

virus. The results also suggest that the constituent in a mixed virus population responsible for reactivation might be a small percentage of undetected wild-type virus.

DISCUSSION

While clinical experience with drug-resistant viruses is limited, the best-studied system of acquired antiviral resistance has been HSV resistant to ACV (4, 13, 32, 45, 50). The mechanisms of ACV resistance and the in vivo pathogenicity of resistant mutants have been determined with laboratory-generated ACV-resistant viruses (10, 24, 28). Mutations in either the virus-encoded TK (TK^D, TK^A, or low-TK-producer phenotypes) or the viral DNA polymerase can confer resistance to ACV (10, 26, 44). Viruses of all three phenotypes have been isolated from patients (2, 12, 18, 42); however, the predominant ACV-resistant phenotype recovered in the clinic has been TK^D (1, 11). While virus with this phenotype is avirulent in patients with normal immunity (16), it can cause significant locally progressive disease in immunocompromised patients (4, 6, 13, 20, 22). The recovery of ACV-resistant virus from immunocompromised patients is not unexpected since these patients usually present with large lesions containing significant amounts of a virus population that may be heterogeneous in terms of susceptibility to ACV (41). These patients usually receive prolonged and repeated doses of ACV therapy (4, 45), which in the laboratory is a method of generating ACV-resistant mutants (19, 23). Since immunocompromised patients cannot depend on their immune system to eliminate ACV-resistant viruses, locally severe herpetic disease can result (3, 4, 45). The emergence of ACV-resistant isolates in immunocompromised patients appears to correlate primarily with treatment failures, but the appearance of ACV-resistant viruses following recurrent disease has been reported (40, 43). Therefore, this patient group is at a greater risk for harboring ACV-resistant virus and for developing significant disease because of it.

Our results demonstrate that an extremely high percentage of ACV-resistant isolates (77 of 100) can be recovered from patients positive for HIV and not responding to ACV therapy. Virus isolates from this HIV-positive patient population that were originally classified as either ACV sensitive or ACV resistant by their ED_{50}s of ACV have TK phenotypes similar to those of ACV-sensitive or ACV-resistant control viruses. These results confirm that the mechanism of ACV resistance for these viruses is due to a defect in the viral TK. This is the most common type of defect seen in ACV-resistant clinical isolates recovered from other patient groups (16).

Coen et al. (8) have described resistance to ACV in terms of the amount of TK expressed and have suggested a cutoff for ACV sensitivity at 40% or above wild-type enzyme levels. Although it has been difficult to extrapolate the TdR-phosphorylating activity of a clinical isolate's TK and the isolate's in vitro ACV susceptibility, our data indicate that ACV-phosphorylating activity may be an accurate indicator of sensitivity to ACV. For example, VLs 10091 and 11680 have roughly equivalent TdR-phosphorylating activities, 46 and 51% of wild-type enzyme activity, respectively. However, there is a more-than-twofold increase (9 to 20%) in ACV-phosphorylating activity of VL 10091 compared with that of VL 11680. Even with this diminished ACV-phosphorylating activity, VL 10091 is ACV sensitive, while VL 11680 is ACV resistant.

When the PFU/MID_{50} ratios of representative isolates are compared with those of control viruses, the results are sufficiently similar to imply that the isolates recovered from patients positive for HIV are of approximately equal pathogenicity in this model. These findings are consistent with previous studies that found that TK^D viruses are in general less virulent than wild type (14, 24).

The neurovirulence PFU/LD_{50} ratios resulting from intracerebral inoculation indicate that differences in the pathogenicities of viruses with different TK phenotypes are greater in this model than in models of skin infection. The TK^D isolates from patients positive for HIV are less pathogenic than TK^+ isolates, which agrees with previous reports with other patient populations (45). However, there is a recent report of the recovery of a neurovirulent TK^D isolate from a patient with AIDS (6). Since the PFU/LD_{50} ratios compare favorably with the neurovirulence values generated by our control viruses and with previously reported isolates, we conclude that isolates from patients positive for HIV are not more neuropathogenic than previously reported clinical isolates (18, 24, 45).

Although all three TK phenotypes can establish latency, TK^D viruses cannot reactivate and cause recurrent disease (9, 49). The amount of TK needed for reactivation by a laboratory-generated TK mutant has been reported to be 10% of wild-type enzyme (8). In the clinic, ACV-resistant viruses have been isolated from patients with recurrent disease (40, 43), and when the TK was analyzed, the virus was TK^A (40). Our latency data also indicate that ACV-resistant virus can reactivate, but these viruses appear to require a TK that can phosphorylate thymidine. While only rarely have ACV-resistant TK^A viruses been recovered from patients (18, 20), the situation in this patient population may necessitate greater vigilance.

This report, which describes 100 HSV isolates recovered from 51 patients positive for HIV, suggests that the emergence of ACV-resistant viruses, in this population, is the result of mechanisms similar to those that have been described for ACV-resistant viruses recovered from other patient groups. Although severe disease can result from ACV-resistant virus in this patient population, such severe disease is presumably the result of the underlying immunodeficiency of the patient and not due to the emergence of mutant HSV strains with enhanced virulence.

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