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Deletion of the herpes simplex virus type 1 ribonucleotide reductase gene alters virulence and latency in vivo

Arit D. Idowu, Elizabeth B. Fraser-Smith, Kimber L. Poffenberger* and Ronald C. Herman

Syntex Research, Palo Alto, California, U.S.A.

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Summary

The role of herpes simplex virus type 1 (HSV-1)-encoded ribonucleotide reductase (RR) has been investigated in mice and guinea pigs using a mutant from which 90% of the large subunit of the enzyme was deleted. The RR mutant was extremely impaired in its ability to induce external vaginal lesions or to cause death in mice following intracerebral, intraperitoneal, or intravaginal inoculation, or in guinea pigs following intraperitoneal or intravaginal inoculation. The RR mutant replicated poorly in the vagina of mice and guinea pigs when compared with the parental virus. Neither infectious nor latent virus was recovered from the trigeminal ganglia of mice or from the dorsal root ganglia of mice and guinea pigs after inoculation with the RR mutant. Using the polymerase chain reaction, RR mutant DNA was, nevertheless, detected in the dorsal root ganglia of guinea pigs. These studies suggest that HSV-1 RR is essential for virulence and may also play a role in the recovery of reactivatable latent virus from ganglia in both mice and guinea pigs.

Herpes simplex virus type 1; Virulence; Latency; Ribonucleotide reductase

**Present address*: Retrovirology Laboratory, Division of Transfusion Sciences, Food and Drug Administration, Bethesda, MD 20892, U.S.A.

Correspondence to: Arit Idowu, 3401 Hillview Avenue, Palo Alto, CA 94304, U.S.A.

Introduction

Herpes simplex viruses synthesize many enzymes upon infection of mammalian cells. These include DNA polymerase (Keir and Gold, 1963), deoxyribonuclease (Keir and Gold, 1963), thymidine kinase (Kit and Dubbs, 1963), DNA uracil N-glycosylase (Caradonna et al., 1987), and ribonucleotide reductase (Cameron et al., 1988; Dutia, 1983). The biochemical properties of these virus-specified enzymes are distinctly different from those of their mammalian counterparts (Averett et al., 1983; Cheng et al., 1981; Ponce de Leon et al., 1977; Spector and Jones, 1985), thus providing a basis for the development of selective antiviral compounds.

Ribonucleotide reductase (RR) catalyzes a unique step in DNA biosynthesis by directly reducing the ribonucleoside diphosphates to their corresponding deoxyribonucleoside diphosphates (Thelander and Reichard, 1979). The herpes simplex virus type 1 (HSV-1) RR enzyme consists of two nonidentical subunits: the large subunit, designated ICP6, has a molecular weight of 140 000, and the small subunit has a molecular weight of 38 000 (Frame et al., 1985; Goldstein and Weller, 1988b). Modifications of the HSV-1 RR gene to produce mutants partially or completely lacking this enzyme may provide a basis for understanding the role that RR plays in virulence, pathogenicity and latency, and thereby provide a strategy for the design of novel antiviral compounds. Recently, an HSV-1 strain KOS RR mutant (HSV-1 ICP6Δ) was constructed (Goldstein and Weller, 1988a) and tested in both mouse and guinea pig infection models. In one study, the HSV-1 ICP6Δ was severely impaired in its ability to grow during acute infection of the eye and trigeminal ganglia following corneal inoculation in mice (Jacobson et al., 1989). However, in another study, HSV-1 ICP6Δ induced cutaneous lesions in guinea pigs which were comparable to those induced by the parental strain after intradermal inoculation (Turk et al., 1989). These studies suggest that, although HSV-1 RR is important for both lytic and latent infections in mice, the enzyme may not be essential for viral replication in other species such as the guinea pig.

The present paper describes studies using the same RR mutant in different animal infection models. We performed intracerebral (i.c.), intraperitoneal (i.p.), and intravaginal (i.vag.) infections in mice as well as i.p. and i.vag. infections in guinea pigs. These results show that the RR mutant has reduced infectivity and replicates less efficiently than the parental virus in both animal species. We also examined dorsal root ganglia in both mice and guinea pigs and trigeminal ganglia in mice. These studies suggest that RR may be important for recovery of reactivatable latent virus from nerve ganglia in vivo.

Materials and Methods

Viruses and cells

HSV-1 ICP6Δ is a mutant virus with a deletion from the *Xho*I site at map coordinate 0.564 to the *Bgl*II site at coordinate 0.585 within the large subunit of the HSV-1 KOS RR gene (Goldstein and Weller, 1988a,b). HSV-1 KOS served as the parental virus. All in vitro experiments were performed using D14 cells, a transformed African green monkey kidney cell line derived by insertion of the *Hpa*1 F fragment of HSV DNA, that express the RR large subunit (ICP6) upon HSV infection (Goldstein and Weller, 1988a). Cells were maintained and passaged in Dulbecco's modified Eagle's medium (DMEM, J.R. Scientific, Woodland, CA) containing 5% fetal bovine serum (J.R. Scientific), and 250 μg of G418 per ml (geneticin, Gibco Laboratories, Grand Island, N.Y.). Viruses and D14 cells were obtained from S.K. Weller, University of Connecticut Health Center, Farmington, CT.

Animals

Female Swiss Webster mice, Crl:CFW® (SW)BR and BK1:(SW) (Charles River Laboratories, Wilmington, MA, and Bantin and Kingman, Fremont, CA), were used for i.c., i.p., and i.vag. infections. Female Balb/c BK1 mice (Bantin and Kingman) were used for the corneal infection model. Female Hartley guinea pigs, Crl:1AF(HA)BR (Charles River), were used for i.vag. and i.p. infections. Experimental groups contained 5 to 20 animals. When required, anesthesia was produced by i.p. injections of 50 mg/kg of nembutal sodium solution (Abbott Labs, Chicago, IL).

Intracerebral and systemic infection models

Mice of 13–15-g weight range (3–4 week-old) were injected in the right cerebral hemisphere under anesthesia with 0.020 ml of serial dilutions of viral suspension, starting at titers of 1.7×10^3 and 4.4×10^6 pfu/mouse for HSV-1 KOS and HSV-1 ICP6Δ, respectively. In addition, mice of 19 to 21 g weight range (5–6 week-old) were inoculated i.p. with 0.5 ml of serial dilutions of viral suspension starting at a titer of 4.3×10^8 and 1.1×10^8 pfu/mouse for HSV-1 KOS and HSV-1 ICP6Δ, respectively. Guinea pigs of 150–200 g weight range (10 day-old) were inoculated i.p. with 0.5 ml of viral suspension at a titer of 3.6×10^8 pfu/guinea pig for both viral strains. Deaths were recorded for 21 days after inoculation, at which time all surviving animals were healthy.

Vaginal infection models

Mice of 19–21-g weight range (5–6 week-old) and guinea pigs of 180–230 gm or 300–350-g weight range (15 day- or 5–6 week-old, respectively) were

inoculated by swabbing the vagina first with 0.1 N NaOH, then dry swabbing, and finally swabbing with a viral suspension at a titer of 8.6×10^8 and 1.1×10^9 pfu/ml for HSV-1 KOS and HSV-1 ICP6Δ, respectively. The severity of the vaginal herpetic lesions was scored on a scale of 0 to 4 from day 4 through 16 after infection.

To assay for the presence of infectious virus in the ganglia, 3–4 dorsal root ganglia from each animal were removed on day 3 after viral inoculation, homogenized and plated on D14 cells. The presence of reactivatable latent virus in the dorsal root ganglia was assayed on day 30 after viral inoculation, at which time all of the surviving animals had cleared the primary infection. Three to four dorsal root ganglia from each animal were explanted into DMEM [containing 5% fetal bovine serum, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of Fungizone (Gibco Labs) per ml] for 5 days at 37°C and 5% CO_2 before homogenizing and plating on D14 cells. Cytopathic effects (CPE) were recorded for 2 to 3 weeks. Cultures with no CPE were freeze-thawed and the resulting suspensions were seeded on fresh D14 cells. CPE were recorded for another 2 to 3 weeks. Controls included ganglia from mock-inoculated mice as well as D14 cells without ganglia.

To assess viral shedding, vaginal secretions were obtained on days 1, 3, 5, 7, and 9 after infection by swabbing with a sterile dacron-tip applicator (Dacroswab, Spectrum Labs, Los Angeles, CA). The applicators were placed into 1.0 ml DMEM supplemented with 2% fetal bovine serum, 100 μg of gentamicin per ml (Gibco Labs), and 0.25 μg of Fungizone per ml. Tubes were capped, mixed thoroughly and stored at -70°C prior to titration of virus.

Plaque titration assay

Virus in vaginal secretions was titrated on confluent monolayers of D14 cells in 12-well plates. Ten-fold serial dilutions in DMEM of each vaginal sample were adsorbed with rocking for 1 1/4 h. After adsorption, the medium was aspirated and an overlay consisting of DMEM, 2% fetal bovine serum, and 0.6% methylcellulose (Sigma Chemicals, St. Louis, MO) was applied. Two wells were used per dilution. After 4 days of incubation at 37°C in 5% CO_2 the overlay was removed, and the cells fixed with methanol for 10 min and stained with 0.05% methylene blue. Plaques were counted at 17 × magnification with a plaque viewer (Bellco Glass, Vineland, NJ).

Bilateral corneal infection model

Mice of 16–18-g weight range (5–6 week-old) were anesthetized before scarifying the corneas with three superficial strokes of a 30 1/2 g hypodermic needle. The scarified eyes were then bathed with 0.020 ml of viral suspension with starting titers of 5.4×10^5 and 1.4×10^7 pfu/eye for HSV-1 KOS and HSV-1 ICP6Δ, respectively. The development of keratitis was monitored daily. Trigeminal ganglia were removed on days 3 and 30 after viral inoculation for

assay of infectious virus and latent virus, respectively, using the procedure described above for the dorsal root ganglia.

Statistical analyses

Differences in the number of survivors or the number of animals with virus were evaluated with the Fisher exact probability two-tailed test (Maxwell, 1961). The Mann-Whitney U test (Hollander and Wolfe, 1973) was used to compare mean lesion scores. Differences in vaginal viral titers were analyzed with a standard *t*-test. Probit analyses (Finney, 1964) were used to calculate the lethal challenge of virus at which 50% of the animals survived (LC_{50}).

Polymerase chain reaction

Dorsal root ganglia were removed at 30 days after infection from mock-, ICP6Δ-, and KOS-infected animals; 2 ganglia from each animal, 5 animals per group, were pooled for these analyses. The ganglia were solubilized overnight in 20 mM Tris-HCl, pH 7.4, 20 mM EDTA, pH 8.0, 0.5% SDS containing proteinase K (100 μg/ml) at 50°C. The DNA was extracted with phenol/$CHCl_3$ and precipitated with ethanol.

Polymerase chain reactions (PCR) were performed using a GeneAmp kit (Perkin Elmer Cetus) under the conditions described by the supplier. Ganglia DNA was boiled for 7 min prior to addition to each tube (amounts indicated in the text) and then amplified for a total of 30 cycles: 94°C for 1 min; 55°C for 2 min; 72°C for 3 min, with the exception that the 72°C step was increased to 7 min during the last cycle. Purified HSV-1 DNA (1 ng) was amplified in parallel as a positive control. Oligonucleotide primer pairs and probes were specific for either the HSV-1 DNA polymerase (pol) or ICP6 gene. The ICP6-specific primers were designed to amplify a region of the genome that had been deleted from the ICP6Δ mutant.

Portions (20-μl) of each reaction mixture were electrophoresed in 2% agarose in Tris/acetate buffer containing ethidium bromide for approximately 2–3 h at 24 V. The gels were soaked for 1 h in 0.5 N NaOH, 1.5 M NaCl, rinsed with dH_2O, and then soaked for 1 h in 0.5 M Tris-HCl, pH 8.0, 1.5 M NaCl.

The gels were blotted onto Duralose (Stratagene) overnight; the filters were dried at room temperature for 30 min and then baked at 80°C for 2 h under vacuum. Prehybridization was in 6 × SSC, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin, 0.04% Ficoll 400, 0.1% SDS, 100 μg of salmon sperm DNA per ml at 37°C for 16 h. Hybridizations were performed at 37°C for 16 h in the same buffer containing the specific probe ($\sim 1 \times 10^6$ cpm/ml), which had been labeled using [γ-^{32}P]ATP. The filters were washed in 2 × SSC, 0.1% SDS at room temperature for 1 h, and then in 6 × SSC, 0.1% SDS at 56°C for 20 min prior to autoradiography.

Results

Virulence

When given i.c., HSV-1 ICP6Δ was 10 000-fold less neurovirulent than the parental virus. The LC_{50} was 2.8×10^6 pfu/mouse for HSV-1 ICP6Δ and 3.7×10^2 pfu/mouse for HSV-1 KOS (20 mice/group, data not shown). Both viruses demonstrated a dose-dependent increase in virulence. The times of death for mice in both groups were comparable, with the range falling between days 3–10 after viral inoculation.

When given i.p., HSV-1 ICP6Δ failed to cause death in either mice or young guinea pigs even at inoculation titers of 1.1×10^8 or 3.6×10^8 pfu per mouse or guinea pig, respectively (20 animals/group; data not shown). On the other hand, HSV-1 KOS demonstrated a dose-dependent increase in virulence with an LC_{50} of 2.6×10^7 pfu/mouse. The times of death ranged from days 6–13 after viral inoculation. HSV-1 KOS also caused death in 8 of 20 (40%) young guinea pigs inoculated i.p. with 3.6×10^8 pfu/animal. The times of death ranged from days 2–3 after viral inoculation.

When given i.vag., HSV-1 ICP6Δ produced vaginal lesions in only 1 of 20 mice (5%), compared to 16 of 20 mice (80%) challenged with HSV-1 KOS (Table 1). HSV-1 ICP6Δ induced vaginal lesions that lasted less than two days and all the animals survived the infection.

Conversely, HSV-1 KOS was invasive, caused 15% mortality (times of death were from days 9–16 after viral inoculation) and the vaginal lesions lasted 2 to 12 days. Neither HSV-1 ICP6Δ nor HSV-1 KOS produced any observable external vaginal lesions in adult guinea pigs (5–6 week-old). However, when given to young guinea pigs (15 day-old) HSV-1 KOS produced lesions in 14 of 19 (74%) animals compared to 0 of 18 (0%) animals inoculated with HSV-1 ICP6Δ (Table 1).

TABLE 1

Lesion score for intravaginal inoculation with HSV-1 ICP6Δ or HSV-1 KOS

Species	Virus	Challenge (pfu/ml)	Overall mean lesion[a] score	Lesion duration (days) mean (range)	Animals with lesions/ total (*n*)	Percent with lesions
Mouse	HSV-1 ICP6Δ	1.1×10^9	0.1[b]	1 (<2)	1/20[c]	5
	HSV-1 KOS	8.6×10^8	1.8	7 (2–12)	16/20	80
Guinea pig[d]	HSV-1 ICP6Δ	1.1×10^9	0.0[b]	0	0/18	0
	HSV-1 KOS	8.6×10^8	0.8	5 (1–14)	14/19	74

[a]Lesions were scored on a scale of 0 to 4 with 0 = no lesions, 1 = one lesion, 2 = 2 to 4 lesions, 3 = 4 or more lesions (with lesions extending halfway to anus in mice), 4 = ulcerating lesions (with extension down to anus in mice).

[b]$P < 0.05$ compared to HSV-1 KOS (Mann Whitney U).

[c]$P < 0.05$ compared to HSV-1 KOS (Fisher exact).

[d]15 days old.

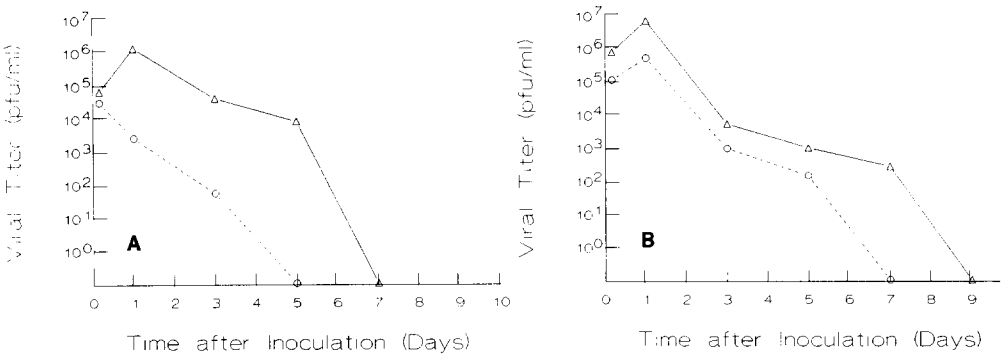

Fig. 1. Viral shedding by mice (A) or guinea pigs (B) inoculated intravaginally with HSV-1 ICP6Δ or HSV-1 KOS (n = 20). Animals were inoculated by swabbing with a viral suspension at a titer of 1.1×10^9 and 8.6×10^8 pfu/ml for HSV-1 ICP6Δ and HSV-1 KOS, respectively. ○ = HSV-1 ICP6Δ; △ = HSV-1 KOS.

After i.vag. inoculation, HSV-1 ICP6Δ was shed for 5 days in mice and for 7 days in guinea pigs whereas HSV-1 KOS was shed for 2 days longer in both species (Fig. 1). There was no net increase in the vaginal viral titer of HSV-1 ICP6Δ in mice and only a small increase in guinea pigs (mean peak titer 5×10^5 pfu/ml) following i.vag. inoculation. In contrast, the parental HSV-1 KOS replicated more efficiently in both mice and guinea pigs (mean peak titers 1.3×10^6 and 6.8×10^6 pfu/ml; respectively).

In addition, fewer animals shed HSV-1 ICP6Δ compared to HSV-1 KOS. On day 3 after viral inoculation, virus was detected in the vaginal secretions of only 8 of 20 (40%) mice and 10 of 15 (67%) guinea pigs inoculated with HSV-1 ICP6Δ compared to 17 of 20 (85%) mice and 14 of 14 (100%) guinea pigs inoculated with HSV-1 KOS (data not shown). On day 5, HSV-ICP6Δ was detected in only 1 mouse of 20 (5%) and 4 of 15 (27%) guinea pigs, compared to 17 of 20 (85%) mice and 14 of 14 (100%) guinea pigs inoculated with HSV-1 KOS. By day 7 none of the mice or guinea pigs inoculated with HSV-1 ICP6Δ and none of the mice inoculated with HSV-1 KOS were positive for virus. On the other hand, 5 of 15 (33%) guinea pigs inoculated with HSV-1 KOS were still shedding virus.

Latency

Despite a high inoculation titer of 10^7 pfu/eye, no detectable virus was recovered from the trigeminal ganglia of mice at 3 or 30 days after inoculation with HSV-1 ICP6Δ (Table 2). In contrast, infectious virus was recovered from the ganglia of 5 of 5 mice (100%) when removed and homogenized on day 3, and from ganglia of 20 of 20 mice (100%) when explanted on day 30 after inoculation with 26-fold fewer pfu of HSV-1 KOS per eye.

Dorsal root ganglia removed from mice and guinea pigs 3 and 30 days after

TABLE 2

Recovery of HSV-1 ICP6Δ or HSV-1 KOS from mouse trigeminal ganglia

Virus	Challenge (pfu/eye)	No. mice with virus/total (%) on day 3	No. mice with virus/total (%) on day 30
HSV-1 ICP6Δ	1.4×10^7	0/5 (0)	0/20 (0)[a]
HSV-1 KOS	5.4×10^5	5/5 (100)	20/20 (100)
	5.4×10^4	ND	18/20 (90)
	5.4×10^3	ND	17/20 (85)

[a] $P < 0.05$ compared to all challenge levels of HSV-1 KOS (Fisher exact). ND = not done.

TABLE 3

Recovery of HSV-1 ICP6Δ or HSV-1 KOS from dorsal root ganglia

Species	Virus	Challenge (pfu/ml)	No. animals with virus/total (%) on day 3	No. animals with virus/total (%) on day 30
Mouse	HSV-1 ICP6Δ	1.1×10^9	0/5 (0)	0/20 (0)[a]
	HSV-1 KOS	8.6×10^8	5/5 (100)	17/17 (100)
Guinea pig	HSV-1 ICP6Δ	1.1×10^9	0/5 (0)	0/20 (0)[a]
	HSV-1 KOS	8.6×10^8	3/5 (60)	18/18 (100)

[a] $P < 0.05$ compared to HSV-1 KOS (Fisher exact).

inoculation with HSV-1 ICP6Δ also failed to yield virus (upon homogenization and explantation, respectively) in any of the animals tested (Table 3). On the other hand, both infectious and reactivatable latent viruses were readily recovered from dorsal root ganglia of both mice and guinea pigs that were removed on days 3 and 30 following inoculation with HSV-1 KOS.

The results presented above indicate that latent HSV-1 ICP6Δ was not recovered from the dorsal root ganglia of guinea pigs at 30 days after inoculation even though the parental KOS strain could be recovered. It was therefore necessary to determine if the ICP6Δ DNA had reached the ganglia using the polymerase chain reaction (PCR).

Viral sequences were amplified by PCR with a primer set specific for the HSV-1 DNA pol gene. A unique pol-specific product was detected by Southern blot hybridization when 2 μg of DNA ($\sim 6 \times 10^5$ cell equivalents) were amplified after extraction from the ganglia of guinea pigs infected with the parental KOS strain. The PCR product comigrated with the product produced when 1 ng of purified HSV-1 DNA was amplified. No products that hybridized to the pol-specific probe were detected when 2 μg of ganglia DNA from either ICP6Δ- or mock-infected animals were amplified in parallel reactions (not shown).

Positive hybridization signals were obtained for both the ICP6Δ-infected and KOS-infected samples when 10 μg of ganglia DNA ($\sim 3 \times 10^6$ cell equivalents) were amplified (Fig. 2 left, lanes C and D, respectively); no signal was detected

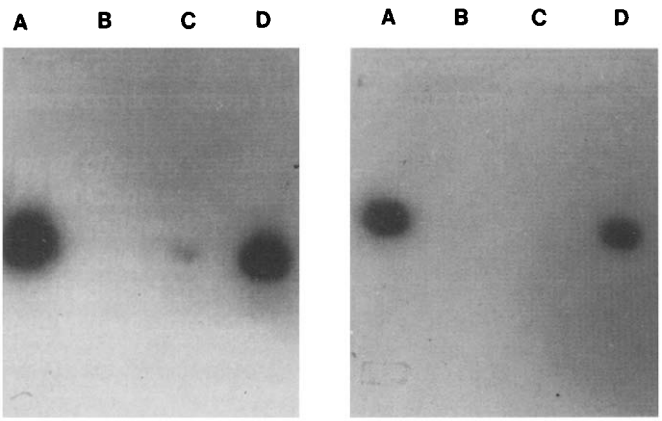

Fig. 2. Southern blot analyses of PCR products. Portions of the PCR reactions (20 μl) were electrophoresed in a 2% agarose gel, blotted onto Duralose, and then probed as outlined in Materials and Methods. The samples were separated from each other by two empty lanes to avoid cross-contamination. The X-ray films were exposed for 1–2 h at room temperature. Left, pol-specific primers and probe; right, ICP6-specific primers and probe. A, purified HSV-1 DNA; B, mock-infected ganglia DNA; C, ICP6Δ-infected ganglia DNA; D, KOS-infected ganglia DNA.

when 10 μg of mock-infected ganglia DNA were amplified even after significant overexposure of the autoradiogram (Fig. 2 left, lane B, and data not shown). Using the autoradiogram as a template, the bands were excised from the filter and counted in a liquid scintillation counter. The results indicated that there was approximately 45 times more radioactivity hybridized to the product of the KOS-infected ganglia DNA than to the product of the ICP6Δ-infected ganglia.

Using a PCR primer set and probe that were specific for the ICP6 gene, a positive signal was detected by Southern blotting when ganglia DNA (10 μg) from KOS-infected animals was amplified (Fig. 2 right, lane D); this product comigrated with that produced when purified HSV-1 DNA (1 ng) was amplified in a parallel reaction (Fig. 2 right, lane A). No PCR products were detected by the ICP6-specific probe when 10 μg of ganglia DNA from either mock-infected or ICP6Δ-infected animals were amplified (Fig. 2 right, lanes B and C, respectively). These results confirmed that viral sequences could be detected in the ganglia of animals infected with the mutant virus and that this DNA retained the original deletion in the ICP6 gene.

Discussion

The studies presented here show that a deletion in the large subunit of the RR gene of HSV-1 KOS greatly reduces the virulence of the virus in mice. Following i.c. inoculation, the neurovirulence of HSV-1 ICP6Δ was reduced by 4 logs when compared to the parental virus, HSV-1 KOS. Following i.p. inoculation, HSV-1 ICP6Δ failed to cause death even at 10^8 pfu per mouse.

These data correlate well with the results obtained by Cameron et al. (1988) showing that mutations in either the large or small subunit of the HSV-1 RR reduced virulence 10^6-fold in mice challenged either i.c. or i.p. when compared to the parental HSV-1 strain 17.

The ability to produce vaginal lesions in mice was also severely compromised by the deletion in the ICP6 gene. Only one animal inoculated i.vag. with HSV-1 ICP6Δ had lesions, whereas the majority of animals inoculated with the parental virus had lesions. Furthermore, the titer of HSV-1 ICP6Δ did not increase in the mouse vagina following i.vag. inoculation. The peak vaginal titer was >500-fold lower than the peak titer of the parental virus.

In addition to showing effects on neurologic, systemic, and vaginal virulence in mice, the present studies also indicate that RR may have a role in the recovery of virus from both the trigeminal and dorsal root ganglia in mice. Following corneal or i.vag. inoculation with HSV-1 ICP6Δ, neither infectious nor latent virus was detected in either the trigeminal or dorsal root ganglia. In contrast, infectious and reactivatable latent virus was readily recovered from the ganglia of all mice inoculated with the parental strain. These results are similar to those reported by Jacobson et al. (1989) for mouse trigeminal ganglia using the same RR deletion mutant.

Recent publications have indicated that HSV-1 ICP6Δ grows poorly in murine cells relative to its growth in guinea pig, simian, or human cells (Jacobson et al., 1989; Turk et al., 1989). Consequently, it has been suggested that the ability of HSV-1 ICP6Δ to replicate, induce lesions, or colonize ganglia may be host species-dependent, and thus, the mouse might not be an appropriate animal model for evaluating the role of RR in virulence and latency. Since HSV-1 ICP6Δ grows well in guinea pig cells (Turk et al., 1989), we evaluated the ability of the RR mutant to replicate and to establish latency using guinea pig vaginal and systemic infection models. Compared with the parental virus, the peak vaginal titer of HSV-1 ICP6Δ was reduced >10-fold; fewer of the adult guinea pigs shed HSV-ICP6Δ virus with time, and viral shedding stopped earlier. Furthermore, unlike the parental strain, HSV-1 ICP6Δ failed to produce either vaginal lesions or death in young guinea pigs when given either i.vag. or i.p., respectively. These data suggest that HSV-1 ICP6Δ has significantly reduced infectivity, virulence, and rate of replication in guinea pigs as well as in mice. Thus, adequate correlations between in vitro and in vivo results using cells from the same animal species may not be possible.

The present results are in contrast to a recent report by Turk et al. (1989) showing that HSV-1 ICP6Δ induces cutaneous lesions in guinea pigs that are comparable to those caused by the parental strain. Perhaps the ability of the RR mutant to replicate and cause disease in the guinea pig is a function of the host tissue type rather than a function of host species per se. Rapidly dividing cells such as those of the skin, may have a sufficiently large pool of deoxyribonucleosides to compensate for the loss of the viral RR, whereas other cell types may be unable to supply ample amounts of these essential precursors. Perhaps quantitative analyses of deoxyribonucleoside pool sizes in cells from

the different tissues would help to clarify this point.

Our PCR analyses demonstrate that a small amount of viral DNA reached the dorsal root ganglia of guinea pigs inoculated with HSV-1 ICP6Δ even though reactivatable latent virus could not be recovered. The use of primers and probes specific for the pol and ICP6 genes confirmed that the viral sequences detected in these ganglia retained the original deletion within ICP6. This point is significant because the HSV-1 ICP6Δ stock was propagated in the D14 helper cell line. D14 cells carry a functional copy of the HSV-1 ICP6 gene (Goldstein and Weller, 1988a), and the possibility that recombination could restore this gene to some of the progeny virions had not been previously ruled out.The PCR results recently published by Katz et al. (1990) for infection of mouse trigeminal ganglia by this ICP6Δ mutant and its parent are consistent with those presented here. Unfortunately, Katz et al. also grew the HSV-1 ICP6Δ stock in D14 helper cells but used only a PCR primer set and probe specific for the HSV-1 thymidine kinase gene to detect viral sequences in ganglia DNA. They are therefore unable to confirm that the DNA detected in the mouse ganglia retained the original ICP6 deletion.

It has recently been suggested that an origin of viral DNA replication lies within the region of the ribonucleotide reductase gene that is deleted from HSV-1 ICP6Δ. This origin can apparently be amplified by host cell factors during transient transfection assays in the absence of all viral gene products (Sears and Roizman, 1990). No evidence, however, exists at the present time to indicate that viral DNA sequences are amplified in neurons from such a site either during latency or reactivation. Although the loss of an origin could potentially affect the ability to recover latent HSV-1 ICP6Δ from ganglia, it is unlikely that deletion of this sequence alone can account for the significant reduction of virulence and pathogenicity of the ICP6Δ mutant.

In summary, the HSV-1 RR gene appears to be necessary for virulence and may also play an important role in the recovery of reactivatable latent virus from the ganglia of both guinea pigs and mice. These data suggest that ribonucleotide reductase is a valid target for antiviral chemotherapy.

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