Antibody Response, Recurrence Patterns and Subsequent Herpes Simplex Virus Type 2 (HSV-2) Re-infection Following Initial HSV-2 Infection of Guinea-pigs: Effects of Acyclovir

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SUMMARY

The production of antibody to specific herpes simplex virus type 2 (HSV-2) polypeptides, the recurrence patterns and the susceptibility to re-infection were studied in the guinea-pig model of genital HSV-2 infection. Further, we defined the effects of acyclovir (ACV) therapy on these parameters of infection. Treatment with ACV reduced the clinical severity of the initial disease but did not affect vaginal viral shedding. Production of neutralizing antibody as well as antibody to the nucleocapsid protein, and glycoproteins B, D and G were all delayed in ACV recipients. ACV treatment of initial infection did not significantly alter the pattern of subsequent recurrence although by several criteria treated animals tended towards decreased recurrences. Re-inoculation with a second strain of HSV-2 resulted in a local cervicovaginal infection but, except for one ACV-treated animal, neural tissue was protected from re-infection.

INTRODUCTION

Although much work has been done to define the serological response to herpetic infection in both man and experimental animals, the temporal response to the individual polypeptides of herpes simplex virus (HSV) is still not well defined. Investigators employing immune precipitation of radiolabelled HSV polypeptides and SDS–polyacrylamide gel electrophoresis have observed a major response to the glycoproteins of HSV (Zweerink & Corey, 1982; Gilman et al., 1981; Ashley & Corey, 1984), an expected finding since these surface glycoproteins appear to represent the immunologically most important viral antigens. More recently, we (Bernstein et al., 1984a) and others (Eberle et al., 1984) have reported on the immune response to lower molecular weight non-glycosylated proteins [ranging from mol. wt. 40000 (40K) to 49K]. We (Bernstein et al., 1984a) and others (Ashley & Corey, 1984) have also reported that oral acyclovir (ACV) therapy of initial genital herpes alters the antibody response to HSV polypeptides. These reports also noted that patients treated with ACV developed earlier and more severe recurrences than placebo-treated patients. Thus, although ACV has been shown to reduce the severity of primary genital HSV infections (Bryson et al., 1983a; Mertz et al., 1984), some concern has been expressed regarding the consequences of the altered immune response with regard to recurrences and re-infections (Bryson, 1983).

We therefore initiated an evaluation of the effect of oral ACV on the temporal antibody response to HSV type 2 (HSV-2) and its relationship to recurrent disease and the susceptibility to re-infection. Because patients are infected with different virus strains of various inoculum sizes, and may be re-infected with the same or differing strains at uncertain and unpredictable intervals, human clinical trials to address these issues are difficult to interpret. For these
reasons, we undertook to investigate the effects of oral ACV in an animal model of HSV infection. Genital HSV infection of the guinea-pig was selected because of the similarities of the natural history of infection to that which occurs in the human and because of the accuracy by which this model predicted the efficacy of oral ACV for initial (Kern, 1982; Pronovost et al., 1982) and recurrent (Kern, 1984) genital disease in the human.

METHODS

Cells and viruses. First- to third-passage rabbit kidney (RK) cells were prepared from the kidneys of 3-week-old pasteurella-free female New Zealand white rabbits (Hazleton-Dutchland, Denver, Pa., U.S.A.) and maintained with Eagle's basal medium containing heat-inactivated (56 °C, 30 min) 10% foetal bovine serum, penicillin (100 units/ml), streptomycin (50 μg/ml) and L-glutamine (2 mm). Rabbit skin, Vero and HEp-2 cells were maintained similarly. Clarified pools of the MS (ATCC VR-540) and 333 (Stanberry et al., 1985) strains of HSV-2 were prepared in RK cells and stored frozen at -70 °C.

Animals. Female Hartley guinea-pigs (Charles River Breeding Laboratories, Wilmington, Mass., U.S.A. weighing approximately 200 g each were used. As previously described (Stanberry et al., 1982), their vaginal closure membrane was ruptured and the vaginal vault swabbed with a pre-moistened calcium alginate-tipped swab (Spectrum Diagnostics, Glenwood, Ill., U.S.A.). One h later the animals were inoculated with 10^5.7 p.f.u. MS strain HSV-2 instilled into the vaginal vault by use of a syringe and 20-gauge plastic catheter. Animals with vaginal viral replication for at least 24 h were considered infected. A similar procedure was used for re-inoculating 10^7.8 p.f.u. strain HSV-2.

Clinical illness scores. Animals were examined daily for external genital skin lesions as previously described (Stanberry et al., 1982). Mean lesion scores are the average of the total of these daily scores over the specified time. Clinical recurrences are reported as the total number of days with any lesion (lesion days), lesion score or number of recurrences (a separate recurrence was scored when a new lesion appeared). Asymptomatic recurrences occurred when vaginal viral shedding was documented in the absence of apparent clinical lesions.

Virus isolation. Cervicovaginal viral cultures were obtained daily employing a pre-moistened calcium alginate-tipped swab (Stanberry et al., 1982). Cultures obtained during the first 10 days after infection or re-infection were frozen at -70 °C, until quantified by plaque assay in RK cells employing a 1% methylcellulose overlay. Virus cultures obtained on all other days were inoculated directly onto RK cells for qualitative virus determination. At sacrifice, supernatants from 10⁻¹ (w/v) tissue homogenates and minced tissue fragments of lumbosacral dorsal root ganglia, spinal cord, and samples of cervix and genital skin were inoculated onto RK cell monolayers (Stanberry et al., 1982, 1985). Productive infection was defined by the detection of HSV by culture of vaginal swabs or cell-free tissue homogenates, while latent infection was defined by recovery of virus only by explant co-cultivation. Cervicovaginal swab and tissue homogenate cultures were observed for 10 days whereas explant cultures were maintained for 6 weeks.

Experimental design (Fig. 1). Eighteen animals were randomly assigned to receive drinking water either with or without 5 mg/ml ACV (kindly provided by Burroughs Wellcome, Research Triangle Park, N.C., U.S.A.) supplied ad libitum. ACV was administered for 21 days beginning 12 h after inoculation. The drinking water was changed daily. Sera were collected by intracardiac puncture prior to infection and on days 14, 21, 28 and 60 following inoculation. On day 31, 11 ACV and eight control animals were re-inoculated with 10^7.8 p.f.u. of a different strain of HSV-2 (333). Four re-inoculated ACV and three re-inoculated control animals were sacrificed between days 34 and 37, i.e. 3 to 6 days after re-inoculation. The remaining animals not re-infected (five ACV and five control) and those re-infected but not sacrificed (six ACV and five control) were followed for recurrent disease and asymptomatic viral shedding until day 61 when all animals were sacrificed.

ACV assays. The ACV sensitivity of the parent virus and isolates obtained during the last week of ACV therapy was measured by a standard plaque reduction method (Collins et al., 1982) in RK cells. Drug levels in plasma were determined by Dr P. deMiranda, Burroughs Wellcome, employing a radioimmunoassay (Quinn et al., 1979).

Plaque neutralization test. Neutralization titres were measured by a plaque reduction assay. Briefly, serial twofold dilutions of heat-inactivated plasma (56 °C, 30 min) were incubated for 1 h at 37 °C with approx. 100 p.f.u. of MS strain HSV-2. Virus–plasma mixtures were then adsorbed for 1 h at 37 °C on rabbit skin cells and then overlaid with 1.0% agarose. Plaques were counted after a 4 day incubation period. Neutralizing titres are expressed as the reciprocal of the highest dilution producing a 50% reduction in the number of plaques.

Immunoblot assay. The immunoblot procedure was a modification of that previously described (Bernstein et al., 1984a, b). Briefly, semi-confluent HEp-2 cells were infected with HSV-2 strain MS at a multiplicity of infection of 10 p.f.u./cell. After almost complete c.p.e. was noted (24 h) the cells were scraped, washed three times in phosphate-buffered saline and then solubilized in electrophoresis buffer containing 0.0625 M-Tris base pH 6.8, 2% SDS, 10% glycerol and 5% 2-mercaptoethanol (except as noted). After boiling for 3 min, the polypeptides were separated on 6 to 12% (w/v) polyacrylamide gels containing SDS and cross-linked with N,N'-diallyltartardiamide.
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Initial intravaginal inoculation
HSV-2 MS strain

ACV (18)
(treated for 21 days)

Intravaginal re-inoculation (11)
HSV-2, strain 333
Sacrifice (4)
Sacrifice (6)
Sacrifice (5)
Sacrifice (3)
Intravaginal re-inoculation (8)
HSV-2, strain 333
Sacrifice (5)
Sacrifice (5)

Control (18)
Sacrifice (5)

Time after initial inoculation (days)

0 0.5 31 33 37 61

Fig. 1. Experimental design. The numbers of animals concerned are in parentheses. The remaining animals in each group died from either the initial disease or bleeding.

Following electrophoresis, the polypeptides were transferred electrically (3 h at 300 mA) to nitrocellulose paper (0.22 μm, Schleicher & Schüll). The sheet of nitrocellulose was then cut into 6 to 7 mm strips, incubated for 2 h at room temperature in Tris–saline–azide buffer containing 5% (w/v) non-fat dry milk (TSAM) and then incubated overnight with test serum diluted 1:50 in TSAM. To identify glycoproteins D and B, rabbit sera obtained from animals immunized with cloned gB and gD (kindly supplied by Dr R. L. Burke, Chiron, Emeryville, Ca., U.S.A.) were assayed in parallel. To identify gG, mouse monoclonal antibody (obtained from Dr M. Zweig, NCI, Frederick Cancer Research Facility and Dr N. Balachandran, University of Florida) was used. The unbound antibodies were removed by extensive washing in TSAM and then incubated with 125I-labelled Protein A in TSAM for 2 h followed by extensive washing over a 4 h period. Autoradiographic images were then made on Kodak XAR-5 film.

Restriction enzyme analysis. Confluent monolayers of Vero cells, washed twice with phosphate-free Eagle’s MEM supplemented with 2% foetal calf serum, were inoculated with viral isolates at a multiplicity of infection of 1 p.f.u./cell. HSV DNA was labelled with 32P (50 μCi/ml), extracted and prepared for restriction analysis by a modification of methods previously reported (Buchman et al., 1978; Lonsdale, 1979). Restriction endonuclease analysis was performed with BamHI under the conditions specified by the manufacturer after preliminary experiments had determined that this enzyme readily differentiated the strains MS and 333. The digested DNA was electrophoresed in horizontal 0.6% agarose gels for 20 h in a 50 V field. The gels were then dried and exposed to Kodak XAR-5 film for 8 h.

Analysis. Two placebo-treated animals did not become infected and were omitted from subsequent analysis. The unpaired Student’s t-test was used except where indicated.

RESULTS

Acute disease

Initial disease was milder in ACV-treated than in placebo-treated animals (Fig. 2). Of the 16 HSV-infected control animals, 15 developed vesiculoulcerative disease (from days 4 to 13) with a mean total lesion score of 10.5, while 12 of the 18 ACV-treated animals developed vesiculoulcerative disease (from days 4 to 12) with a total mean lesion score of 3.9 (P < 0.001) (Fig. 2). Two control animals developed hind limb paralysis and 10 urinary retention, compared to no and three ACV-treated animals, respectively. Two control animals but no ACV-treated animals died. The course of viral replication in the vagina during the initial period (days 1 to 10) was similar in both ACV-treated and control animals (Fig. 2). The mean ACV level was 1.2 μg/ml (range 0.35 to 3.8 μg/ml) in 31 blood samples obtained from 16 animals from day 2 to day 20 of ACV therapy.
Fig. 2. Effect of oral ACV (5 mg/ml in drinking water) on genital HSV-2 infection in weanling Hartley guinea-pigs. (a) Clinical course of external genital skin disease (mean lesion score ± standard error). (b) Course of vaginal HSV-2 replication as estimated by plaque titration of vaginal swab specimens. The standard error of the mean (not shown) ranged from 0.02 to 0.2. □, ACV-treated; ▲, control.

Antibody response

No response to HSV-2 polypeptides was detected using sera obtained on or before day 7. As shown in Fig. 3, the earliest major response observed on day 14 was to two non-glycosylated proteins of mol. wt. 40K and 43K. Sera from all 12 placebo-treated guinea-pigs examined reacted to these polypeptides, while 14 of 16 ACV-treated animals also had antibody to these polypeptides. These bands were not observed at the expected location when analysed under non-reducing conditions, indicating that these proteins contain intermolecular disulphide bonds.

Sera obtained on day 14 from 10 of 12 placebo-treated animals also reacted to a 148K protein believed to be the major nucleocapsid protein, while antibody to this polypeptide was not detected in the sera of the ACV-treated animals unless the immunoblots were exposed for at least twice the standard time. A reaction to gG (mol. wt. 130K), identified by immunoblotting with monoclonal antibody, was detected at this time in sera from five of 12 placebo-treated animals but not in those from any ACV recipients. The overall decrease in antibody production to these specific polypeptides was also reflected in a decrease in the geometric mean titre (GMT) of neutralizing antibody obtained from ACV-treated animals (2.8) compared to placebo controls (5.8) (P < 0.025).

When sera obtained on day 21 were reacted a general increase in the density and the number of polypeptides recognized was detected (Fig. 4). Sera from placebo-treated animals now also reacted to two other polypeptides of mol. wt. between 40K and 50K as did sera from ACV-treated animals. All sera from placebo-treated animals reacted to the nucleocapsid protein while still only six of 16 sera from ACV-treated animals were reactive. A reaction to gG was also observed in all placebo recipients as compared to seven of 16 ACV recipients. At this time a reaction to polypeptides of mol. wt. 80K and 85K was also noted in nine of 10 placebo-treated animals but in only five of 16 ACV recipients. These proteins have mol. wt. values similar to those of gE and gF. A reaction to gB, identified with polyclonal rabbit serum produced against the cloned polypeptide, was seen in four of 12 placebo recipients but in no ACV recipients.
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Fig. 3. Immunoblots of sera obtained on day 14 from either placebo (lanes 1 to 4) or ACV (lanes 5 to 8) treated guinea-pigs. The reciprocal of the neutralizing titre (NT) for each animal and the geometric mean neutralizing titre (GMT) (and number of animals evaluated) for that group of animals are shown below the respective blots. The GMT is significantly reduced in ACV recipients compared to control animals ($P < 0.025$). The positions of the mol. wt. standards ($\times 10^{-3}$) are indicated on the right.

Again, ACV recipients demonstrated a lower GMT of neutralizing antibody (10.5) compared to the placebo group (29.5) ($P < 0.05$).

On day 28, neutralizing titres were still lower for ACV (18.2) recipients than placebo-treated animals (35.5), although the difference did not achieve statistical significance ($P < 0.1$). Representative examples of the reaction of sera obtained on this day are shown in Fig. 4. All but one serum from ACV-treated animals reacted to the 148K polypeptide and all but three sera, all from ACV-treated animals, reacted to gG. A reaction to gB was seen at this time in all placebo recipients but in only six of 11 ACV recipients. The reaction to polypeptides of mol. wt. between 80K and 85K was seen in all but one placebo-treated animal and in all but three of the ACV recipients. A response to gD was seen in six of 11 placebo and two of 11 ACV recipients.

Recurrence patterns

All surviving animals recovered from the initial infection by day 14. From days 15 to 21, while still receiving drug therapy, similar numbers of control (10 of 14) and ACV recipients (10 of 17) had clinical recurrences (Table 1). However, during this period recurrences were less severe in
Fig. 4. Immunoblots of sera obtained on (a) day 21 and (b) day 28 are shown for placebo groups (lanes 1 to 4) and ACV groups (lanes 5 to 8) with data on neutralization as presented in Fig. 3. The GMT is significantly reduced in ACV recipients compared to control animals ($P < 0.05$) on day 21 only. Lanes gB and gD were reacted with polyclonal sera to the cloned purified proteins. Lane gG was reacted with a mouse monoclonal antibody to the gG. The positions of the mol. wt. standards ($\times 10^{-3}$) are indicated on the right.

Table 1. Pattern of recurrent genital HSV-2 infection in guinea-pigs treated for 21 days with ACV compared to control animals

<table>
<thead>
<tr>
<th>Day Group</th>
<th>No. with recurrence</th>
<th>No. of recurrences†</th>
<th>Lesion score†</th>
<th>Lesion days†</th>
<th>No. shedding</th>
<th>Mean days/animal/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–21 ACV*</td>
<td>17</td>
<td>10</td>
<td>0-9</td>
<td>1-2§</td>
<td>1-6</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>10</td>
<td>1-4</td>
<td>2-6§</td>
<td>2-6</td>
<td>2</td>
</tr>
<tr>
<td>22–28 ACV</td>
<td>17</td>
<td>13</td>
<td>1-0</td>
<td>1-3</td>
<td>1-6</td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>9</td>
<td>1-2</td>
<td>1-6</td>
<td>1-9</td>
<td>4</td>
</tr>
<tr>
<td>22–60 ACV</td>
<td>11</td>
<td>11</td>
<td>0-6</td>
<td>0-7</td>
<td>0-8</td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>8</td>
<td>0-9</td>
<td>1-0</td>
<td>1-2</td>
<td>7</td>
</tr>
<tr>
<td>31–60 Re-inoculated</td>
<td>10</td>
<td></td>
<td></td>
<td>9</td>
<td>0-6</td>
<td>0-6</td>
</tr>
<tr>
<td>Not re-inoculated</td>
<td>10¶</td>
<td>10</td>
<td>0-6</td>
<td>0-6</td>
<td>0-7</td>
<td></td>
</tr>
</tbody>
</table>

* ACV animals were treated for 21 days after the initial inoculation.
† Mean per animal per week.
‡ Asymptomatic vaginal viral shedding was defined as recovery of HSV from vaginal swabs in animals without lesions.
§ $P < 0.05$ by Student’s $t$-test.
|| Six ACV, four control.
¶ Five ACV, five control.

ACV-treated compared to placebo-treated guinea-pigs ($P < 0.05$). Asymptomatic vaginal viral shedding occurred in fewer placebo (two of 14) than ACV (five of 17) recipients.

Once ACV treatment was discontinued at 21 days, ACV recipients had similar recurrence rates, viral shedding patterns, and severity of recurrences to control animals. From days 22 to 28, 76% (13 of 17) ACV recipients and 64% (nine of 14) placebo recipients developed clinical
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Recurrences. Mean total lesion scores during this period were 1.3 in ACV recipients and 1.6 in placebo recipients. Asymptomatic viral shedding occurred on an average of 0.9 days (nine of 17 animals) compared to 0.8 days (four of 14 animals) per guinea-pig in ACV-treated and control animals, respectively. Four of the six ACV-treated animals that did not develop vesiculoulcerative disease initially had symptomatic recurrences with viral shedding and one had asymptomatic viral shedding alone. The placebo-treated animal that did not develop clinically apparent initial disease also demonstrated asymptomatic viral shedding during this period.

On day 31, 11 ACV and seven control animals were re-challenged with a different strain of HSV-2 (333). Analysis of the recurrence patterns in the remaining 10 animals (five ACV and five controls) revealed no difference in the mean number of recurrences per week for ACV recipients (0.7) compared to controls (0.9) after the drug was discontinued (days 22 to 60) (data not shown). During the time of peak viral replication in the vaginal vault of re-inoculated animals (days 1 to 5 post-inoculation), lower daily symptom scores were noted when re-inoculated animals were compared to those not re-inoculated (mean daily symptom score 0.05 compared to 0.19 respectively, \( P < 0.001 \)) (data not shown).

However, after this period, re-inoculation did not alter long-term recurrence patterns (Table 1). Therefore, the recurrence patterns of all the animals were examined and no significant differences were found in the mean number of recurrences per week, 0.6 and 0.9 for ACV and placebo recipients respectively, or in the severity of the recurrences as measured by either lesion score or lesion days after ACV was discontinued (days 22 to 60) (Table 1). High rates (\( \geq 7 \)) of recurrences were, however, observed in four of nine controls compared to two of 11 drug recipients. Although not achieving statistical significance, ACV-treated guinea-pigs therefore had fewer recurrences by all parameters we examined except for viral shedding.

In all groups of animals, recurrences decreased in number and severity over time. In the period immediately after the acute infection (days 15 to 30), animals with recurrences had a mean of 2.7 recurrences and a mean total lesion score of 4.0 compared to 1.0 recurrences and a lesion score of 1.3 during the last recorded interval (days 45 to 60) (\( P < 0.001 \) by the paired Student’s \( t \)-test).

**ACV susceptibility of isolates**

Analysis of the sensitivity of the infecting virus (MS strain) to ACV revealed a 50% inhibitory dose (ID\(_{50}\)) of 0.11 \( \mu \)g/ml and an ID\(_{90}\) of 0.32 \( \mu \)g/ml. All isolates obtained from vaginal swabs after the initial replication while the animals were receiving therapy (days 14 to 21) were also examined. The ID\(_{50}\) of these isolates ranged from 0.07 to 0.15 \( \mu \)g/ml while the ID\(_{90}\) ranged from 0.21 to 0.38 \( \mu \)g/ml.

**Re-infection**

After 30 days, 11 ACV-treated and seven control animals were re-challenged with \( 10^7 \) p.f.u. of strain 333 HSV-2. Re-inoculation resulted in vaginal HSV-2 replication with initially similar kinetics to that seen after primary inoculation (Fig. 5). Viral titres peaked at 24 h and were similar up to 72 h but then declined more rapidly after re-inoculation than after primary infection. The pattern of vaginal viral replication was similar in previously ACV-treated and control animals. No animal manifested clinically apparent disease that could be attributed to re-challenge except for the two control animals which did not become infected after the initial inoculation.

To determine whether the re-challenge virus could be isolated acutely from neural tissues, eight animals were sacrificed 3 to 6 days after re-inoculation (Table 2). A productive virus infection was documented by recovery of virus from homogenate cultures of spinal cord obtained from two control animals but was found to be the initial infecting MS strain (Fig. 6, lane 3). Virus was not recovered from homogenate cultures of neural tissues from any previously ACV-treated animal. Latent virus recovered by co-cultivation of dorsal root ganglia from four (two control, two ACV) of the eight animals sacrificed at this time was found to be the MS strain (Fig. 6, lane 4).
Fig. 5. Course of vaginal HSV-2 replication after intravaginal re-inoculation of HSV-2 strain 333. All animals had been inoculated intravaginally 30 days prior to this with HSV-2 strain MS and had either received ACV for 21 days (□) after the primary inoculation or not (▲). Replication of virus in the vagina was measured by plaque titration of vaginal swabs.

Table 2. Isolation of productive or latent HSV-2 from guinea-pig tissues after re-inoculation

<table>
<thead>
<tr>
<th></th>
<th>DRG</th>
<th>Spinal cord</th>
<th>Cervix</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prod.†</td>
<td>Latent</td>
<td>Prod.‡</td>
<td>Latent</td>
</tr>
<tr>
<td>No. of animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>2 (MS)</td>
<td>0</td>
</tr>
<tr>
<td>ACV</td>
<td>5</td>
<td>0</td>
<td>2 (MS)</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0</td>
<td>1 (MS)</td>
<td>0</td>
</tr>
<tr>
<td>ACV</td>
<td>6</td>
<td>0</td>
<td>2 (MS)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Guinea-pigs initially infected with MS strain HSV-2 were re-inoculated with strain 333 HSV-2 then sacrificed 3 to 6 days after re-inoculation. The strain isolated is indicated.
† Productive virus was that detected in cell-free homogenates while latent virus was detected only by explant co-cultivation of minced tissue fragments. DRG, Dorsal root ganglia.
‡ Guinea-pigs infected and re-inoculated as in * were sacrificed 31 days after re-inoculation (61 days after the initial infection).

After 60 days all remaining animals were sacrificed and homogenate and explant cultures were obtained from lumbar and sacral dorsal root ganglia, spinal cord, cervix and genital skin (Table 2). Latent virus was recovered from the explant cultures of the dorsal root ganglia from four animals. Three isolates were the MS strain (Fig. 6, lane 5) while only strain 333 virus was isolated from a fourth animal that had received ACV during initial infection (Fig. 6, lane 6). Isolates from vaginal swabs from this animal obtained on days 41 and 43 were, however, the MS strain (Fig. 6, lane 7). This animal had had clinically apparent disease after the initial infection and a neutralization titre of 1:80 on day 28.

DISCUSSION

In this study, as in previous reports (Kern, 1982; Pronovost et al., 1982), ACV therapy reduced the severity of acute HSV-2 genital infection but had no effect on vaginal virus shedding even though therapeutic ACV levels were achieved. Initial ACV therapy also had no significant impact on long-term recurrence patterns. Despite diminished humoral responses, early recurrences were not more severe in guinea-pigs that had received ACV compared to controls as has been suggested may occur in humans (Ashley & Corey, 1984).

Recurrent diseases occurred in all animals including six ACV-treated animals that had subclinical initial infection. One control animal also did not exhibit vesicular disease after initial infection but subsequently exhibited asymptomatic viral shedding. These data would support
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Fig. 6. Restriction enzyme analysis of the tissue culture-grown HSV-2 strain 333 (lane 1) and strain MS (lane 2). Lane 3, isolate obtained from the homogenate culture of a spinal cord on day 35 of a re-inoculated animal, identified as MS strain HSV; lane 4, isolate obtained by explant co-cultivation of a dorsal root ganglion (DRG) obtained 5 days after re-inoculation with 333 and identified as the initial infecting strain MS; lane 5, isolate obtained from explant co-cultivation of a DRG obtained 30 days after re-inoculation with 333 and identified as MS strain; lane 6, isolate obtained from the explant culture of a DRG from an ACV-treated animal, identified as strain 333; lane 7, isolate obtained from a vaginal swab on day 41 from the same animal as in lane 6 and identified as MS strain.

our previous observation (Bernstein et al., 1984b) that patients with non-primary first episode genital herpes may be experiencing the first clinical recurrence of what had been a subclinical primary infection.

The major initial humoral response of infected guinea-pigs was directed to two unglycosylated proteins of mol. wt. 40K and 43K, while the group of antigens from 40K to 49K included two or three other proteins which also appeared to be major targets of the convalescent antibody response. Previous reports based on immune precipitation of human (Ashley & Corey, 1984; Zweerink & Corey, 1982) or guinea-pig sera (Ashley & Kern, 1984) have not described these as major antigens, while other studies employing immunoblotting techniques (Eberle et al., 1984) have noted findings similar to ours (Bernstein et al., 1984a). This suggests that these polypeptides are either poorly labelled or solubilized and are thus not readily detected by
immune precipitation. These proteins appeared in similar positions to the proteins found in HSV-1-infected cells termed the ICP35 family by Braun et al. (1984) which also appeared to contain intermolecular disulphide bonds (Braun et al., 1983).

The response to the major glycoproteins of HSV-2 did not appear to be as marked in our observations compared to that observed by others in humans (Gilman et al., 1981; Zweerink & Corey, 1982). While possibly a species difference, this may be related to the different assay systems utilized (immune precipitation and immunoblotting). Although it is clear from the reaction of the polyclonal rabbit anti-gB and -gD that both gB and gD are present in adequate quantities on the reacted nitrocellulose, it is possible that some of the antigenic determinants may be irreversibly damaged by denaturation with SDS and boiling. The observation that plasma without an identifiable reaction to gD by immunoblotting was reactive in an ELISA using cloned gD as the antigen (our unpublished results) also suggests that immunoblotting may not be as sensitive as other methods for detection of antibody to this protein. Norrild et al. (1981) also noted that the major reactive polypeptides were not the HSV-1 glycoproteins, although Eberle & Mou (1983) have reported strong responses to gB and gD in patients with recurrent HSV-1 infections in a system similar to ours. Preliminary studies in our laboratory have also suggested that weanling guinea-pigs, such as those used in this study, do not respond as well to gB and gD as do adult guinea-pigs (unpublished data).

ACV treatment delayed the production of antibody to a number of polypeptides including the glycoproteins, as previously reported in patient studies (Ashley & Corey, 1984; Bernstein et al., 1984a). Although it was delayed, we observed the same sequence of antibody production in ACV-treated as in control animals. Because guinea-pigs developed recurrences while receiving therapy, the diminished responses were more evident on days 14 and 21 than on day 28. Thus despite adequate plasma concentrations, ACV therapy did not suppress recurrences in the guinea-pig as efficiently as has been reported from human studies (Douglas et al., 1984; Straus et al., 1984). Recurrences while ACV was present were not related to the development of drug-resistant mutants, as all isolates obtained under these conditions remained sensitive to ACV, with ID₅₀ values below the lowest level of serum ACV detected. These observations suggest that antibody production may be delayed because of diminished antigenic stimulation and that the differences observed are quantitative and not qualitative. Thus, the previously observed failure to detect specific antibodies (Ashley & Corey, 1984; Bernstein et al., 1984a) may represent a further delay in the production of these late-appearing antibodies and not an absolute absence.

Acyclovir therapy of initial infection has also been reported to decrease long-term recurrences (Bryson et al., 1983b). Analysis of long-term recurrence patterns in this study revealed a slight decrease in the number of recurrences and the number of lesion days in ACV- compared to placebo-treated animals, but these differences were not significant. Only 18% (two of 11) ACV-treated compared to 44% control animals had ≥7 recurrences and only 9% of the ACV group compared to 33% of the controls had ≥8 occurrences during the observation period. These findings are similar to those reported for humans in whom 12% of ACV and 39% of placebo recipients had ≥7 recurrences, and 13% compared to 32% respectively had ≥8 recurrences over a much longer period (Bryson et al., 1983b). Thus, the pattern of recurrent disease in guinea-pigs was similar to that seen in humans but occurred over a shorter period of time.

ACV therapy did not alter susceptibility to re-infection. Re-inoculation of both ACV-treated and control animals resulted in similar vaginal virus replication patterns to that seen initially after the primary infection. After the first 3 days, however, viral titres declined more rapidly after re-inoculation than after the primary infection. This suggests that vaccination may not provide complete local protection, as even a prior vaginal infection did not induce complete immunity at this site. Since the re-infecting strain replicated to high titres in the vaginal vault without producing clinical symptoms it is conceivable that unrecognized re-infection in humans could provide a reservoir of virus transmissible to sexual partners.

Ganglionic re-infection has not been detected following HSV-1 re-infection of the rabbit cornea (Centifanto-Fitzgerald et al., 1982), HSV-2 genital re-infection in guinea-pigs (Stanberry et al., 1986) or when sought in sexually active patients at high risk for exposure (Schmidt et al.,
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1984). In this study, we were able to demonstrate ganglionic superinfection only in a single ACV-treated animal. Similarly, ganglionic re-infection by HSV-1 has been demonstrated following corneal re-inoculation of mice previously infected with an HSV-1 × HSV-2 recombinant strain of reduced neurovirulence (Meignier et al., 1983) and in mice treated with phosphonoacetic acid after skin inoculation (Klein et al., 1980). These observations imply that re-infection of neural tissues is a possible but unlikely event except under conditions which alter the pathogenesis of the initial infection.

A surprise to us was the recovery of the initial infecting MS strain from homogenate cultures of spinal cord from two control animals following re-infection with the 333 strain. It would appear that re-infection may have induced reactivation of latent virus at this site. Homogenate cultures of spinal cords obtained this long after the acute primary infection from more than 50 other animals studied in this laboratory have all been culture-negative (unpublished observation).

Oral ACV therapy of initial genital HSV-2 infection in the guinea-pig delayed the production of neutralizing antibody and antibody to specific HSV-2 polypeptides. Treated animals, however, did not manifest severe or more frequent recurrences. Indeed, slight reductions in recurrence patterns were detected. ACV treatment during initial infection did not alter the pattern of local vaginal viral replication during initial or subsequent re-infection but may have increased susceptibility to ganglionic re-infection in one animal.

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REFERENCES


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