Modification of primary and recurrent genital herpes in guinea pigs by passive immunization

Nigel Bourne, Richard B. Pyles, David I. Bernstein and Lawrence R. Stanberry

Children’s Hospital Medical Center, Division of Infectious Diseases, 3333 Burnet Ave, Cincinnati, OH 45229-3039, USA

Guinea pigs were administered antiserum 24 h (As+24) or 72 h (As+72) after intravaginal herpes simplex virus type 2 (HSV-2) challenge. Treatment at either time reduced acute virus replication in the dorsal root ganglia and the overall magnitude of replication in the genital tract. In two studies, As+24 treatment significantly reduced the severity of primary genital skin disease and the frequency of subsequent spontaneous recurrent disease. In contrast, As+72 treatment produced a modest reduction in primary disease severity but did not impact on recurrent disease. Quantitative PCR analysis of dorsal root ganglia DNA from latently infected animals showed that As+24 treatment produced a significantly reduced viral DNA burden, which appeared to correlate with the reduction in recurrent disease. The amount of DNA in the ganglia of As+72-treated animals was not significantly lower than that of controls. These observations have implications for both the dynamics of latency establishment and desirable vaccine characteristics.

Passive immunization is used clinically against varicella-zoster virus for the prophylaxis of immunocompromised patients who are exposed to the virus (Zaia et al., 1983; Kavaliotis et al., 1998) and against cytomegalovirus for prophylaxis in bone marrow, renal and liver transplants, particularly in situations where the recipient is seronegative and the donor seropositive (Snydman, 1990; Sawyer, 2000). However, it has not been widely studied in humans for use against herpes simplex virus (HSV) infections. In contrast, passive transfer has been, and continues to be, explored in mouse models of HSV infection (e.g., Walz et al., 1976; McK Kendall et al., 1979; Morrison et al., 2001). Differences in experimental protocols, e.g. the time of antibody administration relative to the virus inoculum and the challenge route, limit direct comparisons of these studies. However, they have generally shown that if antibody is present at the time of challenge or is administered soon after, acute disease outcome is improved. The results of some studies using explant cocultivation techniques and more recently quantitative PCR analysis of virus DNA have also shown that passive immunization can reduce latent virus infection (Klein, 1980; Morrison et al., 2001). However, it has not been possible to determine whether the reduction in latent virus load is sufficient to produce an impact on recurrent disease, because mice do not develop spontaneous recurrences. Guinea pigs, unlike mice, experience a quantifiable, self-limiting, primary genital skin disease after intravaginal HSV challenge, which is followed by periodic spontaneous recurrent herpetic lesions on the perineum (Stanberry et al., 1982, 1985). This allows the impact of interventional strategies such as vaccines and antivirals on both primary and recurrent disease to be evaluated (Stanberry et al., 1987). Accordingly, in these studies we examined the effect of passive immunization on primary and subsequent recurrent genital herpes in the guinea pig model.

A pool of high-titre antiserum was produced from female Hartley guinea pigs (Charles River Breeding Laboratories, Wilmington, MA, USA) that had recovered from symptomatic primary genital HSV-2 infection. The animals were immunized with HSV-2 antigen prepared from infected Vero cell monolayers, which were harvested, washed and resuspended in PBS. Following freeze–thawing three times, the lysates were sonicated until all cell debris had been disrupted, then centrifuged to remove particulate matter. The supernatant was aliquoted and stored at −20 °C. Protein concentrations were determined by bicinchoninic acid assay (Pierce). For the first immunization, animals received antigen (~200 µg total protein) and complete Freund’s adjuvant into the rear footpads. Subsequent immunizations were given subcutaneously at 4 week intervals with incomplete Freund’s adjuvant. Animals were bled weekly beginning 2 weeks after the initial immunization and the serum stored at −20 °C. Prior to use it was thawed, pooled, aliquotted and refrozen. The ELISA antibody

Author for correspondence: Nigel Bourne. Present address: Sealy Center for Vaccine Development, Department of Pediatrics, The University of Texas Medical Branch at Galveston, 301 University Boulevard, Galveston, TX 77555-0436, USA.
Fax +1 409 7478150. e-mail nibourne@utmb.edu
Table 1. Effect of passive antibody administration on genital HSV

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>No. of infected*/no. of inoculated animals</th>
<th>Primary disease</th>
<th>Recurrent disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence</td>
<td>Severity†</td>
</tr>
<tr>
<td>1</td>
<td>Untreated</td>
<td>12/12</td>
<td>12/12</td>
<td>8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>As + 24</td>
<td>14/15</td>
<td>68/14</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>As + 72</td>
<td>10/16</td>
<td>11/16</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>Untreated</td>
<td>12/12</td>
<td>12/12</td>
<td>7 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>As + 24</td>
<td>10/12</td>
<td>48/10</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>As + 72</td>
<td>11/12</td>
<td>6/11</td>
<td>3 ± 0.4</td>
</tr>
</tbody>
</table>

* Includes animals defined as infected by recovery of virus from vaginal swab sample collected on days 1 and 2 p.i.
† Mean ± SE: severity measured as the area under the lesion score–day curve for animals with primary disease.
‡ Mean ± SE: frequency defined as number of days on which recurrent lesions were observed (Study 1, days 15–63 p.i.; Study 2, days 15–42 p.i. for animals with recurrences).
§ P < 0.005, ¶ P < 0.01. All versus untreated controls. Incidence data compared by Fisher’s exact test. Comparisons of means performed by ANOVA with Bonferroni correction for multiple groups.

Vaginal virus titre (log10 p.f.u./ml) was determined as previously described (Bernstein & Harrison, 1989) and defined as the reciprocal of the highest serum dilution producing an absorbance > 0.1 and twice that of control antigen. The neutralization titre was assayed by HSV-2 plaque reduction on rabbit kidney (RK) cells (Bernstein et al., 1986) and defined as the reciprocal of the serum dilution producing a 50% reduction in plaque number compared with naïve serum. The antiserum pool used in these studies had an ELISA titre of 1:32,000 and a neutralizing titre of 1:800.

In Study 1 we examined the effect of antiserum treatment on virus replication in the genital tract and the course of primary and recurrent genital HSV-2 disease. Forty-three Hartley guinea pigs were inoculated intravaginally with 5.7 log10 p.f.u. of HSV-2 strain MS as previously described (Stanberry et al., 1982). Group 1 (As + 24; n = 15) received 5 ml antiserum per animal by intraperitoneal injection 24 h post-inoculation (p.i.). Group 2 (As + 72; n = 16) received antiserum 72 h p.i., while Group 3 (n = 12) were untreated controls. Vaginal swabs were collected on days 1, 2, 3, 5, 7, and 10 p.i. and stored at −80 °C until virus was quantified by plaque assay on RK cells. Animals were examined daily and primary genital skin disease quantified as previously described (Stanberry et al., 1982). Following recovery from primary disease, animals were monitored daily from day 15 to 63 p.i. for spontaneous recurrent herpetic lesions on the genital skin (Stanberry et al., 1982). Animals that did not develop primary genital skin disease were defined as infected if virus was isolated from vaginal swabs collected within the first 2 days p.i. By this definition 12/12 controls, 14/15 As + 24 and 16/16 As + 72 animals became infected and were included in the analysis. As + 24 treatment profoundly altered the course of genital herpes (Table 1, Study 1). Both the incidence of primary genital skin disease (P < 0.005; Fisher’s exact test) and, severity in symptomatic animals (P < 0.01; ANOVA) were significantly reduced. Furthermore, when spontaneous recurrent disease was examined, As + 24 treatment marginally reduced the number of animals that developed recurrences (P = 0.08; Fisher’s exact test) and significantly reduced the number of days on which those animals experienced recurrent lesions (P < 0.001 ANOVA). As + 72 treatment was less effective, and although the incidence and severity of primary disease were reduced compared with controls, the reductions did not reach significance and the frequency of recurrent genital skin disease was comparable with that seen in controls (Table 1, Study 1).

Peak vaginal virus titres were seen on day 2 p.i. and were not reduced by As + 24 treatment (Fig. 1). However, by day 3 p.i., titres were lower in As + 24 animals than in controls and remained lower on days 5 and 7 p.i., resulting in a significant

Fig. 1. Effect of administration of polyclonal antiserum 24 or 72 h post-intravaginal virus challenge on the course of virus replication in the genital mucosa of guinea pigs.
reduction in the overall magnitude of virus replication in the genital tract during the first 10 days p.i. as measured by the area under the curve of the virus titre–day graph (25·3 ± 0·7 for controls vs 19·2 ± 1·9 for As + 24; P < 0·05). Virus replication in the genital tract was also reduced in As + 72 animals on days 5 and 7 p.i. (Fig. 1) resulting in a significant reduction in the area under the curve compared with untreated controls (19·7 ± 1·3; P < 0·05). Studies in mice have shown that antiserum treatment reduces acute virus replication in the ganglia innervating the inoculation site (Walz et al., 1976; Oakes & Rosemund-Hornbeak, 1978; Klein, 1980). Accordingly, in a separate study we examined the impact of antiserum on virus replication in the dorsal root ganglia (DRG) during primary infection in guinea pigs. Twenty-four animals were inoculated with HSV-2 strain MS and received As + 24 (n = 9), As + 72 (n = 6) or were untreated controls (n = 9). On day 3 p.i., three As + 24 and three control animals were sacrificed. The DRG from each animal were collected and homogenized on ice. The homogenates were centrifuged for 10 min at 6000 r.p.m. at 4 °C, returned to ice and the supernatant immediately titrated for virus on RK cells. The process was repeated on days 4 and 5 p.i. with the addition of three As + 72 animals on each day. The limit of detection of the assay was 1·91 log_{10} p.f.u./g tissue. Virus was recovered from the supernatants of DRG homogenates in a total of 6/9 control animals on days 3–5 p.i. with mean (± SE) titres of 2·66 ± 0·02 log_{10} p.f.u./g tissue on day 3, 3·49 ± 0·33 on day 4 and 2·91 ± 0·00 on day 5. In contrast, virus was detected in 0/15 animals that received antiserum 24 or 72 h p.i. during the same period (P < 0·001; Fisher’s exact test).

It is reasonable to hypothesize that the dramatic reduction in acute virus replication seen in the DRG of As + 24-treated animals would produce a concomitant reduction in the amount of virus returning intraneuronally to replicate in the genital mucosa and so be responsible at least in part for the reduced vaginal virus titres seen in treated animals after day 2 p.i. Furthermore, since primary genital skin disease in the guinea pig model is also thought to be caused by virus returning from the DRG to the periphery (Stanberry et al., 1982), the reduction in virus replication in the DRG may contribute directly to the reduction in primary disease seen in As + 24-treated animals. Support for this hypothesis can be found in ocular HSV challenge studies in mice where the protection against severe ocular damage provided by the administration of antibody is also believed to result from restriction of virus spread from the nervous system back to the periphery (Schimeld et al., 1990). The enhanced protection against acute disease seen in As + 24 compared with As + 72 animals appears to result from an early reduction of acute virus replication, since the impact of treatment in the DRG in As + 24 and As + 72 animals was comparable after day 4 p.i. and vaginal titres were comparable from day 5 p.i.

We and others have shown by quantitative PCR analysis that prophylactic immunization can reduce the magnitude of latent virus DNA in the ganglia of guinea pigs after intravaginal HSV-2 challenge and that this reduction is paralleled by a reduction in recurrent disease (Bourne et al., 1996; Wachman et al., 2001). Thus, the reduced recurrent disease in As + 24 animals could be due to a reduction in the amount of latent virus DNA. To explore this possibility, 36 Hartley guinea pigs were inoculated with HSV-2 strain MS and received As + 24 (n = 12), As + 72 (n = 12) or were untreated controls (n = 12). Vaginal swabs were collected on days 1 and 2 p.i. Animals were examined daily until day 14 p.i. for primary genital skin disease and from days 15–42 for recurrent disease. Table 1, Study 2 shows that the effect of treatment on disease outcome in this study was similar to that in Study 1. As + 24 treatment significantly reducing the incidence (P < 0·005) and severity (P < 0·001) of primary genital skin disease and the incidence (P < 0·01) and frequency (P < 0·05) of recurrent disease. As + 72 treatment again moderated primary genital skin disease with the impact on this occasion reaching significance. However, as in Study 1, delaying treatment until
confirm previous studies in mouse HSV infection models that treatment is possible, for example the post-exposure prophylaxis to produce this effect. In As72 treatment significantly reduced the amount of HSV-2 DNA present in the DRG compared with control animals (P < 0.01). These results confirm previous studies in mouse HSV infection models that have shown that passive antibody transfer can reduce latent HSV-2 infection (Klein, 1980; Morrison et al., 2001) and extend them by showing that, in guinea pigs, the reduction in latent HSV infection can significantly reduce the frequency of spontaneous recurrent disease. However, our results also indicate that the treatment must occur soon after virus challenge to produce this effect. In As + 72 animals, neither the amount of latent virus DNA in the ganglia nor the frequency of recurrent disease were significantly reduced compared with untreated controls. This strongly suggests that, at least in this model, much of the burden of latent virus is established within the first 3–4 days p.i., possibly even before initial symptoms are seen (genital lesions seldom develop prior to day 4 p.i.). If the same is true in humans, then by the time patients are seen at the onset of symptoms it is already too late for antibody therapy or indeed antivirals to impact on the burden of latent virus. Thus, clinical opportunities for therapy to affect latency would be limited to instances where the early initiation of treatment is possible, for example the post-exposure prophylaxis of rape victims and treatment of newborns at risk of developing neonatal herpes following passage through an infected birth canal.

We thank Alisa Reece, Jim Ireland, Frances Childs and Tony Zalar for excellent technical assistance and Toni Cunningham and Tedra Kelly for help in manuscript preparation. Financial support was provided by the National Institute of Health AI-65289 and AI-22667.

References


Received 25 March 2002; Accepted 16 July 2002