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Herpes simplex virus glycoprotein treatment of recurrent genital herpes reduces cervicovaginal virus shedding in guinea pigs

Martin G. Myers[1], David I. Bernstein[1,2], Christopher J. Harrison[1] and Lawrence R. Stanberry[1]

[1]*Division of Infectious Diseases, Children's Hospital Research Foundation and* [2]*James Gamble Institute of Medical Research, University of Cincinnati College of Medicine, Cincinnati, Ohio, U.S.A.*

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Summary

Treatment of previously infected guinea pigs with herpes simplex virus (HSV) glycoproteins reduces the frequency and severity of subsequent genital recurrences. An effective vaccine should also reduce episodes of viral shedding. In this study, HSV glycoproteins B and D treatment of animals experiencing recurrent genital herpes reduced the frequency of both clinical recurrences and cervicovaginal viral shedding. When virus was shed, however, the peak viral titer and duration of shedding was unaltered.

Glycoprotein; Immunotherapy; Recurrent genital herpes

Introduction

Genital herpes simplex virus (HSV) infections are increasingly common. Strategies designed to control genital HSV must address the fact that virus persists and

Correspondence to: M.G. Myers, Division of Infectious Diseases, Children's Hospital Research Foundation, Elland and Bethesda Aves., Cincinnati, OH 45229-2899, U.S.A.

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infection may repetitively recur. While chronically ingested acyclovir alters the frequency of recurrent disease (Strauss et al., 1984; Douglas et al., 1984), effectiveness is limited by cost, compliance and duration of therapy. An alternative strategy might be the use of defined HSV glycoproteins as immunotherapeutic agents for the control of recurrent HSV infections. Thus, we have previously reported that HSV glycoprotein immunogens alter the frequency and severity of subsequent genital herpetic recurrences in guinea pigs when given prior to (Stanberry et al., 1987a) or after (Stanberry et al., 1987b) genital HSV-2 infection.

Both initial and recurrent genital HSV infection may be either symptomatic or asymptomatic. Indeed, asymptomatic shedding of HSV in genital secretions may occur frequently (Nahmias et al., 1970; Ng et al., 1970; Centifanto et al., 1971; Jeansson and Molin, 1974; Rattray et al., 1978; Willmott et al., 1978; Ekwo et al., 1979, McCaughtry et al., 1982; Vontver et al., 1982; Wittek et al., 1984) and has been associated with horizontal (Mertz et al., 1985; Rooney et al., 1986) as well as vertical (Whitley et al., 1980; Yeager et al., 1984) viral transmission. Epidemiologic limitation of this often sexually transmitted disease therefore, will require not only suppression of symptomatic disease but also reduction of potential communicability by diminution in either the frequency or magnitude of viral shedding. In this study, we explored the effects of glycoprotein immunotherapy upon cervicovaginal HSV-2 shedding in guinea pigs with established recurrent genital herpes. The guinea pig model was selected because of the similar pathophysiology of infection to that which occurs in humans (Stanberry et al., 1982, 1985; Bernstein et al., 1986), viral reactivation being manifested by spontaneous symptomatic and asymptomatic recurrences in both.

Materials and Methods

Experimental design

Initial genital disease in 350 to 500 g female Hartley guinea pigs (Charles River Breeding Laboratories, Wilmington, Mass.) was scored daily (Stanberry et al., 1982) following intravaginal inoculation with 5.7 $\log_{10}$ PFU of MS strain HSV-2 (ATCC VR-540). Only animals that exhibited external genital skin disease during initial infection were studied. After recovery from initial infection, animals were examined daily for evidence of recurrent herpetic disease as previously described (Stanberry et al., 1985). One group of animals (N = 31) received no therapy whereas the other group of animals (N = 31) received a mixture of recombinant HSV-1 glycoproteins B (12.5 μg) and D (6.25 μg) (gBgD) kindly provided by Chiron Corporation (Emeryville, CA). Vaccine (150 μl) was administered with an equal volume of complete Freund's adjuvant (CFA) in the hind footpads 21 and again 42 days after intravaginal HSV-2 inoculation. Clinical recurrences are reported as the number of days with any lesion (lesion days) after day 21.

Viral shedding

Twelve untreated and 10 gBgD treated animals were evaluated for cervicovaginal virus shedding. Daily vaginal swab cultures were obtained on days 22–100 following intravaginal infection employing a pre-moistened calcium alginate swab which was transported on ice in 1.0 ml of tissue culture medium (Basal Eagle's medium supplemented with 5% fetal calf serum, 2 mM L-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 1 μg/ml amphotericin B). The unfrozen specimen (0.2 ml) was inoculated within 1 h of collection onto second passage rabbit kidney cells (RK) which were observed for CPE for 10 days. The cultures were interpreted without knowledge of the treatment group. Stored (−70°C) swab samples, from which virus had been recovered, were then titrated in triplicate in RK. Endpoints of titrations (Reed and Muench, 1938) were determined after 10 days incubation but do not incorporate the effects of dilution in transport media. The level of sensitivity of detecting HSV was ≥ 0.5 $\log_{10}$ $TCID_{50}$/ml. Recurrences manifested by cervicovaginal virus shedding are reported as days that virus was shed (shedding days) or as shedding episodes (an interval during which HSV was recovered, preceded and followed by a day without a lesion or virus recovery). Asymptomatic shedding episodes occurred when vaginal viral shedding was documented in the absence of clinical disease within 24 h.

Results

As previously observed (Stanberry et al., 1987b), gBgD treated animals experienced fewer days with clinically apparent recurrences than the untreated animals

TABLE 1

Effect of post infection gBgD therapy upon recurrent genital herpes[a]

	Untreated	Treated
Initial lesion score[b]	10.6 ± 0.8	10.8 ± 0.6
Recurrences[c]		
Lesion days	19.0 ± 1.3	15.2 ± 1.5[d]
Shedding days	1.6 ± 0.3	0.6 ± 0.3[d]
Shedding episodes	1.5 ± 0.3	0.4 ± 0.2[d]
asymptomatic	0.7 ± 0.3	0

[a] Animals were untreated (N = 31) or received 12.5 μg gB and 6.25 μg gD with CFA (N = 31) on days 21 and 42 after intravaginal infection with 5.7 $\log_{10}$ PFU HSV-2. Twelve untreated and 10 treated animals had daily vaginal cultures obtained on days 22 through 100 (one treated animal died on day 40).

[b] Mean ± SE of the area under the initial score curve.

[c] Mean ± SE days 22 through 100 after infection. Recurrences are reported as days with a genital lesion (lesion days), days that virus was detected in cervicovaginal secretions (shedding days) and intervals during which HSV was recovered preceded and followed by a day without a lesion or virus recovery (shedding episodes). Asymptomatic recurrences occurred when virus was detected in the absence of clinical disease within 24 h.

[d] $P<0.05$ by Student's t-test.

TABLE 2

Effect of post infection gBgD therapy upon vaginal HSV shedding

	Untreated (N = 12)	Treated[a] (N = 10)
% Animals that shed HSV[b]		
Days 22–40	50	30
Days 41–100	67	0[c]
Days 22–100	83	30[d]
Days shedding HSV[e]	1.9 ± 0.3	1.7 ± 0.7
Magnitude of shedding[f]	1.22 ± 0.36	1.01 ± 0.26

[a] Animals received 12.5 μg of gB and 6.25 μg of gD with CFA on days 21 and 42 after intravaginal infection with 5.7 $\log_{10}$ PFU HSV-2.

[b] Cervicovaginal cultures were obtained daily from day 22 through day 100.

[c] N = 9; P = 0.002 by Fisher exact test.

[d] P = 0.02 by Fisher exact test.

[e] For animals that shed virus; mean ± SE.

[f] For animals that shed virus; mean ± SE $\log_{10}$ $TCID_{50}$/ml.

(P = 0.03) (Table 1). Similarly, treated animals shed HSV less commonly than untreated control animals, experiencing reduced days (P = 0.04) and episodes (P = 0.01) that virus was detected in cervicovaginal secretions. After day 39, no gBgD treated animals exhibited viral shedding while untreated control animals continued to intermittently shed HSV (Table 2). Indeed, only one treated animal shed virus after day 25. However, the number of days that animals shed virus (for animals that shed virus) and the magnitude of virus shed did not differ between groups.

HSV was detected in cervicovaginal secretions on only 6 of 393 lesion days (2 of 173 in the treated group and 4 of 220 in the untreated group), 18 of 24 shedding days having been lesion free (3 and 15 days, respectively). However, although eight of 22 shedding episodes were asymptomatic, asymptomatic episodes of HSV shedding were not observed among the gBgD treated animals.

Discussion

Because herpesviral glycoproteins reduce the frequency and severity of subsequent recurrent genital herpetic lesions when administered before (Stanberry et al., 1987a) or after (Stanberry et al., 1987b) primary genital HSV infection in animals, these immunogens are being considered as possible therapeutic agents in humans. Indeed, initial uncontrolled studies of HSV glycoprotein vaccines have been reported to affect recurrent disease in man (Meignier, 1985; Hall and Katrak, 1986).

Both symptomatic and asymptomatic HSV shedding in genital secretions are potential epidemiologic sources of virus (Nahmias et al., 1970; Ng et al., 1970; Centifanto et al., 1971; Jeansson and Molin, 1974; Rattray et al., 1978; Willmott and Mair, 1978; Ekwo et al., 1979; McCaughtry et al., 1982; Vontver et al., 1982; Wittek et al., 1984; Mertz et al., 1985; Rooney et al., 1986) which may also place the

newborn at risk of severe infection (Whitley et al., 1980; Yeager et al., 1984). Because post-infection gBgD immunotherapy of animals with established HSV infection can modify the nature of clinically apparent recurrent genital herpes (Stanberry et al., 1987b), it seemed important to define whether such treatment would reduce viral shedding or, conversely, increase the proportion of episodes of asymptomatic shedding by diminishing the severity of recurrences to a level below clinical detection.

The frequency of virus shedding was reduced by gBgD treatment: none of these animals shedding virus after day 39. After vaccine, no recurrent episodes of viral shedding were asymptomatic. Although gBgD immunotherapy reduced the number of animals that shed virus and the frequency that an animal shed virus, when virus was shed there was no difference in either the duration or the magnitude of viral shedding. It seems unlikely that adjuvant alone might have affected virus shedding as we have not observed an effect of adjuvant alone on clinical recurrences (Stanberry et al., 1987a,b; authors' unpublished data).

Women may often experience clinically apparent genital herpetic recurrences without virus being detected in cervicovaginal secretions (Jeansson and Molin, 1974; Rattray et al., 1978; Vontver et al., 1982; Wittek et al., 1984) or shed virus in genital secretions in the absence of clinically apparent symptoms (Nahmias et al., 1970; Ng et al., 1970; Centifanto et al., 1971; Jeansson and Molin, 1974; Rattray et al., 1978; Willmott and Mair, 1978; Ekwo et al., 1979; McCaughtry et al., 1982; Vontver et al., 1982; Wittek et al., 1984). Similarly, in this caviid model, most clinical recurrences were not associated with detectable virus in genital secretions and more than one-third of shedding episodes were not associated with a clinical recurrence within 24 h.

Previously HSV infected animals that were experiencing spontaneous clinical recurrences and cervicovaginal HSV shedding demonstrated a reduction in the frequency of subsequent cervicovaginal viral shedding as well as clinical recurrences after treatment with gBgD.

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