Antiviral Research, 11 (1989) 203–214
Elsevier

AVR 00351

Herpes simplex virus glycoprotein immunotherapy of recurrent genital herpes: factors influencing efficacy

Lawrence R. Stanberry, Christopher J. Harrison, David I. Bernstein, Rae Lyn Burke, Rakesh Shukla, Gary Ott and Martin G. Myers

Division of Infectious Diseases, Children's Hospital Research Foundation, Cincinnati, Ohio; the James N. Gamble Institute of Medical Research, Cincinnati, Ohio; the Division of Biostatistics, University of Cincinnati College of Medicine, Cincinnati, Ohio and the Chiron Corporation, Emeryville, California, U.S.A.

(Received 1 December 1988; accepted 22 February 1989)

Summary

Recombinant herpes simplex virus type 1 (HSV-1) glycoproteins B (gB) and D (gD) were used as immunotherapeutic agents for the treatment of recurrent genital herpes in guinea pigs. Administration of a gBgD vaccine eight to 21 days after intravaginal HSV-2 inoculation significantly increased the titer of anti-HSV antibodies ($P<0.005$) while significantly reducing the frequency of subsequent herpetic recurrences ($P<0.05$). The effectiveness of gBgD immunotherapy was influenced by both the co-administration of adjuvant, the type of adjuvant, and by the timing and route of administration. These data demonstrate that recurrent HSV disease in animals with established latent infection may be favorably altered by the administration of immunogenic viral proteins.

HSV-1; Glycoprotein; Genital herpes; Guinea pig

Correspondence to: L.R. Stanberry, Division of Infectious Diseases, Children's Hospital Research Foundation, Elland and Bethesda Avenues, Cincinnati, OH 45229–2899, U.S.A.

Animals used in this study were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Children's Hospital Research Foundation.

Introduction

Initial herpes simplex virus (HSV) infection results in a host immune response which limits the primary disease but does not prevent the establishment of latency (Corey and Spear, 1986). Recurrent disease, a consequence of reactivation of latent virus, occurs despite measurable anti-HSV humoral and cellular immune responses (Stanberry, 1986). It has been suggested that augmentation of anti-HSV immunity in the latently infected host might result in a reduction in the frequency and/or severity of herpetic recurrences. Indeed, anecdotal reports and uncontrolled human studies have suggested that the administration of immunogenic viral components can reduce recurrent HSV infections (Meignier, 1985; Hall and Katrack, 1986). Using the guinea pig model of recurrent genital herpes (Stanberry et al., 1985a) we recently reported the first controlled experimental evidence that the administration of HSV glycoproteins to animals with established HSV latency not only augmented the host immune response but also reduced the frequency and severity of recurrent HSV disease (Stanberry et al., 1988) and genital virus shedding (Myers et al., 1988). In these earlier studies we emulsified the HSV antigens with complete Freund's adjuvant and immunized animals in the hind footpad. In this study we examined the influence of adjuvant as well as the timing and the route of administration upon the efficacy of HSV glycoprotein immunotherapy in order to explore therapeutic regimens which may be used in human clinical trials.

Methods

Virus

Clarified pools of the MS (ATCC VR-540) strain of HSV-2 were prepared in rabbit kidney cells and stored frozen at −70°C as previously described (Stanberry et al., 1985b).

Animals

Female Hartley guinea pigs (Charles River Breeding Laboratories, Wilmington, Mass, USA) weighing 350–450 g each were used. As previously described (Stanberry et al., 1982), the vaginal closure membrane was ruptured and the vaginal vault swabbed with a pre-moistened calcium alginate tipped swab (Spectrum Diagnostics, Glenwood, IL, U.S.A.). One hour later the guinea pigs were inoculated with $10^{5.7}$ pfu MS strain HSV-2 instilled into the vaginal vault via a syringe and 20-gauge plastic catheter. No antivirals were administered. During the initial infection the severity of genital skin disease was assessed daily by means of a previously described lesion score (Stanberry et al., 1982). The severity of initial clinical disease (severity score) was estimated by calculating the area under the lesion score–day curve. Control and vaccinated groups had similar mean severity scores. After recovery from initial infection animals were evaluated daily for evidence of

recurrent herpetic disease as previously described (Stanberry et al., 1985a). Evaluation was by observers blinded as to treatment.

Glycoproteins and adjuvants

HSV-1 gB and gD were prepared as previously described (Stanberry et al., 1988; Pachl et al., 1987; Sanchez-Pescador et al., 1988). Briefly, the glycoproteins were produced by cloning truncated gB and gD genes into mammalian cell expression vectors with subsequent expression in Chinese Hamster Ovary cells. A mixture of gB (12.5 μg) and gD (6.25 μg) (gBgD) was administered without adjuvant or combined with an equal volume of complete Freund's adjuvant (CFA) or an experimental adjuvant. CFA was obtained from Gibco (Grand Island, NY, U.S.A.). Ribi triple mix was kindly provided by Ribi Immunochem (Hamilton, Mont, U.S.A.) and contained per dose 50 μg of detoxified monophosphoryl lipid A, 50 μg of trehalose dimycolate and 50 μg of bacterial cell wall skeleton presented in 2% squalene and 0.008% Tween 80 (Ribi et al., 1976). The synthetic muramyl dipeptide *N*-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (MDP) was obtained from Ciba-Geigy Corp. (Basel, Switzerland) and emulsified with 40% squalene and 10% Arlacel A (50 μg/dose) (Sigma Chemical Co., St. Louis, MO, U.S.A.). Animals received the gBgD mixture on two occasions 21 days apart. The glycoprotein was administered either via the hindlimb footpad (100 μl per footpad) or intramuscularly into the thigh.

Serology

Antibody to HSV was measured by an ELISA using the recombinant HSV-1 gB or gD as a coating antigen at 5 μg/ml. Samples were evaluated in duplicate using a three-fold dilution series (Pachl et al., 1987). The titer was considered to be the reciprocal of the 20% endpoint. Sera collected prior to infection showed no measurable HSV specific antibody (<3).

Data analysis

Analysis of variance for repeated measures (i.e., lesion days over various time intervals) was done using the multivariate approach (Nelter and Wasserman, 1974). Multiple comparisons among group means were done using Duncan's multiple range test. All the analyses were performed using the Statistical Analysis System (SAS Institute, Raleigh, NC, U.S.A.).

Results

Timing of glycoprotein administration

We explored whether the efficacy of immunotherapy was influenced by the interval between intravaginal HSV-2 challenge and the administration of the gly-

coprotein mixture (Fig. 1 and Table 1). Animals were immunized with the gBgD mixture emulsified with complete Freund's adjuvant and delivered via the footpad during initial infection (day 8), early after recovery from initial infection (day 15) or after the onset of recurrent disease (day 21). A second dose of vaccine was administered 21 days after the first dose. Early initiation of immunotherapy produced the greatest reduction in recurrent disease. Animals treated with gBgD on day 8 exhibited a 45% reduction in recurrences (lesion days) during the first 3-week observation period compared to untreated controls. When these animals were boosted on day 29 the herpetic recurrences were reduced 68% over the second 3-week observation period compared to controls. The number of herpetic recurrences were also significantly decreased ($P<0.05$) when immunotherapy was initiated later after HSV-2 challenge (day 15 or day 21) (range of reduction of recurrences: 33–52%). While early initiation of therapy tended to produce a greater reduction in recurrent disease, the differences between the day 8 versus day 15 or day 21 treatment groups were not significant. After discontinuing glycoprotein therapy (third 3-week observation period), recurrent disease continued to occur at a reduced frequency in all three groups of treated animals, but the rate was not significantly less than that observed among the untreated controls.

Animals treated with gBgD developed significantly higher antibody titers than those observed in the untreated controls (Table 2). However, after discontinuing glycoprotein treatment elevated antibody titers were not sustained but began to decline by day 100. Early initiation of glycoprotein immunotherapy (day 8) resulted in higher anti-gB and anti-gD titers on days 20 and 41 post HSV-2 challenge than were seen in either untreated animals or in guinea pigs treated on days 15 or 21 post infection.

Adjuvant and route of glycoprotein administration

The adjuvant employed and the route of administration influenced both the clinical and humoral response to glycoprotein immunotherapy administered 21 and 42 days post infection. As shown in Figs. 2 and 3 and Tables 3 and 4, intramuscular or footpad administration of gBgD without adjuvant failed to affect herpetic recurrences. Glycoproteins co-administered with adjuvants reduced the frequency of recurrent genital herpes but the efficacy of the adjuvants was dependent upon the route of administration. Thus, footpad administration of gBgD with either CFA or the Ribi adjuvant on days 21 and 42 significantly decreased the number of days animals exhibited recurrent HSV disease over a 9-week period ($P<0.05$), while the MDP adjuvant given by this route was ineffective (Table 3). In contrast, the MDP adjuvant was effective ($P<0.05$) by the intramuscular route but the Ribi adjuvant was not (Table 4). Successful immunotherapy after footpad administration occurred in the first two study periods but did not persist, whereas successful suppression of recurrent disease after intramuscular vaccine administration occurred later.

Both the type of adjuvant and route of administration affected the magnitude of humoral immune responses to both gB and gD (Table 5). Footpad administra-

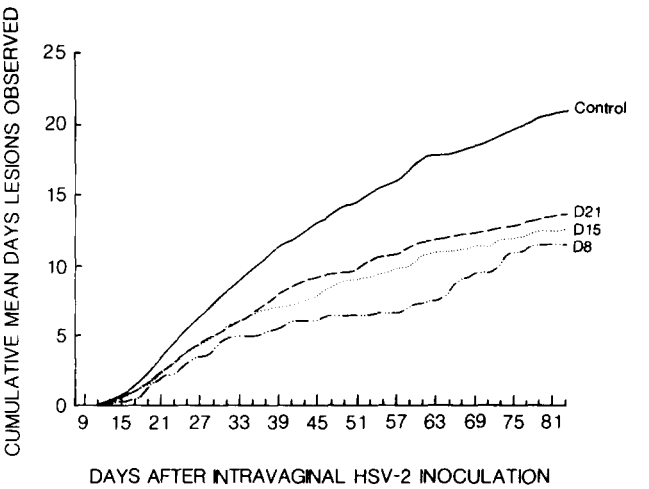

Fig. 1. The cumulative mean number of days when recurrent herpetic lesions were noted. These experimental groups were immunized twice with a vaccine containing gBgD + CFA administered via the hind footpad as follows: Day 8 and 29; day 15 and 36; day 21 and 42. The control group received no treatment. Animals were evaluated daily from day 8 through day 84.

TABLE 1

Glycoprotein immunotherapy of recurrent genital herpes: effect of the timing of gBgD administration

Treatment[a]	Time[b]	*N*	Mean lesion days per three-week observation period[c]			
			1st 3 wks	2nd 3 wks	3rd 3 wks	9 wks total
			Days 9–29	Days 30–50	Days 51–71	Days 9–71
Untreated control	–	19	7.3±0.8	7.4±0.8	3.6±0.6	18.2±1.8
gBgD + CFA	8,29	5	4.0±1.0[d]	2.4±0.6[f]	3.2±1.1	9.6±2.3[d]
			Days 16–36	Days 37–57	Days 58–78	Days 16–78
Untreated control	–	19	9.1±1.0	6.3±0.6	4.3±0.7	19.7±1.7
gBgD + CFA	15,36	9	6.1±1.7	3.0±1.0[e]	2.4±0.9	11.6±3.3[d]
			Days 22–42	Days 43–63	Days 64–84	Days 22–84
Untreated control	–	19	8.7±0.8	5.8±0.7	3.3±0.6	17.8±1.3
gBgD + CFA	21,42	22	5.8±0.7[e]	3.0±0.7[e]	2.1±0.5	10.9±1.7[e]

[a]Animals were treated with gB (12.5 μg) + gD (6.25 μg) with complete Freund's adjuvant (CFA) administered via the hind footpad, or were untreated. The same 19 untreated animals served as controls for each study group with the mean lesion day values calculated for the corresponding time intervals used for each treatment group. All animals experienced at least one recurrence except for one guinea pig in the day 15, 36 group.

[b]Days after intravaginal HSV-2 challenge when gBgD administered.

[c]Mean lesion days/animal ± SE. Lesion days were defined as days on which recurrent lesions were observed. Data calculated for three week intervals beginning the day after glycoprotein administration.

[d]Different from control ($P < 0.05$).

[e]Different from control ($P < 0.01$).

[f]Different from control ($P < 0.001$).

TABLE 2

Antibody to HSV glycoprotein B or D in guinea pigs intravaginally inoculated with HSV-2 and treated with HSV gBgD vaccine

Treatment[a]	Time[b]	*N*	Anti-gB[c]				Anti-gD[c]			
			Day 20	Day 41	Day 62	Day 100	Day 20	Day 41	Day 62	Day 100
Untreated control	–	10	517±97	851±93	691±69	618±60	12±3	18±5	35±9	61±8
gBgD + CFA	8,29	5	5310±1975	50797±4883	ND	8127±980[d]	490±238	9175±2809	ND	3737±1184[d]
gBgD + CFA	15,36	8	793±230	22284±5254	34109±9220	22499±2382[d]	74±18	5261±906	5297±949	5260±1134[d]
gBgD + CFA	21,42	10	194±41	26581±3453	40690±8403	23208±2947[d]	9±4	3770±748	11658±1775	6727±1232[d]

[a]Untreated or gB (12.5 μg) + gD (6.25 μg) administered with complete Freund's adjuvant (CFA), via the hind footpad.
[b]Days post-HSV-2 inoculation when gBgD administered.
[c]ELISA titers (20% endpoint) geometric mean±SE using either HSV-1 gB or gD as the capture antigen.
[d]Significantly different from untreated control $P < 0.005$. Statistical comparisons were analyzed only for day 100 blood samples.
ND, not done.

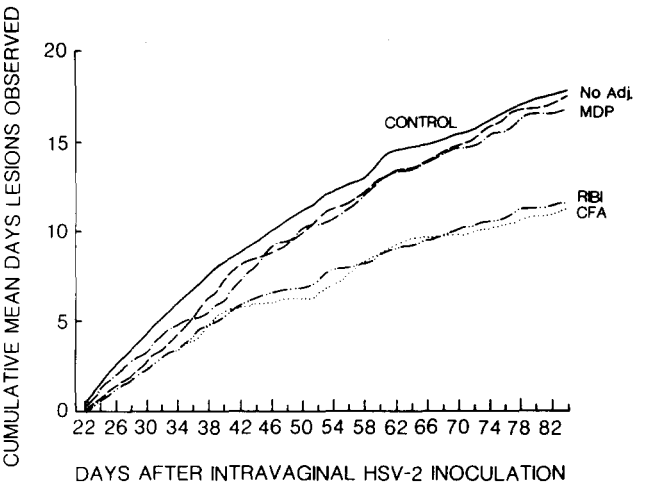

Fig. 2. The cumulative mean number of days when recurrent herpetic lesions were noted. Effect of gBgD with or without adjuvant given via footpad administration. Adjuvants: CFA, complete Freund's adjuvant; Ribi, Ribi triple mix; MDP, muramyl dipeptide. The control group received no treatment. Vaccine was administered on day 21 and again on day 42. Animals were evaluated for recurrences from day 22 through day 84.

tion of gBgD with any experimental adjuvant resulted in significantly higher anti-gB and anti-gD antibody titers than observed when gBgD was administered without adjuvant ($P<0.005$) and the antibody responses to gBgD plus the Ribi or MDP

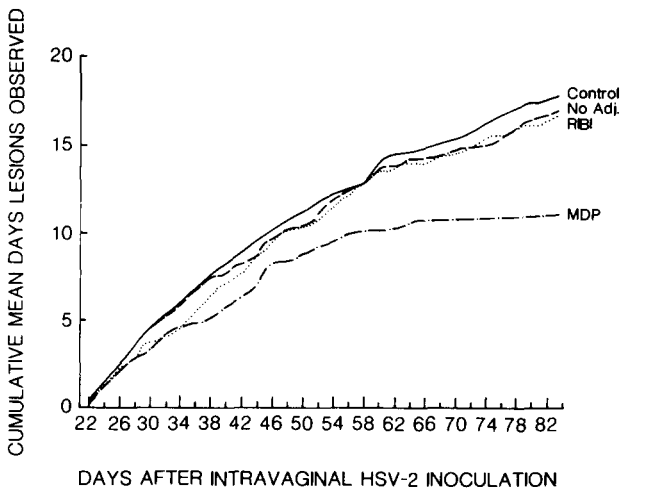

Fig. 3. The cumulative mean number of days when recurrent herpetic lesions were noted. Effect of gBgD with or without adjuvant given via intramuscular administration. Adjuvants: Ribi, Ribi triple mix; MDP, muramyl dipeptide. The control group received no treatment. Vaccine was administered on day 21 and again on day 42. Animals were evaluated for recurrences from day 22 through day 84.

TABLE 3

Glycoprotein immunotherapy of recurrent genital herpes: Effect of gBgD plus adjuvant given via footpad administration

Treatment[a]	*N*	Mean lesion days[b]			
		1st 3 wks Days 22–42	2nd 3 wks Days 43–63	3rd 3 wks Days 64–84	9 wks Days 22–84
Untreated control	19	8.7±0.8	5.8±0.6	3.3±0.6	17.8±1.7
gBgD + no adjuvant	9	7.4±0.5	6.1±1.0	4.0±0.8	17.6±1.7
gBgD + CFA	22	5.8±0.7[c]	3.0±0.7[c]	2.1±0.5	10.9±1.7[c]
gBgD + Ribi	11	5.6±1.0[c]	4.0±0.9	2.0±0.6	11.6±2.0[c]
gBgD + MDP	8	8.3±1.0	5.3±1.3	3.3±0.9	16.9±2.3

[a]Untreated or gB (12.5 μg) + gD (6.25 μg) administered with or without adjuvant on days 21 and 42. Adjuvants: CFA, complete Freund's adjuvant; Ribi, Ribi triple mix (see text); MDP, muramyl dipeptide. All animals experienced at least one recurrence between days 22–84.

[b]Mean lesion days/animal ± SE. Lesion days were defined as days on which recurrent lesions were observed. Data calculated for the three week intervals beginning the day after glycoprotein administration.

[c]Different from control ($P < 0.05$).

adjuvant were generally less after intramuscular injection than after administration via the footpad. When antibody was measured on day 100, animals that received gBgD without adjuvant by either route exhibited no significant increase in anti-gB antibody titer and only a modest increase in anti-gD antibody titer compared to the HSV-2 infected, untreated controls.

Discussion

We previously reported that the frequency of both clinically apparent recurrent genital herpes and recurrent cervicovaginal HSV-2 shedding was reduced in ani-

TABLE 4

Glycoprotein immunotherapy of recurrent genital herpes: effect of gBgD plus adjuvant given via intramuscular administration

Treatment[a]	*N*	Mean lesion days[b]			
		1st 3 wks Days 22–42	2nd 3 wks Days 43–63	3rd 3 wks Days 64–84	9 wks Days 22–84
Untreated control	19	8.7±0.8	5.8±0.7	3.3±0.6	17.8±1.7
No adjuvant	9	7.6±1.1	6.3±0.7	3.3±0.6	17.2±1.7
gBgD + Ribi	11	8.3±1.0	5.7±1.0	2.8±0.5	16.8±2.1
gBgD + MDP	7	6.1±1.1	4.0±0.8	0.7±0.4[c]	10.9±2.0[c]

[a]Untreated or gB (12.5 μg) + gD (6.25 μg) administered with or without adjuvant on days 21 and 42. Adjuvants: CFA, complete Freund's adjuvant; Ribi, Ribi triple mix (see text); MDP, muramyl dipeptide. All animals experienced at least one recurrence between days 22–84.

[b]Mean lesion days/animal ± SE. Lesion days were defined as days on which recurrent lesions were observed. Data calculated for three week intervals beginning the day after glycoprotein administration.

[c]Different from control ($P < 0.05$).

TABLE 5

Antibody to HSV glycoprotein B and D in guinea pigs intravaginally inoculated with HSV-2 and treated with HSV gBgD vaccine

Treatment[a]	Route[b]	*N*	Anti-gB[c]				Anti-gD[c]			
			Day 20	Day 41	Day 62	Day 100	Day 20	Day 41	Day 62	Day 100
Untreated control	–	10	517±97	851±93	691±69	618±60	12±3	18±5	35±9	61±8
gBgD + no adjuvant	FP	9	232±87	ND	ND	852±91	7±3	ND	ND	189±38
gBgD + no adjuvant	IM	9	284±34	ND	ND	893±126	13±7	ND	ND	184±40
gBgD + CFA	FP	10	194±41	26581±3453	40690±8403	23208±2947[d]	9±4	3770±748	11658±1775	6727±1232[d]
gBgD + Ribi	FP	10	252±63	4323±646	8969±1262	2686±458[d]	14±3	580±142	2362±520	766±142[d]
gBgD + Ribi	IM	11	287±65	2464±423	2960±387	1250±196[d]	16±5	138±21	495±84	317±61[d]
gBgD + MDP	FP	9	198±48	3079±829	15380±3173	4988±1130[d]	12±4	176±75	1758±317	815±160[d]
gBgD + MDP	IM	7	232±46	2768±381	5545±904	4334±465[d]	14±5	204±49	900±159	658±83[d]

[a]Untreated or gB (12.5 μg) + gD (6.25 μg) administered with or without adjuvant on days 21 and 42. Adjuvants: CFA, complete Freund's adjuvant; Ribi, Ribi triple mix (see text); MDP, muramyl dipeptide (see text).

[b]FP, footpad; IM, intramuscular.

[c]ELISA titers (20% endpoints) geometric mean±SE using either HSV-1 gB or gD as the capture antigen.

[d]Significantly different from untreated control $P < 0.005$. Statistical comparisons were analyzed only for the day 100 blood samples.

ND, not done.

mals with established HSV latency by the footpad administration of HSV glycoproteins emulsified with complete Freund's adjuvant (Stanberry et al., 1988; Myers et al., 1988). Similarly, Ho et al. (1988) observed that intravenous administration of HSV-1 gD with adjuvant in a liposome formulation reduced genital HSV recurrences in guinea pigs by 75% while intramuscular administration of gD plus adjuvant in a traditional oil emulsion produced a 40–50% reduction in recurrent disease. Our present study confirms the previous observations that treatment with HSV glycoproteins given after primary infection favorably alters the natural history of recurrent genital HSV-2 infections. In addition, we have extended the earlier studies by demonstrating that the efficacy of HSV glycoprotein immunotherapy was affected by the timing of immunization after HSV-2 challenge, by the adjuvant used and by the route of vaccine administration. We observed that early initiation of glycoprotein immunotherapy during initial infection (day 8) tended to induce the greatest reduction in recurrences. Future studies will need to address whether the simultaneous initiation of acyclovir therapy and glycoprotein immunotherapy during the initial infection will result in fewer subsequent recurrences.

Effective glycoprotein immunotherapy was adjuvant and route of administration dependent. No effect was observed in the absence of adjuvant. Similar to our previous findings (Stanberry et al., 1988; Myers et al., 1988), in this study glycoproteins emulsified with CFA and administered via the footpad effectively reduced herpetic recurrences. While CFA is a potent immuno-stimulating agent it can not be used in humans, therefore we evaluated two other experimental adjuvants, a Ribi adjuvant containing detoxified microbial extracts (Ribi et al., 1976, 1987) and a synthetic muramyl dipeptide adjuvant presently being used in human trials (Jones et al., 1988). In the footpad, the vaccine containing Ribi adjuvant was equivalent to vaccine containing CFA but vaccine with MDP adjuvant did not reduce the frequency of recurrences. In contrast, the MDP-gBgD vaccine given by the intramuscular route was effective while the Ribi-gBgD vaccine administered intramuscularly did not reduce recurrences. We did not examine the effect of MDP or Ribi adjuvant alone on recurrent disease because we had previously observed that administration of the more potent immunostimulator, CFA, did not alter the course of recurrences in the guinea pig (Stanberry et al., 1988). A recent report by Berman et al. (1988) failed to observe a reduction in recurrent disease when HSV-1 or HSV-2 gD adsorbed to alum was administered subcutaneously to guinea pigs after recovery from primary infection. The failure of these investigators to confirm the effect of postinfection immunotherapy upon subsequent recurrences may be a consequence of the adjuvant used, the route by which vaccine was administered or the immunogenicity of the glycoprotein vaccine. Using similar HSV subunit vaccines, we and others have observed that alum is a poor adjuvant compared to CFA and that HSV glycoprotein/alum vaccines provide less protection against primary infection when administered subcutaneously, vis-à-vis via the footpad (Sanchez-Pescador et al., 1988; Thomson et al., 1983; Berman et al., 1985; Stanberry et al., 1986).

The mechanism by which glycoprotein-mediated immune modulation alters HSV recurrences is undefined. Although the protection provided by pre-infection pro-

phylaxis with HSV subunit vaccines appears to correlate with anti-HSV antibody titers (Sanchez-Pescador et al., 1988; Thomson et al., 1983; Stanberry et al., 1987), the data from this study indicate that the ability to induce high titer antibody responses to the vaccine components does not correlate with reduction in herpetic recurrences. For example, gBgD given with the MDP or Ribi adjuvant induce similar antibody titers but discordant clinical responses. It is likely that suppression of recurrent disease is mediated by HSV specific cellular immune responses (Rouse et al., 1988). Preliminary studies suggest that gBgD given with CFA in the footpad induces enhanced lymphoproliferative, cytolytic, and lymphokine responses in HSV-2 infected guinea pigs (Harrison et al., 1987) and induced antibody dependent cell cytotoxicity which was protective in the lethal neonatal murine model (Bernstein et al., 1988).

We have established that HSV glycoprotein immunization of animals with latent HSV infection can favorably modify the frequency of recurrent genital HSV-2 infection although the effect is adjuvant and route of administration dependent. While time after infection affects efficacy, glycoprotein immunotherapy was effective when initiated during or after primary infection. It is possible that the use of immunogenic HSV glycoproteins co-administered with potent adjuvants may provide a new strategy for controlling recurrent HSV infections in humans.

Acknowledgements

This work was supported in part by grants AI-22667, AI-23482 and HD-22214 from the National Institutes of Health and from Chiron Corporation. We thank Alisa Black, Jean Carter and Frances Childs for technical assistance; Sandra Bauer for manuscript preparation.

References

Berman, P.W., Gregory, Y., Crase, D. and Lasky, L.A. (1985) Protection from genital herpes simplex virus type 2 infection by vaccination with cloned type 1 glycoprotein D. Science 227, 1490–1492.

Berman, P.W., Vogt, P.E., Gregory, T., Laskey, L.A. and Kern, E.R. (1988) Efficacy of recombinant glycoprotein D subunit vaccines on the development of primary, recurrent and latent genital infections with herpes simplex virus type 2 in guinea pigs. J. Infect. Dis. 157, 897–902.

Bernstein, D.I., Loo, L.S. and Kohl, S. (1988) Antibody to cloned HSV glycoproteins B and D plus adult human lymphocytes protect neonatal mice from lethal HSV challenge. Antiviral Res. 10, 279–288.

Corey, L. and Spear, P.G. (1986) Infections with herpes simplex virus. N. Engl. J. Med. 314, 686–691.

Hall, M.J. and Katrack, K. (1986) The quest for a herpes simplex virus vaccine: background and recent developments. Vaccine 4, 138–150.

Harrison, C.J., Bernstein, D.I., Stanberry, L.R. and Myers, M.G. (1987) Cell mediated immune responses induced by cloned glycoprotein B (gB) and D (gD) immunotherapy of recurrent genital HSV-2 infection. Third International Conference on Immunobiology and Prophylaxis of Human Herpesviruses. Marco Beach, Florida, (Abstract).

Ho, R.J.Y., Burke, R.L. and Merigan, T.C. (1988) Effect of liposome encapsulated recombinant herpes simplex glycoprotein in controlling recurrent herpes genitalis. Antiviral. Res. 9, 153 (Abstract).

Jones, W.R., Judd, S.J., Ing, R.M.Y., Powell, J., Bradley, J., Denholm, E.H., Mueller, U.W., Griffin, P.D. and Stevens, V.C. (1988) Phase I clinical trial of a World Health Organization birth control vaccine. Lancet 1, 1295–1298.

Meignier, B. (1985) Vaccination against herpes simplex virus infections. In: B. Roizman and C. Lopez (Eds.), The Herpesviruses, Vol. 4. Immunobiology and Prophylaxis of Human Herpesvirus Infections, pp. 265–296. Plenum Press, New York.

Myers, M.G., Bernstein, D.I., Harrison, C.J. and Stanberry, L.R. (1988) Herpes simplex virus glycoprotein treatment of recurrent genital herpes reduces cervicovaginal virus shedding. Antiviral. Res. 10, 83–88.

Nelter, J. and Wasserman, W. (1974) Applied Linear Statistical Models; Regression, Analysis of Variance, and Experimental Design, pp. 214–267. Richard Irwin, Homewood, IL.

Pachl, C., Burke, R.L., Sanchez-Pescador, L., Van Nest, G., Masiarz, F. and Dina, D. (1987) Expression of cell-associated and secreted forms of herpes simplex virus type 1 glycoprotein B in mammalian cells. J. Virol. 61, 315–325.

Ribi, E., Takayama, K., Milner, K., Gray, G.R., Goren, M., Parker, R., McLaughlin, C. and Kelly, M. (1976) Regression of tumors by an endotoxin combined with trehalose mycolates of differing structure. Cancer Immunol. Immunother. 1, 265–270.

Ribi, E., Ulrich, J.T., Mashi, K.N. (1987) Immunopotentiating activities of monophosphoryl lipid A. In: J.A. Majde (Ed.), Immunopharmacology of Infectious Disease: Vaccine Adjuvants and Modulators of Non-Specific Resistance, pp. 101–112. Alan R. Liss, Inc, New York.

Rouse, B.T., Norley, S. and Martin, S. (1988) Antiviral cytotoxic T lymphocyte induction and vaccination. Rev. Infect. Dis. 10, 16–33.

Sanchez-Pescador, L., Burke, R.L., Ott, G., Van Nest, G. (1988) The effect of adjuvants on the efficacy of a recombinant HSV glycoprotein vaccine. J. Immunol. (In Press).

Stanberry, L.R. (1986) Herpesvirus latency and recurrence. Prog. Med. Virol. 67, 61–77.

Stanberry, L.R., Kern, E.R., Richards, J.T., Abbott, T.M. and Overall, J.C., Jr. (1982) Genital herpes in guinea pigs: pathogenesis of primary infection and description of recurrent disease. J. Infect. Dis. 146, 397–404.

Stanberry, L.R., Kern, E.R., Richards, J.T. and Overall, J.C., Jr. (1985a) Recurrent genital herpes simplex virus infection in guinea pigs. Intervirology 24, 226–231.

Stanberry, L.R., Kit, S., Myers, M.G. (1985b) Thymidine kinase deficient herpes simplex virus type 2 genital infection in guinea pigs. J. Virol. 55, 322–328.

Stanberry, L.R., Stephanopoulos, D.E., Burke, R.L., Pachl, C.A. and Myers, M.G. (1986) The efficacy of herpes simplex virus glycoprotein vaccines [abstract no. 266]. In: Program and Abstracts of the 11th International Herpesvirus Workshop. University of Leeds, Leeds, United Kingdom.

Stanberry, L.R., Bernstein, D.I., Burke, R.L., Pachl, C. and Myers, M.G. (1987) Vaccination with recombinant herpes simplex virus glycoproteins: protection against initial and recurrent genital herpes. J. Infect. Dis. 155, 914–920.

Stanberry, L.R., Burke, R.L. and Myers, M.G. (1988) Herpes simplex virus glycoprotein treatment of recurrent genital herpes. J. Infect. Dis. 157, 156–163.

Thomson, T.A., Hilfenhaus, T., Moser, H. and Morahan, P.S. (1983) Comparison of effects of adjuvants on efficacy of virion envelope herpes simplex virus vaccine against labial infection of Balb/c mice. Infect. Immun. 41, 556–562.