Soluble Glycoprotein D Blocks Herpes Simplex Virus Type 1 Infection of Rat Eyes

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Received 24 February 1992/Accepted 15 May 1992

Herpes simplex virus type 1 (HSV-1) ocular infection in rats was blocked by treating the eyes with UV-inactivated virions containing glycoprotein D (gD) prior to ocular challenge. In contrast, rats treated with UV-inactivated virions lacking gD were not protected. A soluble, truncated form of HSV-2 gD (gD-2t) also protected against ocular infection. Treatment with gD-2t not only reduced mortality but also restricted progression of pathology and reduced the amount of viral antigen in the cornea. Host antibody or alpha/beta interferon responses to the gD-2t treatment were not detected. These results are similar to those observed in cell culture (D. C. Johnson, R. L. Burke, and T. Gregory, *J. Virol.* 64:2569–2576, 1990). The *in vivo* effect of exogenous gD is consistent with blocking of a cell surface gD receptor or with an inhibitory interaction of gD with virions.

Herpes simplex virus type 1 (HSV-1) is capable of infecting diverse cell types from many animal species. To have such a broad host range, HSV-1 must utilize mechanisms of adsorption and penetration that are effective across a wide spectrum of cell types. As yet, these mechanisms are not completely understood, in part because of the relatively large number of HSV-1 glycoproteins. Of the glycoproteins that have been characterized, only three (gB, gD, and gH) have been shown to be essential for productive infection *in vitro* (6, 19, 20, 32). In cell culture, these glycoproteins are required for virion penetration (2, 6, 19, 20, 32), which occurs by fusion of the virion envelope with the cell plasma membrane (26). In addition, a fourth glycoprotein, gC, appears to be the primary mediator of virion adsorption (12, 18), which involves binding to heparan sulfate proteoglycans on the cell surface (21, 37). In the absence of gC, other glycoproteins, probably including gB, may function in a similar manner (12). HSV-1 mutants lacking any one of the essential glycoproteins can adsorb to cells, but fusion of the viral envelope with the cell membrane does not occur (2, 6, 7, 19, 20, 32).

Of the essential glycoproteins, the requirement for gD is perhaps the best characterized. Antibodies specific for gD are strongly neutralizing (5, 8, 9, 13, 29) and can block the fusion of infected cells (22, 27). Viral mutants unable to produce gD, such as F-gD β and F-US6kan, produce only noninfectious virions when grown on wild-type cells, although they can be propagated on complementing cell lines (16, 19). Additionally, cell lines that constitutively express gD are resistant to infection with HSV (3, 17). Infection with live challenge virus can also be blocked by prior treatment of cells with UV-inactivated wild-type virus. However, treatment with virions lacking gD does not block superinfection, indicating that gD is required for blocking (16). In addition, cells treated with exogenous soluble gD are protected from subsequent HSV infection (15). The unique protective effect of gD-containing particles and soluble gD may be related to the role of gD in penetration or possibly in preventing superinfection. However, comparatively little is known about the function of gD in herpetic disease. This study

demonstrates that the inhibitory effect shown by gD in cell culture also occurs in an animal model of HSV-1 ocular infection.

MATERIALS AND METHODS

Animals. Female Fischer 344 rats (150 g) were purchased from Charles River (Wilmington, Mass.). Following viral infection, rats were maintained in cages with filter bonnets.

Viruses and cells. HSV-1 wild-type strains KOS321 and McKrae were passaged either in normal fibroblasts isolated from xiphisterna of Fischer 344 rats or in Vero cells. Virus passaged in rat fibroblasts was used to infect animals. F-gD β was provided by David C. Johnson (McMaster University, Hamilton, Ontario, Canada) and passaged in VD60 cells (19). Virions lacking gD were produced by infecting Vero Cells with F-gD β . To quantify virions lacking gD, DNA slot blots of F-gD β and KOS321 virus preparations were compared by using an HSV-1-specific ³²P-labelled probe. KOS321 and F-gD β virions were UV inactivated by exposure to a UV lamp (18.4 W, 254 nm) at a distance of 15 cm for 1 h. For interferon (IFN) studies, vesicular stomatitis virus (VSV), Indiana strain, was provided by V. Fay Righthand (Wayne State University, Detroit, Mich.). Rat NRK-52E cells were purchased from the American Type Culture Collection (Rockville, Md.).

Proteins and glycoproteins. The soluble form of recombinant HSV-2 gD (gD-2t) was the generous gift of Genentech, Inc. (South San Francisco, Calif.) (1). The truncated gD-2t contains the first 301 amino acids of the protein, the majority of the 324-amino-acid extracellular domain (1). Tissue culture-grade bovine serum albumin (BSA; fraction V, low in endotoxin, and essentially globulin free) was purchased from Sigma (St. Louis, Mo.). Fetuin, from fetal calf serum, was obtained from Calbiochem (La Jolla, Calif.). All proteins were diluted in sterile phosphate-buffered saline (PBS; pH 7.0) and used at either 0.5 or 1.0 mg/ml.

***In vivo* blocking and infection.** Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (3.25 mg per rat). The right eye was abraded three times with a 26-gauge needle, and tear fluid was blotted from the medial canthus. In experiments in which UV-irradiated virions were tested for blocking activity, rats received 2×10^7 PFU (or

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equivalent as measured by slot blot analysis of HSV DNA) of UV-inactivated KOS321 or F-gD β 2 h prior to challenge with McKrae. Rats received a challenge dose of McKrae (1.5×10^4 to 4.5×10^4 PFU per rat, the equivalent of 10 to 30 50% lethal doses [LD₅₀]) at 0 h. For experiments in which gD-2t, BSA, or fetuin was tested, 10 μ l of glycoprotein solution was dropped onto the ocular surface and massaged into the eye with the eyelid. These rats were challenged at 0 h with McKrae suspended in the protein solution.

Evaluation of ocular pathology. Rats were lightly anesthetized with ether, and their eyes were examined with a slit-lamp biomicroscope (Kowa SL-5; Keeler Instruments, Broomall, Pa.). Eyes were clinically evaluated for corneal ulceration, opacity, vascularization, and uveitis. Eyes were also enucleated, fixed, and processed for routine histologic examination. Median sections of whole globe were stained with hematoxylin and eosin.

Immunostaining of eyes for viral products. The method for immunostaining whole eyes has been previously described (36). Briefly, eyes from uninfected, infected, and gD-2t-treated and infected rats were enucleated and fixed for 30 min with 1.5% glutaraldehyde in sterile PBS (pH 7). Eyes were washed with PBS overnight at room temperature (RT). Next, they were incubated for 1 h at RT with the primary antiserum, a polyclonal rabbit anti-HSV-1 diluted 1:500 in 3% BSA. After incubation, eyes were washed with PBS and preincubated for 30 min with a 1:10 dilution of normal goat serum in PBS. Eyes were then incubated for 1 h at RT with the secondary antibody, goat anti-rabbit immunoglobulin G (IgG) (Fc)-peroxidase conjugate (Promega, Madison, Wis.) diluted 1:1,000 in 3% BSA. Peroxidase activity was localized by incubating the eyes for 15 min at RT with a 4-chloro-1-naphthol solution (Promega). Eyes were photographed by using a Wild dissecting microscope equipped with a 35-mm camera.

Sample collection. Tears were collected by placing calibrated 5- μ l micropipets in the medial canthus of each eye. The volume of tears collected from each rat was recorded (usually 7 to 15 μ l), and the tears were expelled into 100 μ l of PBS-1% BSA. Serum was obtained from 1 ml of blood collected from the lateral tail vein. Equal volumes of individual samples from each experimental group were pooled and frozen at -20°C until assayed for HSV-1-specific antibodies or interferons.

RIA for detection of HSV-1-specific antibodies. Pooled tear and serum samples were assayed for anti-HSV antibodies by solid-phase radioimmunoassay (RIA). PVC microtiter plates (Falcon, Oxnard, Calif.) were coated with KOS321 passaged in Vero cells. The plates were washed, twofold serial dilutions of the sample or polyclonal reference standard were added to the wells, and the plates were incubated overnight at RT. The plates were washed, and ^{125}I -labelled rabbit anti-rat isotype-specific antibody, provided by Jane V. Pappard (CIBA-GEIGY Corporation, Summit, N.J.), was added to the appropriate wells. The plates were incubated at RT for 5 h and washed, and bound radioactivity was determined by using an LKB Multigamma 1260 gamma counter (Pharmacia LKB Nuclear, Inc., Gaithersburg, Md.). The quantity of bound antibody was determined by using standard curves generated from polyclonal reference standards included in each assay.

Reference standards were produced and quantified as previously described (30). Standards were HSV-1-immune serum (for IgG and IgM) and immune bile (for IgA). Polyclonal IgG and IgM anti-HSV-1 antibodies were produced by injecting UV-inactivated KOS321 virions intraperitoneally

into rats. Blood was collected by cardiac puncture at days 4 and 14, and the sera were pooled. Polyclonal IgA-specific antibodies were elicited by injecting UV-inactivated KOS321 into rat Peyer's patches (10). The common bile ducts of the immunized rats were cannulated at day 13, and bile was collected overnight (28). Serum and bile were tested by RIA for anti-HSV activity. The concentrations of anti-HSV antibody in the polyclonal standards were determined to be 2.8 μg of IgG per ml and 0.7 μg of IgM per ml in the serum pool and 0.5 μg of IgA per ml in the bile.

Assay for IFN- α/β . Serum samples were assayed for IFN by the VSV plaque reduction assay. Confluent NRK-52E cell monolayers in 24-well flat-bottom tissue culture plates (Falcon) were treated with twofold dilutions of serum samples or rat IFN- α/β standards (Lee BioMolecular Research Laboratories, Inc., San Diego, Calif.) for 24 h at 37°C . After incubation, the samples were removed and the cells were infected with 100 to 150 PFU of VSV in 100 μ l of Dulbecco modified Eagle medium (GIBCO, Grand Island, N.Y.) supplemented with 4% fetal calf serum (HyClone, Logan, Utah) and antibiotics. Plates were incubated for 40 min at 37°C to permit viral adsorption and then overlaid with 1% methylcellulose in supplemented medium. After 42 h, the cell monolayers were stained with crystal violet and the number of plaques per well was determined. Percent plaque reduction was converted to quantitative values (international units per milliliter), using standard curves generated with the rat IFN- α/β standards included in each assay.

RESULTS

Blocking of HSV-1 infection in vivo by using UV-inactivated whole virus. Initially, we sought to determine whether viral entry into ocular tissues could be blocked by UV-inactivated virus and, if so, whether the blocking activity had the same requirement for gD observed in tissue culture. To do so, we treated the abraded corneas of one group of rats (five rats per group) with UV-inactivated virions of wild-type strain KOS321. The dose of virus used was 2×10^7 PFU per rat and was determined from the titer prior to UV inactivation. A second group was treated with a similar quantity of UV-inactivated F-gD β virions produced in Vero cells. The concentration of F-gD β virions was determined by comparison of the relative concentrations of viral DNA in the cell-free F-gD β and KOS321 stocks. A third group received only PBS. Two hours later, all rats were infected with 1.5×10^4 PFU (10 LD₅₀) of the pathogenic HSV-1 strain McKrae. Typically, rats infected on the abraded corneas with 10 to 30 LD₅₀ died from neurologic disease within 10 to 14 days of infection (unpublished results). The survival curves show that treatment with UV-inactivated KOS321 virions, but not F-gD β virions, protected rats from ocular challenge with a pathogenic strain of HSV-1 (Fig. 1). This finding suggested that the F-gD β virions lacked a component necessary for blocking infection with the challenge virus.

The mutant virus, F-gD β , has been well characterized (19). The virus is unable to produce gD and the nonessential glycoprotein gI. In cell culture, it has been shown that F-US6kan, a mutant which fails to produce gD but does produce gI, behaves similarly to F-gD β with respect to blocking activity (19). This finding suggested that the failure of F-gD β virions to block infection in rat eyes was due to a lack of virion gD. However, to verify that F-gD β virions lacked gD yet contained wild-type quantities of the other major viral glycoproteins, gB and gC, ^{35}S -labelled KOS321 and F-gD β were produced in Vero cells and purified on

FIG. 1. Blocking of HSV-1 infection in vivo, using whole virus. The abraded eyes of two groups (five rats per group) were treated with 2×10^7 UV-inactivated PFU of KOS321 or the equivalent of UV-inactivated F-gD β . The third group (five rats) received only PBS. Rats were challenged 2 h later with 1.5×10^4 PFU (10 LD₅₀) of McKrae strain HSV-1, and data were plotted as percent survival versus days postinfection.

dextran gradients (33). Monoclonal antibodies were used to immunoprecipitate gB, gC, and gD from Nonidet P-40 extracts of virions (14). The immunoprecipitates and extracts were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Glycoprotein bands on the gels were quantified directly, using an AMBIS autoradiographic imaging system. The yield of mutant virions was approximately fivefold lower than that of KOS321. Normalizing for this difference, we found that the quantity of gD in F-gD β virions was reduced at least 30-fold, while levels of gB and gC varied by at most 2-fold. In addition, comparison of the total glycoprotein profiles did not reveal any unexpected difference in the minor glycoprotein bands between the two virion types (data not shown).

Blocking of HSV-1 infection in vivo by using soluble gD. A recombinant, truncated form of gD from HSV-2 (gD-2t) has been shown to be effective in blocking both HSV-1 and HSV-2 infection in vitro (15). To determine whether a similar blocking effect could occur in vivo, gD-2t was tested in the rat eye model. A 10- μ l volume of a solution containing 0.5 mg of gD-2t per ml was placed on scarified eyes of anesthetized rats 1 h prior to viral challenge (–1 h), at the time of viral challenge (0 h), and at 1, 2, and 3 h after infection. Control rats received only PBS at the same time points. Both groups were challenged with a lethal dose of McKrae at 0 h. The rats that received gD-2t treatment had a significantly higher rate of survival than did the control rats (Table 1, experiment 1).

To determine the optimal course of the gD-2t treatment, the number and timing of gD-2t doses were varied. Each dose contained 5 μ g of the glycoprotein. The use of two doses of soluble gD-2t, administered at –1 and 0 h, was as effective at blocking infections as were five hourly doses (Table 1, experiment 2; Fig. 2A). Successful blocking of infection required that gD-2t be present prior to and at the time of infection (Table 1, experiment 3; Fig. 2B). In contrast, rats treated with a single dose either 1 h before or 1 h after lethal dose challenge were not protected. These data show a significant reduction in mortality when gD-2t was present before and during virus adsorption to the eye.

The final experiments in this series were designed to document the specificity of the gD-2t protective effect. To rule out the possibility that the effect was the result of nonspecific protein binding, the ability of the commonly used nonglycosylated blocking agent BSA and the glycoprotein fetuin to either block infection or inhibit the protective effect of gD-2t treatment was determined. Rats received 5 μ g of BSA either alone or together with 5 μ g of gD-2t at –1 and 0 h and were challenged with 30 LD₅₀ of McKrae at 0 h. Experiments using fetuin were performed similarly. Again, the rats receiving the gD-2t treatment had a significantly higher survival rate than did those treated with only BSA or fetuin (Table 1, experiments 4 and 5; Fig. 2C and D). The percent survival in the groups treated with gD-2t plus BSA or fetuin was comparable to that observed in previous experiments even though the rats received a higher dose of

TABLE 1. Blocking of HSV-1 infection in vivo, using soluble gD-2t

Expt. no.	No. of animals/group	Treatment ^a	Treatment time (h) ^b	% Survival	P ^c
1	18	PBS	–1, 0, 1, 2, 3	22.2	<0.025
	18	5 μ g of gD-2t	–1, 0, 1, 2, 3	66.7	
2	17	PBS	–1, 0	11.8	<0.005
	17	5 μ g of gD-2t	–1, 0	88.2	
	17	5 μ g of gD-2t	–1, 0, 1, 2, 3	88.2	
3	12	PBS	–1, 0	0	<0.05
	13	5 μ g of gD-2t	–1, 0	46.2	
	15	5 μ g of gD-2t	–1	13.3	
	13	5 μ g of gD-2t	+1	7.7	
4	15	5 μ g of BSA ^e	–1, 0	0	<0.005
	15	5 μ g of BSA + 5 μ g of gD-2t	–1, 0	53.3	
5	18	5 μ g of fetuin	–1, 0	0	<0.025
	18	5 μ g of fetuin + 5 μ g of gD-2t	–1, 0	38.9	

^a All treatments were administered to abraded corneas.

^b All rats received a lethal dose of McKrae at 0 h. In experiments 1 to 3, rats were challenged with 20 LD₅₀ of McKrae. In experiments 3 and 4, rats received 30 LD₅₀.

^c Significance of gD treatment compared with the control (PBS, BSA, or fetuin), as determined by the chi-square test for contingency tables (38).

^d NS, not significant by the chi-square test.

^e Fraction V, low endotoxin, and essentially globulin free.

FIG. 2. Blocking of HSV-1 infection in vivo, using soluble gD-2t. Survival curves of rats treated with gD-2t were plotted against time. Panels A through D represent experiments 2 through 5 in Table 1.

challenge virus. These results indicate that HSV-1 entry into ocular tissues can be specifically blocked by treatment with soluble gD-2t.

Effect of gD-2t on ocular pathology. Randomly selected rats from several experiments were examined for the development of HSV-1-induced ocular disease. The eyes of lightly anesthetized rats were examined twice weekly for 2 weeks. Of the 22 control rats that received only a lethal dose of McKrae, all developed severe uveitis prior to death. Eleven of twenty-five gD-2t-treated rats also developed severe ocular disease and died. Of the remaining rats, 11 were clinically normal and 3 had mild corneal opacities. Histologic examination of gD-2t-treated severely diseased eyes revealed pathologic lesions similar to those in the untreated controls. These results confirm that gD-2t treatment protected rats by limiting viral entry into the ocular tissues.

Immunostaining of eyes. To determine whether treatment of the eye with gD-2t actually inhibited infection of the ocular surface, eyes from uninfected rats, infected rats, and infected rats receiving gD-2t treatment were stained for the presence of HSV-1 antigens by using a rabbit anti-HSV-1 antiserum. Infected rats were challenged with 20 LD₅₀ of HSV-1 McKrae. Rats that were given gD-2t received 5 μ g at -1 and 0 h prior to challenge. In two separate experiments, the eyes from a total of four uninfected rats, six rats challenged with McKrae alone, and seven rats treated with gD-2t prior to challenge were examined 48 h after infection.

As reported for the mouse eye (36), a nonspecific peroxidase reaction occurred around the conjunctiva. The eyes of uninfected animals displayed no specific staining, even within the scarifications (Fig. 3A). Eyes from rats receiving only McKrae, but not those receiving gD-2t and McKrae, had strongly stained regions on the corneal epithelium (Fig. 3B). These regions were roughly circular in shape, with the most intensely stained area around the periphery, and resembled plaques formed in tissue culture. Eyes from rats treated with gD-2t had essentially no stained regions (Fig. 3C). The lack of positive staining on the eyes of gD-2t-treated rats indicates that gD-2t blocked infection of the cornea and confirms the protective effect of gD observed in our other experiments.

Host responses to gD-2t treatment. Ocular antigen administration has been shown to elicit both local and systemic antibody responses (23-25, 30). Cells of the cornea produce IFN- α/β (35), which has been shown to protect against ocular infection and to reduce invasiveness of some strains of HSV-1 (11, 34). To determine whether either of these host responses were induced by application of gD-2t, we examined the production of antibodies in tears and serum as well as IFN in serum of rats treated only with gD-2t.

The abraded cornea of six rats received 5 μ g of gD-2t every hour for 5 h. Tear and serum samples were collected twice weekly for 2 weeks then analyzed by solid-phase RIA for the presence of HSV-1-specific antibodies in tears (secre-

FIG. 3. Light micrographs of whole Fischer 344 rat eyes stained for HSV-1 antigens 48 h after application to abraded corneas of PBS (A), 20 LD₅₀ of McKrae (B), and 5 µg of gD-2t at -1 h and 5 µg of gD-2t plus 20 LD₅₀ of McKrae at 0 h (C). The arrow denotes a region of strong specific staining. The bright white circle is an artifact of the camera lighting.

tory IgA) or serum (IgG and IgM). Minor fluctuations in the amount of HSV-1-specific antibodies were noted. However, the levels did not increase dramatically above the pretreatment levels (Fig. 4). In studies of the rat immune response to ocular herpetic infection, rats receiving HSV-1 on abraded corneas began showing increased secretory IgA tear and IgG serum antibody levels on day 8. These levels increased approximately 5-fold by day 14 (unpublished data). Additionally, when the gD-2t-treated rats were challenged with a lethal dose of McKrae 43 days after receiving gD-2t, none survived. Thus, the protection from lethal dose challenge by soluble gD-2t application was not due to the development of an antibody response directed against gD-2t.

Serum was obtained from six rats before and at 4, 8, and 24 h after receiving two 5-µg doses of gD-2t. Serum was also collected from control rats that received only 20 LD₅₀ of McKrae. The serum samples were analyzed for IFN by inhibition of VSV plaque formation. Ocular infection with McKrae resulted in a serum IFN response, whereas treat-

ment of the eye with gD-2t did not elicit a detectable IFN response (Table 2). These data suggest that the protection by soluble gD-2t treatment did not result from the development of an IFN-α/β response.

DISCUSSION

The work described here shows that lethal encephalitis resulting from HSV-1 infection of scarified rat corneas was blocked by pretreatment with UV-inactivated wild-type HSV-1 virions but not by F-gDβ, a mutant unable to produce gD. Ocular infection was also blocked by application of gD-2t, a soluble truncated form of HSV-2, shortly before and during challenge. The effectiveness with which gD-2t blocked subsequent infection was demonstrated not only by the survival of the infected animals but also by the lack of virus-induced ocular lesions and the inability to detect viral antigens in the eyes of gD-2t-treated rats. These results indicate a large reduction in virus replication at the portal of entry, the abraded cornea, thereby reducing virus spread, the probability of viral invasion of the central nervous system, and the incidence of encephalitis. The transient nature of the blocking effect suggested that the gD-2t treatment protocol did not elicit an immune response. This conclusion was confirmed by the inability of gD-2t, under these experimental conditions, to induce a detectable increase in antiviral antibodies. Further, exposure to gD-2t did not elicit detectable IFN-α/β. The fact that soluble gD

FIG. 4. Antibody response to gD-2t treatment. Five micrograms of gD-2t was applied to the abraded corneas of six rats every hour for 5 h. Tear and blood samples were collected twice weekly for 2 weeks. Samples were analyzed by solid-phase RIA for the presence of HSV-1-specific antibodies and plotted as micrograms per milliliter versus days after gD-2 treatment.

TABLE 2. IFN-α/β induced in serum by gD-2t treatment

Treatment ^a	IFN-α/β (IU/ml) induced ^b at:			
	0 h ^c	4 h	8 h	24 h
gD-2t ^d	<1	<1	<1	<1
McKrae ^e	<1	73	54	340

^a Administered to anesthetized rats with abraded corneas.

^b IFN-α/β levels were determined by inhibition of VSV plaque formation and expressed as international units per milliliter of serum calibrated, using a rat IFN standard. The standardization of rat IFN units approximates that for humans, mice, and rabbits.

^c Time after treatment.

^d Two 5-µg doses per rat administered 1 h apart.

^e 3×10^4 PFU (20 LD₅₀) per rat administered in 10 µl.

protected when present at the time of challenge with virus but did not protect when added 1 h after virus infection suggested that the protective effect occurred early in the viral replication cycle. Initial viral adsorption to cells is known to be mediated by gC (12); therefore, the effect of soluble gD must be at a later stage, presumably at the level of virus entry.

The results described here are similar to those obtained by Johnson and coworkers in cell culture (15, 16). In those studies, it was found that UV-inactivated wild-type virions, but not virions lacking gD, could block superinfection. Similarly, soluble truncated forms of HSV-1 and HSV-2 gD also blocked infection. Those investigators proposed a model of HSV-1 entry in which virions initially adsorb to cells by a mechanism not requiring gD, probably by the binding of gC to cell surface heparan sulfate (12, 21, 37), followed by an essential interaction of gD with a cell surface receptor. Johnson et al. have presented evidence for the binding of gD to a titratable cell surface structure (15). The structure remains unidentified, and the binding appears to be of relatively low affinity. This low affinity may explain why relatively high concentrations of soluble truncated gD were needed to block plaque formation (15) and lethal ocular infection (this study). The large number of gD molecules in a virion, estimated at approximately 500 (31), should greatly increase the avidity of the virion for the putative receptor, making it difficult to completely block the receptor with a soluble monomeric competitor. This difference may account for the inability to achieve protection in 100% of the gD-treated animals.

A model in which gD binds to a cell surface receptor is consistent with the resistance of cells constitutively expressing gD to infection with HSV-1 (3, 17). However, an alternative model of gD blocking, in which gD present in the plasma membrane of cells interacts with gD in the envelope of virions to limit virus entry into cells, has been proposed (4). Such an effect on viral entry could be a normal function of gD, preventing reinfection of already infected cells. This model does not preclude the existence of a cellular receptor for gD. While the data presented in this report do not discriminate between these two models, they do establish that the previously observed blocking effect of gD was not an artifact of cell culture. These findings document that the gD blocking effect extends to an animal model of herpetic ocular disease and emphasize the need for a more complete understanding of gD function and HSV-1 entry.

ACKNOWLEDGMENTS

We thank Harriet J. Davidson and William J. Karpus for technical assistance, Jane V. Peppard for helpful discussions and reagent preparation, and Ronald Barrett and James Hazlett for assistance with photography. We also are grateful to David C. Johnson for the gD mutant virus (F-gD β) and VD60 cells and Timothy Gregory (Genentech, Inc.) for gD-2t.

This work was supported by the Michigan Eye Bank and by grants AI-22162, AI-00875, and EY-05122 from the National Institutes of Health. L.B.M. was supported by training grant EY-07093.

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