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Glycoprotein D Protects Mice Against Lethal Challenge with Herpes Simplex Virus Types 1 and 2

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Glycoprotein D is a virion envelope component of herpes simplex virus types 1 and 2. Sets of mice were immunized with purified gD-1 or gD-2 and were challenged with a lethal dose of herpes simple virus, either type 1 or type 2. All or virtually all of the immunized mice survived challenge with either agent, whereas challenge of sham-immunized mice was almost always fatal. Serum samples taken before challenge contained gD-specific antibodies which had 50% neutralization titers ranging from 1:16 to 1:512 against homologous and heterologous virus types. We conclude that either gD-1 or gD-2 is a potential candidate for a subunit vaccine against herpetic infections.

Herpes simplex viruses (HSV) are the causative agents of a number of human diseases, including cold sores, encephalitis, and eye and genital infections (14). The herpes simplex virion envelope and the plasma membrane of HSV-infected cells contain a series of glycoproteins designated gB, gC, gD, and gE (26), all of which appear to be involved in the immune response (15, 27). An additional glycoprotein, gA, is probably a modified form of gB (7). Our laboratory has focused much of its effort on studies of gD. Recently, we described a simple method for purifying gD (9), and we therefore considered the possibility of testing its protective capacity in a mouse model system. A number of studies have shown that gD is a reasonable choice for such a study. gD is a type-common component whose polypeptide and carbohydrate structure appears to be the same in different strains of HSV type 1 (HSV-1) (Cohen and Eisenberg, unpublished data) and is highly conserved between HSV-1 and HSV-2 (8, 18). Purified gD stimulates high titers of complement-independent, type-common virus-neutralizing antibodies in animal systems (4, 5, 9, 19). In addition, gD and other HSV glycoproteins participate in antibody-dependent complement-mediated (1, 16) and antibody-dependent cell-mediated (1, 16, 22) cytotoxic reactions. Passive immunization with monoclonal antibodies directed against gD as well as other HSV glycoproteins is highly effective in protecting mice against challenge by a lethal dose of HSV (10, 16, 22). These studies document the involvement of gD in the immune response to HSV and suggest that gD is an important candidate for a potential subunit vaccine. Live virus, killed virus, and subunit vaccines consisting of a mixture of HSV glycoproteins have been shown in protection studies to be effective (3, 11, 12, 20, 25, 28). More recently (23), purified gC of HSV-1 has been shown to protect mice against a challenge by a lethal dose of HSV-1 but not against HSV-2. The present study evaluates the capacity of purified gD-1 and gD-2 to protect mice against lethal HSV challenge by the homologous and heterologous virus types.

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The intraperitoneal route in mice was chosen for both immunization and lethal virus challenge (see Table 1, footnote *a*, for details). Animals were immunized with affinity-purified gD (9) suspended in complete Freund adjuvant (CFA) because this route-and-adjuvant protocol produced the highest titers of virus-neutralizing antibody. In a preliminary experiment designed to determine the dose of gD needed to stimulate production of neutralizing antibody, groups of mice were given five injections of gD-1 ranging from 0.005 to 2 μg per injection. At a dose of 0.05 μg per injection, two of four mice (50%) responded by producing virus-neutralizing antibody specific for gD (9). On the other hand, 100% of mice receiving doses ranging from 0.5 to 2.0 μg per injection responded by producing gD-specific neutralizing antibody. Based on these data, a regimen of six injections with a total of 6 μg of gD-1 or gD-2 over the course of immunization was chosen for subsequent challenge experiments.

In the first challenge experiment, mice were immunized with gD-1 purified from KB cells infected with strain HF of HSV-1 (9) and were challenged with either the Patton strain of HSV-1 or the 186 strain of HSV-2. In this and the subsequent challenge experiment, serum samples were obtained before virus challenge to be assayed for virus-neutralizing antibody by the 50% plaque reduction method (4, 5, 9) and to be assayed for gD specificity by radioimmune precipitation followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4, 5, 9). Neutralization titers of the immunized mice to be challenged ranged from 1:32 to 1:128 against HSV-1 (the homologous virus) and from 1:16 to 1:128 against HSV-2 (the heterologous virus). All of the immunized mice in this experiment displayed gD-1-specific antibody (data not shown). The sera of mice sham immunized with CFA or with saline showed neither neutralizing antibody nor immunoprecipitating activity with gD-1.

The immunized mice were arranged into two challenge groups, each of which represented the range of neutralization titers against HSV-1 and HSV-2. Table 1 illustrates the capacity of purified native gD-1 (9) to protect mice against lethal challenge with HSV-1 or HSV-2. All of the immunized mice survived the challenge with either HSV-1 or HSV-2. No survivors remained in the mice sham immunized with saline. However, there were some survivors (18 to 33%) among the CFA-immunized mice. These results indicate that gD-1 was effective in protecting mice, including those animals with low neutralization titers (1:16, 1:32), against

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TABLE 1. Protection of mice against lethal challenge by HSV-1 or HSV-2 after immunization with purified gD-1 or gD-2

Immunization group[b]	Challenge virus[c]	Survivors[a]	
		No./total tested	%
Expt 1			
gD-1/CFA	HSV-1 (Patton)	10/10	100
CFA	HSV-1	1/3	33
NaCl	HSV-1	0/9	0
gD-1/CFA	HSV-2 (186)	8/8	100
CFA	HSV-2	2/11	18
Expt 2			
gD-1/CFA	HSV-2 (186)	5/6[d]	83
gD-2/CFA	HSV-2	6/6	100
CFA	HSV-2	0/6	0
NaCl	HSV-2	0/6	0

[a] Survivors are those alive 21 days after challenge.

[b] In both experiments, mice were immunized with a total of 6 μg of purified gD (9). In experiment 1, BALB/c mice were immunized six times at biweekly intervals by intraperitoneal injection of 1.0 μg (dosage and protocol previously established to yield anti-gD responses in 100% of immunized animals) of purified native gD-1 (HF strain) emulsified in 50% CFA. In experiment 2, BALB/c mice were immunized five times, first with 3.0 μg of gD-1 (HF strain) or gD-2 (Savage strain) then twice with 1.0 μg of gD-1 or gD-2 emulsified in 50% CFA, once with 0.5 μg of gD-1 or gD-2 emulsified in 50% CFA, and once with 0.5 μg of gD-1 or gD-2 in 0.15 M NaCl. All immunizations were given at biweekly intervals by intraperitoneal injection. The purification of gD-1 and gD-2 by means of an affinity column containing monoclonal reagents has been detailed previously (9). Control mice were sham immunized with CFA or 0.15 M NaCl alone. Seven days after the final injection in experiment 1, serum was obtained from each animal, and individual samples were tested for virus-neutralizing antibodies (4, 5, 9). In experiment 2, similar procedures were followed, except that serum samples from each immunization group were pooled before assay (see legend to Fig. 1 for SDS-PAGE analysis of pooled serum samples in experiment 2). Two months after the challenge, serum samples were obtained from surviving animals in experiment 2, pooled, and retested by immunoprecipitation and SDS-PAGE (8, 9, 26).

[c] Mouse groups were challenged intraperitoneally with HSV 14 days after final immunization or sham immunization as indicated above. A dosage of four 50% lethal doses was previously determined to yield 100% killing of sham-immunized BALB/c mice for both HSV-1 (Patton strain, 4×10^6 PFU) and HSV-2 (186 strain, 1×10^6 PFU) within 7 to 10 days and was used for experiment 1. A dosage of 20 50% lethal doses of HSV-2 (186 strain, 5×10^6 PFU) was employed for experiment 2.

[d] One animal died on day 1 after challenge.

challenge by either the homologous or heterologous virus. It should also be noted that the homologous virus used for challenge was a strain of HSV-1 different that used as the source of gD-1.

The presence of survivors in the CFA-sham-immunized group led us to increase the challenge dose of virus in the next experiment and to sham immunize all of the control animals with 50% CFA. In this experiment, animals were immunized with 6 μg of either gD-1 or gD-2. Serum samples from mice in each of the immunization groups were again obtained at the end of the immunization course, pooled, and assayed for neutralizing antibody and gD specificity. The neutralization titers ranged from 1:128 to 1:512. None of the CFA-sham-immunized mice possessed neutralizing antibodies against HSV.

The gD specificity of the pooled antisera is demonstrated in Fig. 1. The cell extract used to assess the antibodies present in the serum samples was prepared from HSV-1- or HSV-2-infected cells that were labeled with [^{3}H]arginine for 15 min at 6 h postinfection. We had previously shown that under these conditions, the precursor forms of gD (pgD-1 and pgD-2) as well as other viral glycoproteins were extensively labeled (4, 8, 9). Lanes 1 and 2 of Fig. 1 represent control immunoprecipitations of HSV-1- and HSV-2-infected cell extracts, respectively, made by using a previously prepared monospecific anti-gD-1 serum (4, 9). Lanes 3 and 4 represent immunoprecipitations of HSV-1- and HSV-2-infected cell extracts carried out with a pooled serum sample taken from mice immunized with gD-1. Lanes 5 and 6 represent a similar immunoprecipitation carried out with a pooled serum sample obtained from mice immunized with gD-2. Lanes 7 and 8 represent immunoprecipitations made by using a pooled serum sample obtained from sham-immunized mice. The results show that animals immunized with gD-1 or gD-2 responded by producing gD-specific antibodies. These data also confirm the purity of the gD-1 and gD-2 preparations used for immunization and further document the cross-reactivity of gD-1 and gD-2 antibodies (9).

Table 1 shows that none of the sham-immunized animals in experiment 2 survived the challenge. In contrast, all of the animals immunized with gD-2 and five out of six animals immunized with gD-1 survived the HSV-2 challenge. It should be noted that this one animal died within 1 day after challenge, whereas all of the sham-immunized animals died 5 to 8 days after challenge. It is thus possible that the one death among gD-immunized animals was due to reasons not directly related to a lack of protection. In any event, the results of the second experiment show that both glycoproteins appeared to be effective in protection against challenge by HSV-2. Similar experiments with affinity-purified gD-1 as the immunogen have been repeated independently; all 10 gD-1-immunized mice survived an HSV-2 challenge, whereas

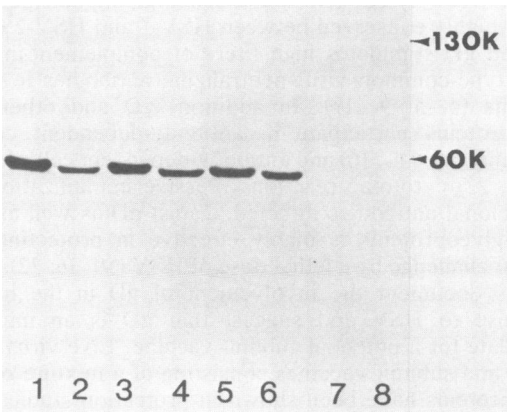

FIG. 1. SDS-PAGE analysis of antisera to gD-1 and gD-2 obtained before virus challenge. Serum samples from immunization groups (Table 1, experiment 2) were obtained 7 days after completion of the immunization cycle and 7 days before challenge. Control samples or pooled samples from immunization groups were tested by immunoprecipitation of cytoplasmic extracts obtained from HSV-1- or HSV-2-infected cells that were pulse-labeled with [^{3}H]arginine for 15 min at 6 h postinfection (4, 8, 9). Lanes 1, 3, 5, and 7 contain extract from HSV-1-infected cells; lanes 2, 4, 6, 8 contain extract from HSV-2-infected cells. Lanes 1 and 2, Rabbit anti-gD-1 control (19); lanes 3 and 4, pooled serum from mice immunized with gD-1 in CFA (Table 2, experiment 2); lanes 5 and 6, pooled serum from mice immunized with gD-2 in CFA (experiment 2); lanes 7 and 8, serum from mice sham immunized with CFA (experiment 2).

none of 10 sham-immunized mice survived (G. Cerini, personal communication).

Two months after the challenge, serum samples were obtained from the surviving gD-1- and gD-2-immunized animals in experiment 2. Samples from each group were pooled and retested by immunoprecipitation and SDS-PAGE (Fig. 2). The serum samples continued to exhibit a major response to gD (compare Fig. 1 and 2) but also displayed an increased heterogeneity, in that antibodies to other viral components, including viral glycoproteins, were present after challenge. This heterogeneity might simply be a response to the infecting dose of virus, or it might be a response to virus which replicated after infection. Further experiments should be done to clarify this point.

The current investigations show that active immunization with gD purified from HSV-1- or HSV-2-infected cells confers protection against lethal challenge with both homologous and heterologous virus types. Moreover, the protective effect is correlated with the presence of gD-specific antibodies. Recovery from HSV infections appears to involve both humoral and cell-mediated immunity (2, 10, 13, 17, 21, 24, 29). It was recently shown that gC of HSV-1 induces type-specific protective immunity in the absence of detectable antibodies (23). However, studies showing conferral of protection after the passive transfer of monoclonal anti-gD antibodies (1, 6, 10, 22) are entirely consistent with the premise that antibodies to gD can exert a significant protective effect. Regardless of the immune mechanism responsible, it is clear from the present investigation that purified gD is an important candidate for a subunit vaccine potentially effective against herpetic infections. It remains to be seen whether such a subunit vaccine will protect against establishment of latency or recurrent infection.

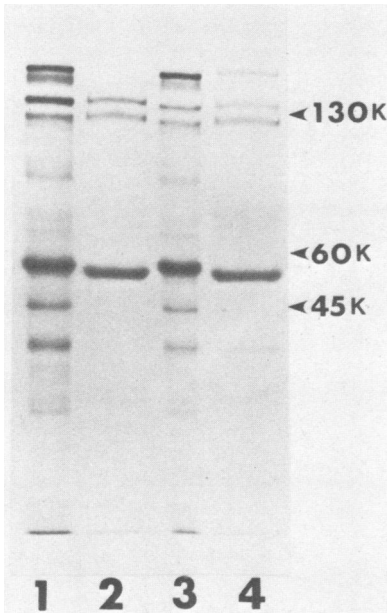

FIG. 2. SDS-PAGE analysis of pooled serum samples taken from immunized mice (Table 1, experiment 2) 2 months after challenge with HSV-2 strain 186. The cytoplasmic extracts are the same as those shown in Fig. 1. Lanes 1 and 3 contain extract from HSV-1-infected cells; lanes 2 and 4 contain extract from HSV-2-infected cells. Lanes 1 and 2, Pooled serum from animals immunized with gD-1; lanes 3 and 4, pooled serum from animals immunized with gD-2.

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