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Pathogenicity in Mice of Herpes Simplex Virus Type 2 Mutants Unable To Express Glycoprotein C

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Herpes simplex virus type 2 (HSV-2) mutants that were unable to express glycoprotein C (gC-2) were isolated. Deletions were made in a cloned copy of the gC-2 gene, and recombinant viruses containing these deletions were screened by using an immunoreactive plaque selection protocol. The viruses did not display a syncytial phenotype. Intravaginal inoculation of BALB/cJ mice with one of the HSV-2 gC-2$^-$ viruses produced local inflammation followed by a lethal spread of the viral infection into the nervous system in a manner identical to that produced by parental HSV-2 strain 333. Similarly, intracerebral inoculation of DBA-2 mice with the gC-2$^-$ virus produced a lethal neurological disease paralleling that caused by HSV-2 strain 333. These results indicate that gC-2 is not required for the spread of HSV-2 infections in mice.

The envelopes of herpes simplex virus (HSV) types 1 and 2 (HSV-1 and HSV-2, respectively) are composed of a cell-derived lipid bilayer and a number of virus-specified glycoproteins. At least five HSV-1 glycoproteins, designated gB, gC, gD, gE, and gH, have been mapped in the viral genome (3; reviewed in reference 46). HSV-2 counterparts for gB, gC, gD, and gE have been described (10, 34, 35, 43, 50), as has an HSV-2 glycoprotein, gG (31, 38). These polypeptides are exposed on the surfaces of virions and infected cells and are believed to play important roles in the adsorption and entry of viruses into cells and in the cell-to-cell spread of virus. In addition, viral glycoproteins most probably play central roles in tissue tropism and recognition of viral infections by the immune system.

It has been very difficult to study the activities of individual HSV glycoproteins principally because very few mutations in the glycoprotein genes have been identified. Studies involving the HSV-1 mutants *ts*B5 and *ts*J12 have suggested a role for gB in the entry of virions into cells (27, 40) and in cell-cell fusion (15, 30). Furthermore, evidence supporting the role of gB and gD in virus attachment to cells and cell-cell fusion has come from experiments with reconstituted viral glycoproteins (23) and monoclonal antibodies (13, 33). Spontaneous HSV-1 gC$^-$ (gC-1) mutants have emerged in cell cultures (4, 18, 19), suggesting that gC-1 is not required for viral replication in these cells. In many cases, these spontaneous HSV-1 gC$^-$ mutants were recognized because of their syncytial (*syn*) or fusion-inducing phenotype. Subsequently, the gC$^-$ and *syn* phenotypes were genetically separated (21, 26, 39) although, for unknown reasons, these phenotypes often coincide. *syn* mutations have been mapped to at least four locations, all of which are removed from the HSV-1 gC gene (7, 28, 36, 39). More recently, a mutant of HSV-2 which does not express HSV-2 gC (gC-2) was isolated (51). This mutant is fully able to replicate in cultured monkey and human cells and expresses a *syn* phenotype.

Since gC-1 and gC-2 appear to be dispensable for the replication of HSV-1 and HSV-2 in cultured cells, it is reasonable to suggest that these glycoproteins play a role in the spread of viral infections in their human hosts. To test this hypothesis, we examined the role of gC-2 in the replication of HSV-2 in murine models involving the female genital tract (32) and the brain (25). Mutant viruses in which a sizeable, genetically engineered deletion was made in the gC structural sequences were isolated. These viruses did not display the *syn* phenotype and replicated normally in cultured cells. After intravaginal inoculation of mice, one of the gC-2$^-$ mutants produced a local infection accompanied by an inflammatory response and followed by a lethal spread of virus into the central nervous system (CNS), a disease pattern identical to that observed with wild-type virus. In addition, the gC-2$^-$ mutant and parental HSV-2 produced very similar patterns of lethal neurological disease after intracerebral inoculation of mice. We conclude that gC-2 does not play a central role in the replication of HSV-2 in mice.

MATERIALS AND METHODS

Cells and viruses. African green monkey (Vero) cells were obtained from the American Type Culture Collection, Rockville, Md., and grown in α-minimal essential medium (GIBCO Laboratories, Burlington, Ontario, Canada) containing 10% fetal bovine serum, penicillin, and streptomycin. HSV-2 strain 33 [HSV-2 (333)] was obtained from P. Spear, University of Chicago, Chicago, Ill., and propagated in Vero cells.

Plasmids. pTYL302, which contains the *Bam*HI A fragment of HSV-2 cloned into pBR322, was obtained from P. Spear. pDJ218 and pDJ1013 were derived from pTYL302 by cleaving the DNA with *Bgl*II, digesting the DNA with nuclease Bal31, and religating the plasmids after an end-filling reaction with the Klenow fragment of *Escherichia coli* DNA polymerase I.

Preparation of viral DNA for transfections. HSV-2 (333) viral DNA was isolated from cytoplasmic nucleocapsids as described elsewhere (44). Briefly, infected Vero cells were washed with phosphate-buffered saline, suspended in 0.01 M Tris hydrochloride buffer (pH 7.8) containing 0.01 M EDTA and 0.5% Triton X-100, held on ice for 10 min, Dounce homogenized, and centrifuged at a low speed to pellet the

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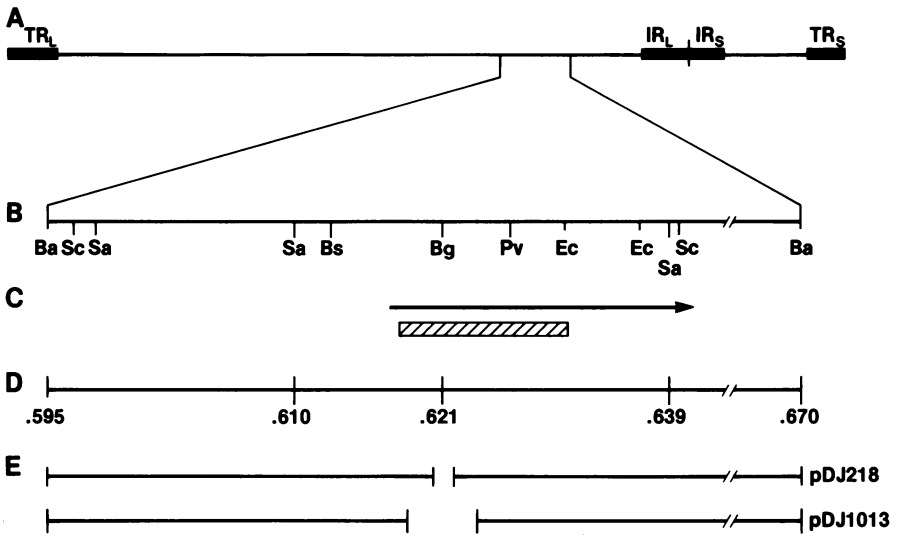

FIG. 1. Organization of the region of the HSV-2 genome encoding gC-2 and locations of deletions made in this region. (A) Location of the *Bam*HI A fragment in the HSV-2 genome. (B) Restriction map of the *Bam*HI A fragment. Abbreviations: Ba, *Bam*HI; Bg, *Bgl*II; Bs, *Bst*EII; Ec, *Eco*RI; Pv, *Pvu*II; Sa, *Sal*I; Sc, *Sac*I. (C) Location of gC-2 mRNA (arrow) and protein-coding region (hatched box) from Swain et al. (47). (D) Map coordinates of HSV-2. (E) Plasmids pDJ218 and pDJ1013 containing 130- and 550-bp deletions, respectively.

nuclei. The cytoplasmic fraction was centrifuged at 80,000 × *g* for 1 h, and the pellet containing the viral nucleocapsids was suspended in 0.01 M Tris hydrochloride buffer (pH 7.5) containing 0.15 M NaCl, 0.01 M EDTA, and 0.5% sodium dodecyl sulfate. The preparation was extracted twice with phenol and dialyzed against TE buffer (0.01 M Tris hydrochloride buffer [pH 7.5], 0.001 M EDTA).

Isolation of gC⁻ viruses by using an immunoreactive plaque assay. Subconfluent monolayers of Vero cells were cotransfected with mixtures of HSV-2 (333) viral DNA and either pDJ218 or pDJ1013 plasmid DNA by the procedure of Graham and van der Eb (14) as modified by Wigler et al. (49). Progeny viruses were plated on Vero cells growing in 60-mm petri dishes, and plaques were stained by using an in situ enzyme immunoassay modified from that described by Holland et al. (20). Briefly, the cells were washed and incubated for 30 min in phosphate-buffered saline containing 0.001 M $CaCl_2$, 0.001 M $MgCl_2$, and 1% (wt/vol) bovine serum albumin (black plaque buffer) at 4°C. Monoclonal antibodies 17αA2 and III-188, both specific for gC-2 (1 ml of a 1:100 dilution of ascites fluid in black plaque buffer) were added, and the cells were incubated for 1 h at 4°C. The monolayers were washed three times with black plaque buffer and incubated with 1 ml of biotinylated rabbit anti-mouse immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.) diluted with 1:250 in black plaque buffer. The cells were washed three times with black plaque buffer and incubated with Vectastain ABC reagent (Vector Laboratories, Burlingame, Calif.) for 1 h at 4°C. The monolayers were washed three times with black plaque buffer, incubated with peroxidase substrate (0.01% [wt/vol] 4-chloro-1-naphthol [Sigma], 0.003% [vol/vol] H_2O_2) for 10 min at room temperature, and washed three times with black plaque buffer. Plaques that did not stain with the insoluble grey-black precipitate derived from 4-chloro-1-napthol were touched with a sterile wooden rod, and viruses were transferred to 2.2-cm^2 monolayers of Vero cells. Virus isolates were subjected to a second round of plaque purification by the same method.

Extraction of viral DNA, Southern blot transfer, and hybridization. Viral DNA was extracted from infected Vero cells growing in 25-cm^2 flasks by the method of Hirt (17). Viral or plasmid DNA was digested with restriction enzymes, electrophoresed on 1% agarose gels, and transferred to nitrocellulose paper (Schleicher & Schuell, Inc., Keene, N.H.) by the procedure of Southern (45). Nitrocellulose filters were hybridized to a ^{32}P-labeled DNA probe and washed as described by Maniatis et al. (29).

Antibodies and immunoprecipitations. Monoclonal antibody 17αA2, specific for gC-2 (1), was provided by S. Bacchetti and W. Rawls, McMaster University, Hamilton, Ontario, Canada. Rabbit antiserum R 71, prepared against HSV-2 gC (50), and monoclonal antibody III-188, directed against gC-2 (50), were kindly provided by P. Spear. Immunoprecipitations were performed as previously described (22) with extracts from virus-infected Vero cells that had been labeled with [^{35}S]methionine from 3 h until 12 h after infection, and the precipitated products were analyzed on 8.5% sodium dodecyl sulfate-polyacrylamide gels cross-linked with *N,N'*-diallyltartardiamide (16).

Intravaginal inoculation of mice and tissue processing. Strains of HSV-2 were inoculated intravaginally into 6-week-old BALB/cJ mice (Health Research Inc., West Seneca, N.Y.) as previously described (32). Groups of 10 mice were scored for the severity of vaginal inflammation, hind limb paralysis, and mortality each day after inoculation. Dorsal spinal ganglia and spinal cords were surgically removed from some animals 5 or 6 days after inoculation with 10^6 PFU of HSV-2 (333) or the gC-2⁻ virus, C2-4, as previously described (48). Explanted tissues were rinsed in media, homogenized, sonicated, and clarified by centrifugation, and virus titers were assayed on Vero cells (32).

Intracerebral inoculation of mice. Groups of eight, 6-week-old female DBA-2 mice (Jackson Laboratory, Bar Harbor, Maine) were inoculated with five doses of HSV-2 (333) of the gC-2⁻ virus, C2-4, suspended in 10 μl of phosphate-buffered saline as previously described (41). The doses ranged from $10^{0.5}$ to 10^3 PFU per animal. Mortality was recorded daily, and the experiments were terminated after 20 days. The 50% lethal dose (LD_{50}) was calculated by the method of Reed and Muench (37).

RESULTS

Construction of recombinant viruses. Plasmid pTYL302, which contains the *Bam*HI A fragment of HSV-2 (333) cloned into pBR322, was used in the construction of deletions of the gene for gC-2. pTYL302 has a single *Bgl*II site at a position corresponding to approximately 0.621 map units in the HSV-2 genome (Fig. 1). Dowbenko and Lasky (8) and Swain et al. (47) found this *Bgl*II site to be 525 base pairs (bp) downstream from the transcription start site of gC-2 mRNA within the structural sequences of the polypeptide. Sequences flanking the *Bgl*II site in pTYL302 were deleted with nuclease Bal31. Two plasmids derived from Bal31-digested pTYL302 were isolated; pDJ218, to which contains a 130-bp deletion in the *Bst*EII-*Pvu*II fragment (map units 0.613 to 0.626) containing the *Bgl*II site, and pDJ1013, which contains a 550-bp deletion in the *Bst*EII-*Pvu*II fragment (Fig. 1), were chosen for further study. HSV-2 (333) DNA and either pDJ218 or pDJ1013 DNA were transfected into Vero cells, and the viral progeny were screened for gC-2$^-$ mutants by using an immunoreactive plaque screening protocol modified from that described by Holland et al. (20). Cell monolayers containing virus plaques were stained by incubation with a gC-2-specific monoclonal antibody, 17αA2, biotinylated rabbit anti-mouse immunoglobulin G, peroxidase-coupled avidin, and peroxidase substrate. Plaques derived from viruses lacking the 17αA2 epitope did not stain with the insoluble grey-black precipitate (black plaque), and viruses could be isolated from these plaques. Approximately 10^3 virus plaques derived from transfections with pDJ218 DNA were screened. Ten plaques were found which did not stain with 17αA2 or, subsequently, with monoclonal antibody III-188, both of which are specific for gC-2 (1, 50). However, the viral progeny from transfections with pDJ1013 DNA did not yield any gC-2$^-$ plaques, although over 10^4 plaques were screened.

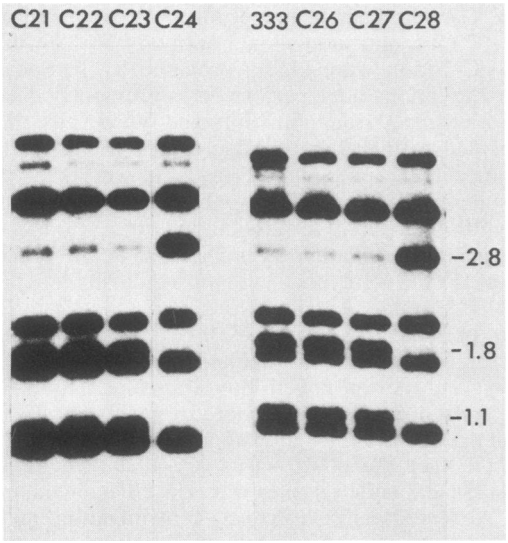

FIG. 2. Southern blot hybridization analysis of DNAs from viruses isolated from cells transfected with pDJ218 DNA and HSV-2 (333) DNA and selected by using the immunoreactive plaque selection protocol. Viral DNA extracts were digested with *Bgl*II and *Sal*I, electrophoresed on 1% agarose gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled pTYL302 DNA. The positions of the 1.1-kb *Sal*I-*Bgl*II fragment (0.610 to 0.621 map units), the 1.8-kb *Bgl*II-*Sal*I fragment (0.621 to 0.639 map units), and a novel 2.8-kb band detected in the mutants and in pDJ218 are shown. Virus isolates C2-1, C2-2, etc., are denoted C21, C22, etc.

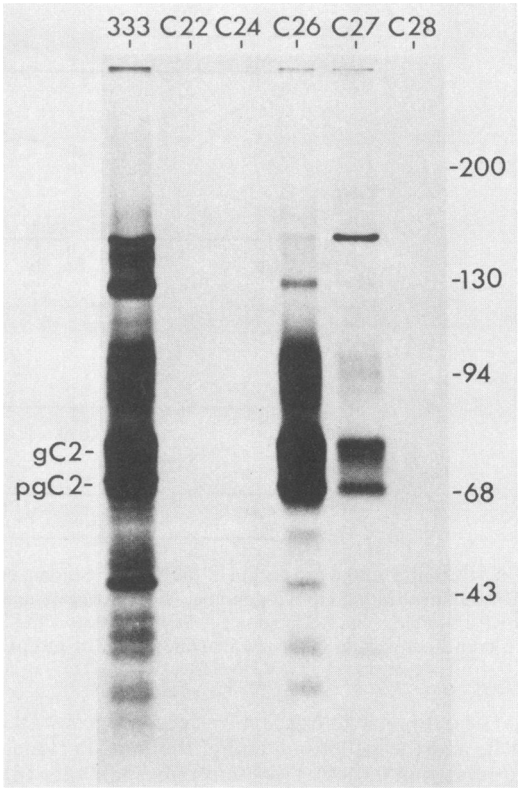

FIG. 3. Polypeptides precipitated by anti-gC-2 antibodies from extracts of Vero cells infected with HSV-2 (333) or viruses isolated by using the immunoreactive plaque selection protocol. Infected cells were labeled with 100 μCi of [^{35}S]methionine per ml from 3 to 12 h after infection, and extracts of the cells were made. The extracts were immunoprecipitated with a mixture of anti-gC-2 serum R 71 and monoclonal antibodies 17αA2 and III-188, and the precipitated products were analyzed on sodium dodecyl sulfate-polyacrylamide gels. The precursor of the immature form of gC-2 is denoted pgC2. The numbers on the right side of the figure refer to molecular weight markers ($\times 10^3$).

The DNAs of viruses selected by the immunoreactive plaque assay were digested with *Bgl*II and *Sal*I and analyzed by Southern blot analysis with ^{32}P-labeled pTYL302 as a probe. Two virus isolates (C2-4 and C2-8) had a 2.8-kilobase (kb) fragment not present in HSV-2 (333) and lacked the 1.1-kb *Sac*I-*Bgl*II fragment (0.610 to 0.621 map units) and the 1.8-kb *Bgl*II-*Sac*I fragment (0.621 to 0.639 map units) (Fig. 2). Other restriction analyses confirmed the presence of a 130-bp deletion at the *Bgl*II site in these viruses (data not shown).

Analysis of gC-2 expression in virus isolates. To determine whether the viruses isolated by the immunoreactive plaque screening protocol expressed gC-2, we immunoprecipitated [^{35}S]methionine-labeled infected cell extracts with a mixture of rabbit anti-gC-2 serum R 71 (50) and monoclonal antibodies III-188 and 17αA2. Virus isolates C2-4 and C2-8, which contained deletions in gC-2 structural sequences, did not express gC-2 (Fig. 3) but did express gD, as assayed by immunoprecipitations with monoclonal antibody 17βA3, specific for HSV-2 gD (data not shown). Another of the virus isolates, C2-2, did not express gC-2, although viral DNA from this isolate did not contain a detectable deletion surrounding the *Bgl*II site (Fig. 1 and 2). It is possible that C2-2

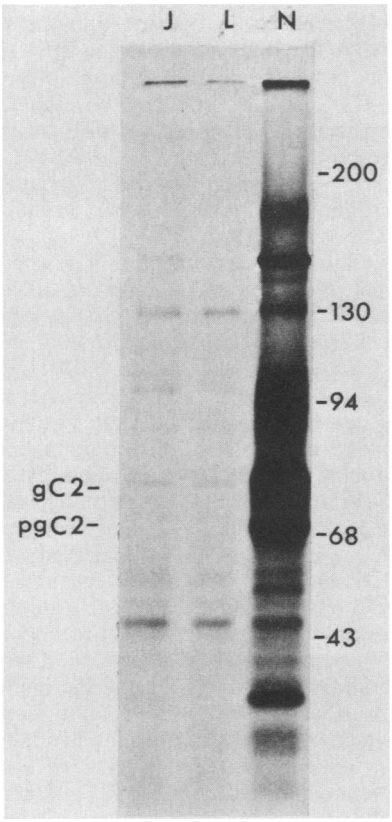

FIG. 4. Polypeptides precipitated by anti-gC-2 antibodies from extracts of Vero cells infected with viruses recovered from mice. Samples of homogenized dorsal spinal ganglia from animals J and L inoculated with the gC-2⁻ virus C2-4 and from animal N inoculated with HSV-2 (333) were used to infect 35-mm dishes of Vero cells. The infected cells were labeled with 100 μCi of [^{35}S]methionine per ml from 4 to 20 h after infection, and extracts of the cells were immunoprecipitated with a mixture of monoclonal antibodies 17αA2 and III-188.

acquired the gC-2⁻ phenotype through a point mutation, as has been described for HSV-1 strain MP (9). None of the isolates described above exhibited the capacity to fuse Vero or 143 cells.

Intravaginal inoculation of a gC-2⁻ virus. One of the gC-2⁻ virus recombinants, C2-4, was arbitrarily chosen for use in subsequent experiments. A total of 10 6-week-old BALB/cJ mice were inoculated intravaginally with 10^3 PFU of C2-4, and another 10 mice were inoculated with 10^3 PFU of HSV-2 (333). McDermott et al. (32) found that, for mice in this age group, the LD_{50} of HSV-2 (333) was from 10^3 to 10^4 PFU per animal. After 5 to 7 days, 7 of 10 mice inoculated with the gC-2⁻ virus and 6 of 10 mice inoculated with HSV-2 (333) displayed vaginal inflammation accompanied by mucus discharge, followed by hind limb paralysis at 7 to 8 days and death between 9 and 18 days. The mice which survived in each group were inoculated intravaginally with 10^6 PFU of C2-4 or HSV-2 (333), respectively, and all died 7 to 8 days later.

Since the death of mice after inoculation with HSV is primarily due to the spread of virus into sensory ganglia and the brain (2, 6), we attempted, in a separate experiment, to detect viruses in nervous tissue taken from mice inoculated intravaginally with HSV-2 (333) or the gC-2⁻ virus. In all but one animal inoculated with HSV-2 (333), homogenized dorsal spinal ganglia or spinal cord or both yielded plaques after 2 days in Vero cell monolayers (Table 1). In most animals, we observed relatively higher levels of virus in the spinal cord than in the ganglia; however, the amount of tissue in the spinal cord homogenates was often greater than that in the ganglion homogenates, and undiluted samples of homogenates from ganglia were sometimes toxic to Vero cells. Viruses isolated from the ganglia of animals J and L inoculated with the gC-2⁻ virus and animal N inoculated with HSV-2 (333) were propagated on Vero cells and analyzed for the expression of gC-2 immunoprecipitation of [^{35}S]methionine-labeled infected cell extracts with a mixture of monoclonal antibodies III-188 and 17αA2 (Fig. 4). As expected, viruses recovered from animals inoculated with the gC-2⁻ virus did not express gC-2, and viruses recovered from the animal inoculated with the wild-type virus did express gC-2. Thus, this phenotype of the viruses remained constant in the mice.

TABLE 1. Determination of virus titers in homogenized spinal cord and dorsal spinal ganglia from mice inoculated with HSV-2 (333) or a gC-2⁻ virus

Animal	Inoculated virus	Days post-inoculation	PFU in:	
			Spinal cord	Ganglia
A	C2-4	5	1,100	200
B	C2-4	5	350	60
C	C2-4	5	9,200	170
D	C2-4	5	10	80
E	333	5	2,100	0
F	333	5	1,440	12
G	333	5	230	320
H	333	5	2,400	19
I	C2-4	6	7,800	18
J	C2-4	6	6,300	320
K	C2-4	6	1,320	90
L	C2-4	6	710	270
M	333	6	0	0
N	333	6	2,150	270
O	333	6	810	22
P	333	6	38	0

Intracerebral inoculation of a gC-2⁻ virus. Intracerebral inoculation of mice with a gC-2⁻ virus, C2-4, and HSV-2 (333) was performed to test the relative ability of the viruses to replicate when inoculated directly into CNS tissue. Two experiments, each including 80 6-week-old DBA-2 mice, were performed. In each case, the LD_{50} of the gC-2⁻ virus was very similar to that of HSV-2 (333) (Table 2). In addition, the kinetics of mortality of animals inoculated with

TABLE 2. Pathogenicity of HSV-2 (333) and a gC-2⁻ virus in mice after intracerebral inoculation

Expt[a]	Virus	LD_{50}[b]
1	HSV-2 (333)	2
	C2-4	12
2	HSV-2 (333)	12
	C2-4	35

[a] Two independent experiments were performed in which 40 DBA-2 mice were inoculated with either HSV-2 (333) or C2-4 (a gC-2⁻ virus) in five doses, as described in Materials and Methods.

[b] The LD_{50} of each virus was determined after intracerebral inoculation of wild-type or mutant virus, as described in Materials and Methods. The results shown represent the mean of two independent experiments.

the gC-2$^-$ virus was not different from that of animals inoculated with HSV-2 (333) (data not shown).

DISCUSSION

We report here the isolation of HSV-2 mutants which do not express the glycoprotein gC-2. These mutants were screened, by using an immunoreactive plaque assay, from pools of virus derived from transfections involving wild-type viral DNA and mutagenized copies of the gC-2 gene. Recombinant viruses containing a 130-bp deletion in the gC-2 gene (flanking the *Bgl*II site at 0.621 map units) were isolated at a frequency of approximately 10^{-2}. However, we were unable to rescue into virus a fragment containing a 550-bp deletion. It is possible that this 130-bp deletion in some way affects the synthesis of the 18-kilodalton polypeptide which maps to the right of gC-2 (8, 47) or the 56-kilodalton polypeptide which maps in the *Bgl*II N fragment (0.585 to 0.621 map units) to the left of the gC-2 structural sequences, although these sequences would likely be over 1 kb from the *Bgl*II site. Alternatively, this deletion may affect the synthesis of potential reading frames which overlap the gC gene region and were first described by Frink et al. (12) in the gene for gC-1 and by Dowbenko and Lasky (8) in the gene for gC-2.

Viruses which contained the 130-bp deletion did not express gC-2 or truncated forms of the polypeptide detectable by monoclonal and polyclonal antibodies, most probably because the deletion produced a frameshift in the gC-2 structural sequences. It seems highly unlikely that an in-phase deletion resulting in a truncated form of gC-2 occurred, because such a deletion would produce a polypeptide with greater than 90% of the gC-2 structural sequences. At least a fraction of the antibodies used here would be expected to recognize such a polypeptide. The mutant viruses isolated by this strategy did not display the *syn* phenotype associated with a number of gC-1$^-$ mutants (4, 18) and with the gC-2$^-$ mutant described by Zezulak and Spear (51). Therefore, as is the case with gC-1, the lack of expression of gC-2 is not directly responsible for the *syn* phenotype. The relationship between these two phenotypes is intriguing and will hopefully be clarified upon closer examination of the *syn* mutations.

Although gC$^-$ mutants of HSV-1 and HSV-2 arise spontaneously in cultured cells, they have not been observed in clinical isolates. These observations suggest a role for gC-1 and gC-2 in the survival of HSV-1 and HSV-2 in humans. It appears, however, that whatever functions are carried out by gC during the spread of HSV infections in humans, they are not grossly visible in animal models of HSV infections. The MP strain of HSV-1, which does not express gC-1 and is mutated at two *syn* loci (36, 39), causes a lethal disease involving the nervous system in mice inoculated in the cornea or subcutaneously (24) and in rabbits inoculated in the cornea (5). In this study, intravaginal inoculation of mice with HSV-2 (333) or a gC-2$^-$ mutant derived from HSV-2 (333) led to identical local inflammation, swelling, and hemorrhage. Local infections produced by the parental and gC-2$^-$ viruses usually led to neurological disease, hind limb paralysis, and death. The death of the animals was presumably caused by viral replication in the CNS, since we were able to recover infectious virus from the ganglia and spinal cords. Mice which did not succumb after the first inoculation with 10^3 PFU of virus died after a second inoculation with 10^6 PFU of virus. The first inoculation of these mice may not have produced mucosal penetration, since we know that animals inoculated with 10^3 PFU of ΔTK-HSV-2, a thymidine kinase-deficient virus, became immune to a second inoculation with 10^6 PFU of wild-type HSV-2 (32). In a similar fashion, direct inoculation of the wild-type and gC-2$^-$ viruses into CNS tissue produced a lethal neurological disease. The results of experiments with this HSV-2 gC$^-$ mutant differed from those reported for HSV-1 gC-specific, monoclonal antibody-resistant mutants, including a gC-1$^-$ virus; it was found that the mutants had a reduced pathogenicity for the nervous system (25).

Our results allow us to conclude that if gC-2 plays any role in local vaginal infections and the spread of virus into the CNS in mice, it is a subtle role. Some slight selective advantage conferred on viruses expressing gC-2 may be sufficient to maintain the gC-2 gene. HSV-1 gC has been found to act as a receptor for the C3b complement component, and it has been suggested that virus-induced C3b receptor activity may inhibit the complement activation pathway, reducing the efficiency of antibody-complement-mediated lysis of infected cells (11). Although gC-1 and gC-2 share a great deal of homology in their primary amino acid sequences (7, 12, 47) and might be expected to share some common activities, C3b receptors were not detected on HSV-2-infected endothelial cells (11). Alternatively, the gC-2 polypeptide may play a much more important role in the spread of virus or in latency in humans. Encephalitis and death are a common feature in the murine model of genital infection with HSV-2, whereas the adult human disease, encephalitis and death are rare, and a latent state is common. Nevertheless, since HSV-1 and HSV-2 gC$^-$ mutants can replicate in human epithelial (18, 39, 51) and neuroblastoma (42; D. C. Johnson, unpublished results) cell lines, we suggest that it is unlikely that either of these polypeptides plays an essential role in the entry of virus into epithelial or neuronal cells, either in mice or in humans.

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