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Herpes Simplex Virus Type 1 Glycoprotein E Is Not Indispensable for Viral Infectivity

H. NEIDHARDT, C. H. SCHRÖDER, AND H. C. KAERNER*

German Cancer Research Center, Institute for Virus Research, 6900 Heidelberg, Federal Republic of Germany

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A mutant of the herpes simplex virus type 1 Angelotti was isolated in which 87% of the coding region of glycoprotein E (gE) was deleted and replaced by a functional neomycin resistance gene of the Tn5 transposon. The mutant was characterized by restriction enzyme analyses and Southern blotting. Western blotting of proteins and immunofluorescence assays revealed that gE was completely absent and that the Fc receptor was not expressed in cells infected with the mutant. The fact that this mutant was viable and that it replicated to a slightly lower titer than did the wild-type virus suggests that the presence of gE is not a prerequisite of viral infectivity in tissue culture.

Herpes simplex virus type 1 (HSV-1) encodes four major glycoproteins termed glycoprotein B (gB), gC, gD, and gE, of which gB, gC, and gD are the most prominent immunogenic viral antigens associated with mature virions and with the membranes of infected cells (for a review, see reference 26). Only gB, however, has been shown to play an indispensable role in HSV infectivity, in that it is obligatorily involved in the penetration of the host cell membrane by the virus during infection (16, 23). The fact that there exist viable HSV-1 mutants which are unable to produce gC suggests that this glycoprotein is not essential for virus replication in cell culture (3, 10, 12, 19, 29). Several functions of gD have been reported. It is an inherent constituent of the virion envelope which gives rise to high titers for virus-neutralizing antibody (4–6, 8). Little is known of the role of gE in the reproductive cycle of HSV, except that this glycoprotein exhibits Fc-binding activity and presumably is identical with the Fc receptor expressed in HSV-infected cells (1, 21).

To get more information about the biological function of gE, we constructed a deletion mutant of HSV-1 Angelotti (ANG) in which the gE gene was completely removed and replaced by the neomycin resistance (Neo^r) gene from the Tn*5* transposon (7) as a screening marker. For this purpose, the region of viral DNA containing the gE gene was cloned into a pBR322-derived plasmid. Using partial digestions with the restriction enzymes *Sst*I and *Sst*II, we partially deleted the 1.5-kilobase fragment from the *Sst*II site at nucleotide 8530 (20) upstream of the TATA sequence to the *Sst*I site at nucleotide 10068 (20). The deletion thus comprised 87% of the gE-coding region. The deleted sequence was replaced by the Neo^r gene flanked by simian virus 40 promoter and polyadenylation sequences. The resulting deletion-insertion plasmid, p622(524), is represented in Fig. 1a.

BSC-1 cells were then cotransfected (9) with HSV-1 ANG wild-type (wt; standard) DNA and plasmid p622(524) DNA (9, 11). The virus offspring (individual plaques) of the transfections were assayed for recombinant clones by slot-blot hybridization (18) of lysates of infected cells. Hybridization with a ^{32}P-labeled Neo^r probe revealed that about 50% of the original plaques contained different amounts of virus associated with Neo^r-specific sequences. Strongly positive clones were subcloned twice by repetition of the procedure described above. About 80% of the first subclones were negative for the presence of Neo^r sequences. The second subcloning of the remaining (positive) clones resulted in the isolation of about 25% of the number of the first subclones as stable clones containing Neo^r sequences, i.e., about 2.5% positive clones from the total offspring of the transfection assay. These clones were then assayed for the presence of gE sequences by slot-blot hybridization with the ^{32}P-labeled gE gene as a probe. None of the clones showed a reaction with our gE probe, which contained the viral DNA sequences deleted in p622(524). All of the clones proved to have lost the majority of their gE sequences corresponding to the region deleted by insertion of the Neo^r sequences, as will be shown below. One of the recombinant clones designated E3/3 was further analyzed.

For the genome analysis, a stock of clone E3/3 was used which had been passaged twice on RC-37 cells at a multiplicity of infection of 0.01 PFU per cell. The restriction fragment patterns generated by five endonucleases (*Bam*HI, *Kpn*I, *Eco*RI, *Bgl*II, and *Xba*I) of purified E3/3 mutant DNA were compared with the corresponding patterns of the parental standard DNA. The agarose gels displaying restriction fragment patterns were stained with ethidium bromide and photographed under UV light. The gels were then blotted on nitrocellulose filters, and the filters were subjected to Southern blot hybridization (25) with a gE-specific sequence as the first probe (Fig. 1a). This probe comprises those gE sequences which were deleted in plasmid p622(524), which was used in the recombination experiments. As a second probe, we used a *Bgl*II-*Bam*HI fragment specific for Neo^r sequences (Fig. 1a). The results of the analyses with enzymes *Bam*HI and *Xba*I are shown in Fig. 1b. The differences between the individual wt and E3/3 patterns are explained with the help of physical maps of the fragments of the corresponding restriction endonucleases (Fig. 1a). With *Bam*HI-J of E3/3, an increment of 630 base pairs was observed, and homology with the gE gene changed to homology with Neo^r sequences. With *Xba*I, evidently the joint fragments were involved in the recombination event. Apparently a new *Xba*I cleavage site has been introduced in the U_S parts of wt fragments A and B, giving rise to the new E3/3 joint fragments A*, B*, G + Sa, and G + Sb and to the new S-terminal fragments Sa and Sb, respectively (Fig. 1c). The blot hybridizations demonstrated again that the gE sequences evidently had been replaced by Neo^r sequences.

* Corresponding author.

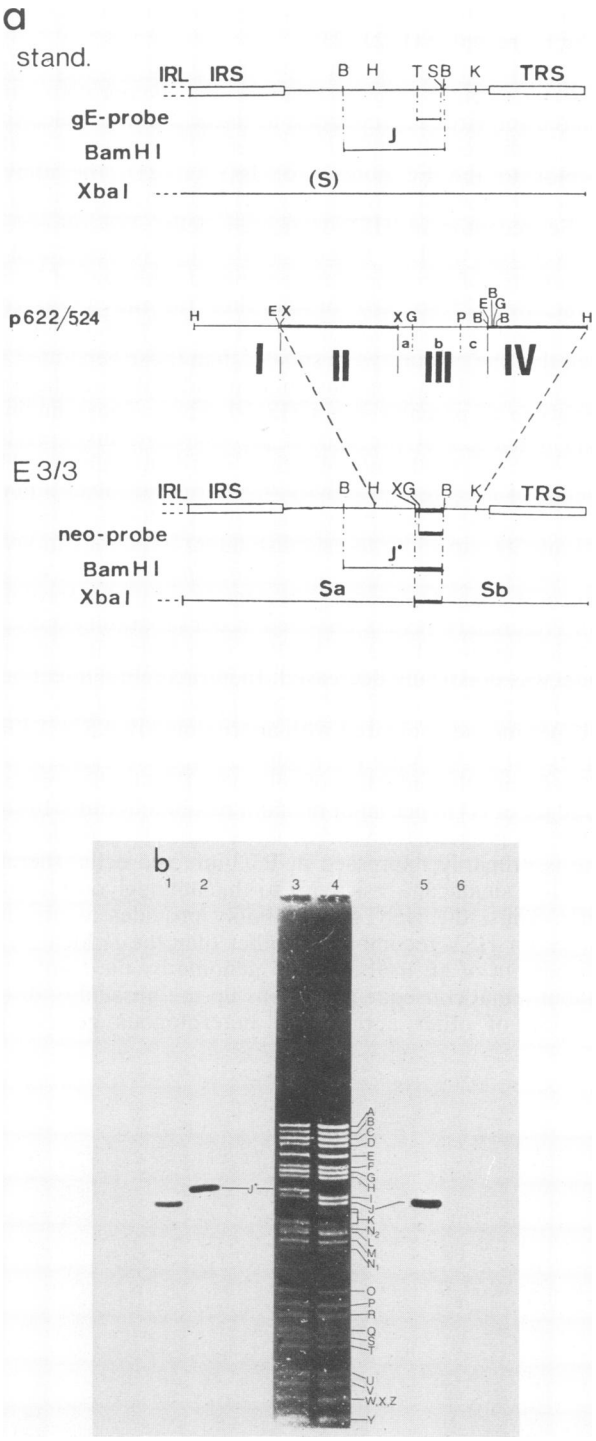

FIG. 1. Restriction enzyme and Southern blot analysis of HSV-1 ANG wt and mutant E3/3 DNAs. (a) Physical maps of the S regions of HSV-1 ANG wt DNA, mutant E3/3 DNA, and linearized plasmid p622(524) DNA. Restriction sites are indicated as follows: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sst*I; T, *Sst*II; and X, *Xba*I. J is *Bam*HI-J. Only the restriction fragments affected by the deletion-insertion mutation are shown. ▬, Tn*5* sequences in the mutant. The precise localization of the gE gene and of the different restriction enzyme recognition sites has been described by McGeoch et al. (20). Regions of the HSV-1 genome are as follows: IRL, inverted repeat of the long region; IRS, inverted repeat of the short region; TRS, terminal repeat of the short region. The sequence arrangement of plasmid p622(524) is as follows: I, pBR322 sequences from the *Nde*I cleavage site at nucleotide 2297 (converted to a *Hin*dIII site) to the *Eco*RI cleavage site at nucleotide 4361; II, HSV-1 DNA sequences mapping from nucleotide 5740 (*Hin*dIII site, converted to an *Xba*I site) to nucleotide 8530 (*Sst*II site, converted to an *Xba*I site); IIIa, simian virus 40 origin of replication from the *Kpn*I cleavage site at nucleotide 294 (converted to an *Xba*I site) to the *Hin*dIII cleavage site at nucleotide 5171 (converted to a *Bgl*II site); IIIb, Tn*5* Neo^r-coding sequences from the *Bgl*II cleavage site at nucleotide 1515 to the *Pst*I cleavage site at nucleotide 2651 (2); IIIc, simian virus 40 polyadenylation signal from the *Pst*I cleavage site at nucleotide 3204 to the *Bam*HI cleavage site at nucleotide 2533; IV, HSV-1 DNA sequences mapping from nucleotide 10068 (*Sst*I site, converted to an *Eco*RI site) to nucleotide 12293 (*Kpn*I site, converted to a *Hin*dIII site). pBR322 and simian virus 40 nucleotide numbers are from GENBANK 7.0 (1 April 1983), and the HSV-1 nucleotide numbers are from McGeoch et al. (20). (b and c) Analysis of standard and mutant DNAs in *Bam*HI (b) and *Xba*I (c) digests. Capital letters designate restriction fragments. Lanes 1 and 2, Result of double probing with the gE probe and the Neo^r probe; lanes 3 and 4, ethidium bromide-stained agarose gels; lanes 5 and 6, corresponding blot hybridizations obtained with the gE probe alone; lanes 2, 3, and 6, E3/3 DNA; lanes 1, 4, and 5, wt viral DNA.

The sequence arrangement of the E3/3 mutant as derived from the above analyses is shown in Fig. 1a.

To confirm that the deleted sequences involved the immunologically defined gE, we analyzed extracts of HSV-1 ANG wt- and E3/3-infected cells by Western blotting which was performed essentially by the method of Snowden and Halliburton (24). A monoclonal mouse antiserum (II-481 B; 15) and a monospecific rabbit antiserum (S24; 21) against HSV-1 gE were used. The results are shown in Fig. 2. As expected from the genetic analysis described above, gE was not expressed in E3/3-infected cells.

The expression of the Fc receptor was investigated in HSV-1 ANG wt- and E3/3-infected cells by immunofluorescence testing by the methods of Watson and Coons (28) and Spendlove et al. (27). Figure 3 shows the indirect staining of HSV-1 ANG wt- and E3/3-infected RC-37 cells with rabbit immunoglobulin G (IgG) and fluorescein isothiocyanate-labeled antirabbit F(ab')$_2$ antibody 7 h after infection. To keep the newly synthesized Fc receptor available for the rabbit antibody, the IgG-containing serum was removed

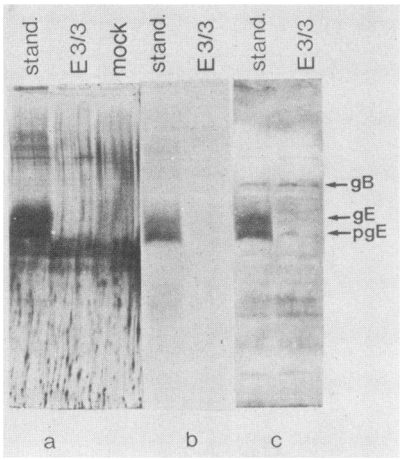

FIG. 2. Western blot analysis of HSV-1 ANG wt- and E3/3-infected cell extracts. (a) First antibody, rabbit monospecific anti-gE antiserum S24 (21, 22; kindly provided by P. G. Spear). Second antibody, goat anti-rabbit IgG, coupled to horseradish peroxidase. (b) First antibody, mouse monoclonal anti-gE antibody II 481 B (15; kindly provided by P. G. Spear). Second antibody, goat anti-mouse IgG $F(ab')_2$ coupled to horseradish peroxidose. (c) The blot described and shown in panel b was reprobed with monoclonal anti-gB antibodies B3 and B5 (13) as first antibodies (kindly provided by J. C. Glorioso) and goat anti-mouse IgG $F(ab')_2$ (horseradish peroxidase coupled) as the second antibody. The immunostain demonstrates that (i) the lanes were loaded with equal amounts of protein (80 μg each), (ii) no anti-gE immunoreactive material was present in E3/3-infected cells, and (iii) gB was expressed at normal levels in E3/3-infected cells.

before virus infection, and the infection was allowed to occur in fetal calf serum-free medium. This manipulation had no influence on the viral reproductive cycle during examination of the cells. The results demonstrate that, in parallel with the lack of gE expression, Fc receptor activity was absent in E3/3-infected cells. This finding strongly confirms earlier data, which suggested that gE is identical with or part of the Fc receptor (1, 21, 23).

The fact that a gE-negative mutant of HSV-1 was viable and stable strongly suggests that this viral glycoprotein is not required for virus reproduction in tissue culture. Since this study confirmed that gE is an essential component of the receptor to the Fc domain of IgG (1, 21), the above-mentioned possibility for gE also holds for the expression of the Fc receptor in HSV-1-infected cells. We observed, however, that the E3/3 mutant of HSV-1 ANG replicated to a lower titer of virus progeny compared with that of its wt parent, i.e., 25 to 30% of the titer for the wt parent. Regarding the biological properties of E3/3, it should be noted that the plaques were smaller than those generated by the wt virus. However, the mutant did not lose the Syn phenotype of parental strain HSV-1 ANG. These findings could indicate that the loss of gE somehow involves the burst size of viral multiplication in tissue culture. The absence of Fc receptor activity, furthermore, raises a number of questions concerning the neurovirulence of mutant E3/3 and its capability to establish latent infections in vivo. Alterations in virulence have been reported as a consequence of changes in single epitopes of viral glycoproteins (14). Preliminary results obtained in our laboratory indicate that the neurovirulence of E3/3 in vivo tested in a mouse model was drastically decreased. In intracerebral infections, the 50% lethal dose of E3/3 was approximately 1.4×10^5 PFU per mouse compared with approximately 0.5×1 PFU of parental strain ANG per animal.

Our finding that infection of cells with E3/3 in the presence of 700 μg of G418 per ml of medium resulted in titers 10-fold higher for progeny than for wt virus suggests that the Neo^r gene is primarily expressed in E3/3-infected cells, thereby providing significant resistance to this aminoglycoside antibiotic. Thus, the Neo^r gene possibly provides a selection marker for HSV recombinants other than the *tk* locus. The replacement of gE in the HSV-1 genome by the Neo^r gene without lethal consequences opens up the possibility of the insertion of other appropriate heterologous genes into

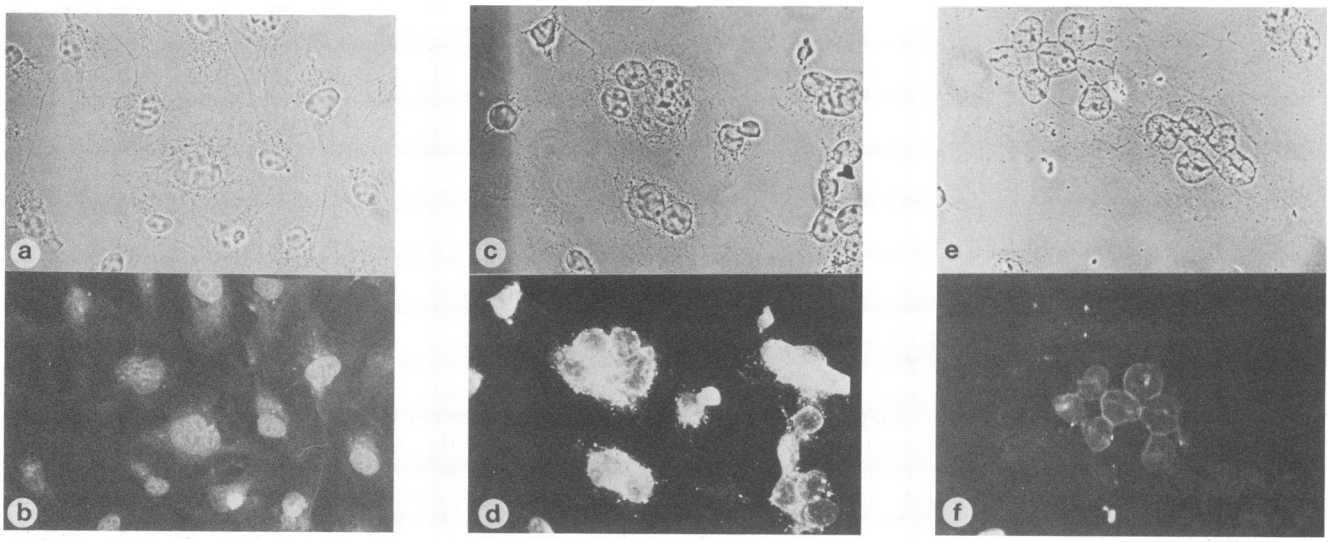

FIG. 3. Immunofluorescence analysis of wt HSV-1 ANG- and E3/3-infected cells (7 h postinfection; multiplicity of infection, 10). Panels a, c, and e were taken with phase-contrast optics, and panels b, d, and f show fluorescein isothiocyanate immunofluorescence of the respective areas. Magnification was 720-fold. (a and b) Mock-infected cells; (c and d) wt-infected cells; (e and f) mutant E3/3-infected cells.

HSV-1 at this position and makes possible the study of gene influences on the viral reproduction process.

ADDENDUM

After completion of this manuscript, a paper by Longnecker and Roizman (17) was published which describes an HSV-1F deletion mutant lacking several genes, including that for gE. The description of an HSV-1 ANG mutant with a deletion confined to the gE gene confirms that gE expression is not essential for in vitro growth of the virus.

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