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# The Herpes Simplex Virus-1 Glycoprotein E (gE) Mediates IgG Binding and Cell-to-Cell Spread through Distinct gE Domains

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**Herpes simplex virus-1 (HSV-1) glycoprotein E (gE) is a multifunctional protein capable of both binding the Fc portion of IgG and mediating cell-to-cell spread of HSV-1. Here we report that the domain on gE involved in IgG binding is distinct from the domain involved in mediating cell-to-cell spread. To do this we have used five mutants of the HSV-1 strain NS: NS-$gE_{null}$, a gE deletion virus; rNS-$gE_{null}$, a gE rescued virus; NS-$gE_{339}$, a gE mutant virus with a four amino acid insert at position 339; rNS-$gE_{339}$, a gE rescue of NS-$gE_{339}$; and NS-$gE_{406}$, a gE mutant virus with the same four amino acids inserted at position 406. Using IgG coated sheep red blood cells in rosetting assays, we show that the NS-$gE_{339}$ does not bind IgG, yet retains the ability to mediate normal cell-to-cell spread. These results demonstrate that the gE domain involved in IgG binding differs from the domain involved in cell-to-cell spread.** 

Herpes simplex virus-1 (HSV-1) expresses eleven glycoproteins on the viral envelope. Glycoproteins gB, gD, gH, gK, and gL are essential for virus entry (1-4). Glycoproteins gC, gE, gI, gG, gJ and gM are non-essential in vitro, yet these glycoproteins have been evolutionarily maintained which suggests that they mediate important functions (5-7). The HSV-1 glycoprotein, gE, is capable of binding IgG and has been described as a low affinity Fc receptor (8-10). The domain on gE involved in IgG binding has been mapped to amino acids 235-380 (11, 12). The ability of gE to bind to IgG may be part of a mechanism through which HSV-1 avoids clearance by the host immune system and may explain the difficulty in developing an effective vaccine for HSV-1 infection. For example, gE has been shown to play a role in protecting HSV-1 infected cells from complement-enhanced antibody neutralization, antibody-dependent cellular cytotoxicity (ADCC) and Fc-dependent attachment of granulocytes in vitro (13-15). While this protective activity has been demonstrated for gE in vitro, a role for gE in protecting the virus in vivo has not been demonstrated.

In addition to Fc receptor activity, gE also plays a role in spread of HSV-1 from cell-to-cell (16-18). HSV-1 spreads from epithelial cells to neurons where it travels to the ganglion (19). HSV-1 mutants which have a gE deletion are defective in their ability to spread from cell-to-cell in vitro and to spread transneuronally after infection of rat eyes in vivo (17, 18). A decreased ability to spread and travel along nerve pathways has also been noted in Pseudorabies virus deficient in the gE homolog and this virus is attenuated for virulence (19-21).

Thus, gE has at least two well studied activities. One is the ability to bind the Fc portion of IgG and the other is the facilitation of viral spread from cell to cell and in nerves. While the role of gE in cell-to-cell spread has been demonstrated in vivo, a role for the ability of gE to protect HSV-1 from the host immune response has not been demonstrated in vivo. In order to demonstrate a role for gE in protecting the virus, it is important to develop a virus which spreads normally, but cannot bind IgG. This virus could then be tested in vivo for virulence and susceptibility to IgG-mediated clearance.

## MATERIALS AND METHODS

*Cells and viruses.* African green monkey kidney cells (Vero) and human epidermal keratinocyte (HaCaT) cells were grown in $\alpha$-minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (Hyclone Laboratories, Inc., Logan, Utah), gentamicin, amphotericin B, vitamins, and N-2 hydroxyethylpiperzine-N′-2-ethanesulfonic acid (HEPES) buffer solution (22).

Wild-type HSV-1 strain NS is a low passage clinical isolate and was used for the generation of gE mutant viruses. To construct a gE null virus, gE was deleted and replaced with a lacZ reporter gene (23). The recombinant gE rescue and gE linker insertion mutants were prepared by recombination of NS-$gE_{null}$ with wild-type gE (NS DNA) or gE with linker inserts (plasmid DNA) (23) and the genomic configuration of the null, rescued and recombinant viruses was verified by Southern blot (23).



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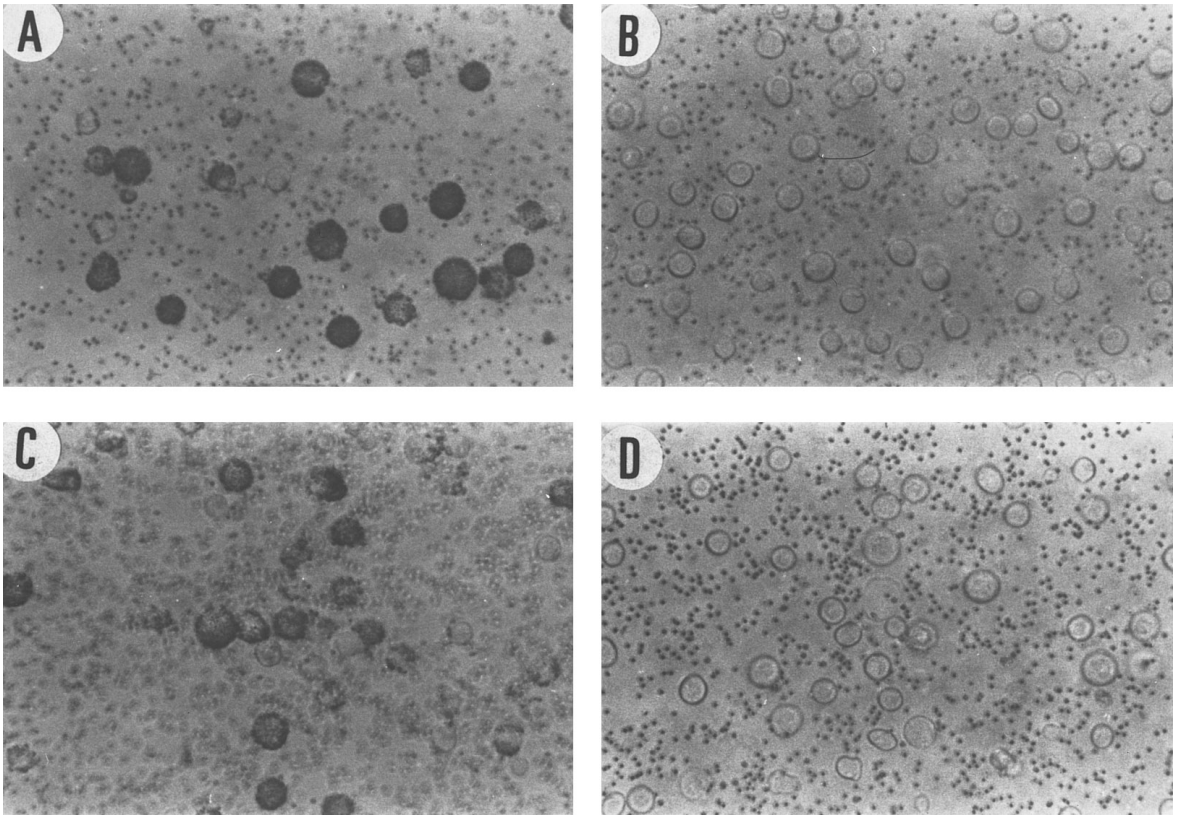


**FIG. 1.** Rosetting of sheep red blood cells by wild-type and mutant virus. Vero cells were infected with NS (A), NS-gE$_{null}$ (B), rNS-gE$_{null}$ (C), and NS-gE$_{339}$ (D). Cells were photographed at a 100× magnification.

*Rosetting assay.* Sheep erythrocytes (ICN Biochemicals Inc.) were sensitized with subagglutinating concentrations of goat anti-sheep erythrocyte antibodies (Life Technologies Inc.) (24). Vero cells were infected with the viruses to be tested at a multiplicity of infection (MOI) of 5 for eighteen hours. The infected cells were then incubated at 37°C for two hour with the IgG coated sheep red blood cells. In order to remove most of the free sheep red blood cells and make the infected cells more easily viewed, the cells were subjected to three washes by low speed centrifugation and resuspended in phosphate buffered saline (PBS). The cells were then viewed at 100× magnification, 100 infected cells were counted and the number of those which had bound five or more sheep red blood cells were considered positive for rosetting and this number was used as the measure of the % of infected cells which rosetted.

*Cell-to-cell spread.* Confluent monolayers of HaCaT cells in 6-well plates were inoculated with 100 p.f.u. of virus. After one hour incubation at 37°C, the viral inoculum was removed and the cells were washed once with PBS. Fresh medium supplemented with 1 % (v/v) human gamma globulin (HGG) (Gammar, Armour Pharmaceuticals) was added to neutralize the spread of extracellular virus. After 48 hours incubation at 37°C, HaCaT cells were fixed and stained with Giemsa for 60 seconds and washed with PBS. Plaque size was determined using an ocular micrometer in an inverted light microscope.

*Single-step growth curves.* Vero cells were infected with virus at an MOI of 5 for 1 hour at 37°C. The inoculum was removed and cells were washed three times with PBS and fresh medium added (zero time point). At various time intervals (0, 4, 8, 16, 20 and 24 hours) medium and cells were harvested separately and frozen at −70°C. Samples were then thawed, cell pellets were sonicated, and infectious virus in both pellets and media were quantified by agarose overlay plaque assay on Vero Cells.

## RESULTS

In order to determine if the gE deleted virus and the linker insertion mutants were altered for IgG binding, the ability of infected cells to bind IgG Fc domains was tested in a rosetting assay using IgG-coated sheep

**TABLE 1**

Rosetting of IgG Coated Sheep Red Blood Cells by NS, Mutant, and Rescued HSV-1 Viruses

| Virus | No. of cells counted | % of infected cells rosetted |
|---|---|---|
| NS (no IgG) | 100 | 0 |
| NS | 100 | 76 |
| NS-gEnull | 100 | 0 |
| rNS-gEnull | 100 | 64 |
| NS-gE339 | 100 | 3 |
| rNS-gE339 | 100 | 63 |
| NS-gE406 | 100 | 72 |

Vero cells were infected with an MOI of 5.0 with the indicated viruses for 18 hours and then incubated for 2 hours at 37°C with IgG coated sheep red blood cells. A total of 100 cells for each condition were counted and the number of cells which displayed five or more bound sheep red blood cells was determined and used as the percent of infected cells rosetted.

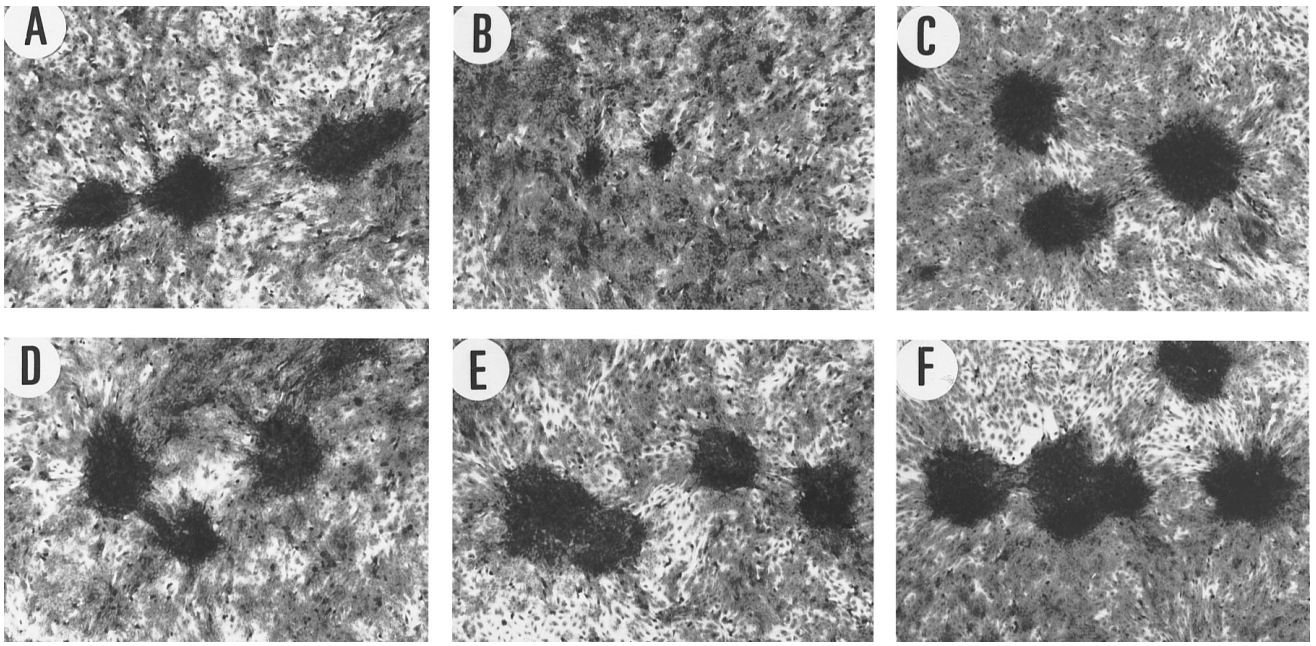


**FIG. 2.** Morphology of foci formed in human epidermal keratinocyte cells, HaCaT, by wild-type, mutant and rescued viruses. Monolayers of HaCaT cells in 6 well plates were infected with 100 p.f.u. and cultured in the presence of 1% HGG for 48 hours. The cells were then stained with Geimsa and photographed at a magnification of 40×. (A) NS, (B) NS-$gE_{null}$, (C) NS-$gE_{339}$, (D) NS-$gE_{406}$, (E) rNS-$gE_{null}$, and (F) rNS-$gE_{339}$.

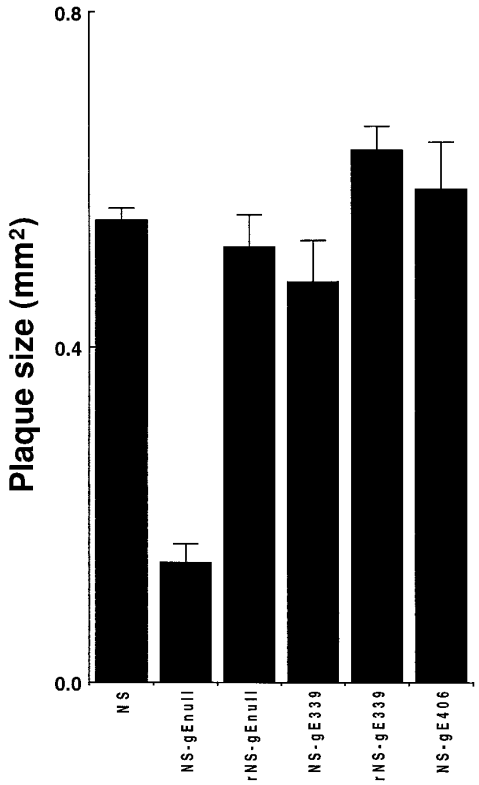


**FIG. 3.** Cell-to-cell spread of wild-type and mutant viruses in human epidermal (HaCaT) cells. Cells were infected at 100 p.f.u. and then cultured in the presence of 1% HGG for 48 hours. The area of the plaques was measured using an ocular micrometer. For each well, 25 plaques were counted in duplicate wells (50 plaques per virus). Plaque size for NS-gEnull was significantly different from all other viruses ($p < 0.01$, analysis of variance using the Scheffe-F test).

erythrocytes. As expected NS-$gE_{null}$ failed to rosette any IgG coated sheep red blood cells while 76% of the NS wild-type infected cells rosetted (Fig. 1). Only 3% of the NS-$gE_{339}$ infected cells rosetted while 63% of the cells infected with rNS-$gE_{339}$ formed rosettes which suggests that both NS-$gE_{null}$ and NS-$gE_{339}$ viruses have lost their ability to bind IgG (Table 1). When cells were infected with the other mutants, 64% - 72% of cells rosetted (Table 1).

In order to determine the ability of the mutant viruses to spread, human epidermal keratinocyte cells (HaCaT) were infected with virus for one hour and then overlaid with medium supplemented with pooled HGG to neutralize the spread of extracellular virus. NS-$gE_{null}$ produced plaques that were eight-fold smaller than wild-type (Figs. 2 and 3). The gE rescued virus, rNS-$gE_{null}$, and the linker insertion mutants NS-$gE_{339}$ and NS-$gE_{406}$ formed plaques similar in size to wild-type (Figs. 2 and 3). These data demonstrate that a four amino acid linker insertion mutation in gE at amino acid 339 within the IgG binding domain does not interrupt the ability of the virus to spread normally in epidermal cells.

To determine if the reduced ability of NS-$gE_{null}$ to spread was the result of decrease replication rates, the replication kinetics of gE mutants were compared with wild-type virus by infecting Vero cells at an MOI of 5 and separately harvesting cells supernatant fluids for viral titers (Figs. 4A and 4B). Each mutant was similar to wild-type indicating that the replication rates of NS, NS-$gE_{null}$, linker insertion mutants and rescued viruses are essentially the same.

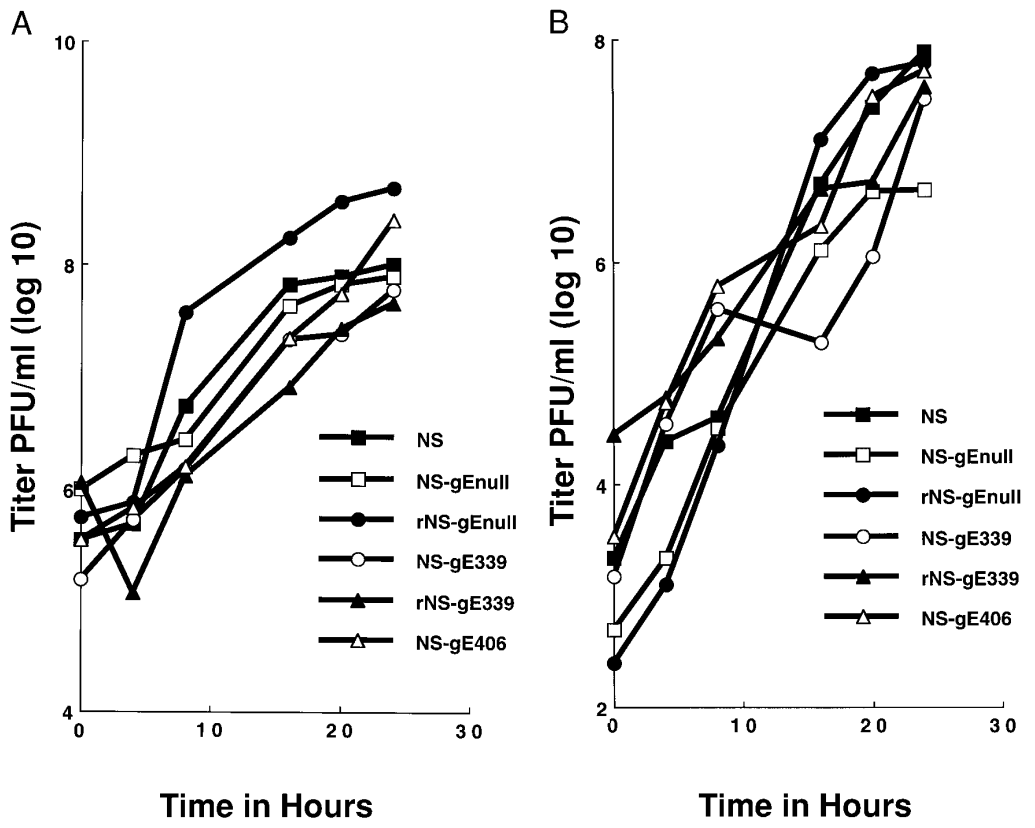


**FIG. 4.** Single-step growth curves of wild-type and gE mutant viruses. Vero cells were infected at an MOI of 5.0 and the cell pellets (A) and culture supernatants (B) were harvested at 1 hour post-infection (zero time point) and at 4, 8, 16, 20, and 24 hours later. Viral titers were determined by plaque assay on Vero cells.

## DISCUSSION

HSV -1 expresses a glycoprotein E which forms an Fc receptor (FcR) that binds to human IgG and blocks Fc-mediated effector functions in vitro (25, 26, 13-15). It has been suggested that through such mechanisms the HSV-1 FcR protects the virus from the host immune system and may render vaccines ineffective (9, 11, 14). However the role of the HSV-1 FcR in vivo has been difficult to demonstrate because glycoprotein E is multifunctional and also mediates viral cell-to-cell spread (16-18). Therefore the lack of virulence observed in gE null viruses (16, 17) could be either due to the inability of these viruses to spread or due to their susceptibility to the host immune system. The purpose of the present study was to develop a gE mutant virus which lacks FcR activity, yet maintains the normal ability to spread from cell to cell. This virus can then be tested in animal models and the role of the FcR studied separately from the contribution of gE to viral spread mechanisms.

The mutant virus NS-$gE_{339}$ presented here does not have FcR activity and is able to spread from cell to cell. This demonstrates that the IgG binding domain on gE is distinct from the domain on gE which mediates cell-to-cell spread. While the domain on gE which binds to IgG and contributes to the FcR has already been mapped to amino acids 235-380 (11, 12), the domain on gE which mediates HSV-1 cell-to-cell spread has yet to be defined. Our studies more narrowly define the gE domain involved in IgG binding and clearly demonstrate that the domain on gE which mediates cell-to-cell spread is distinct from active sites in the HSV-1 FcR.

We are now prepared to test the virulence of virus NS-$gE_{339}$ in mice and determine if the FcR protects the virus from antibody mediated-attack in vivo. Our in vitro data suggest that it does and if so this observation will have an important impact on strategies for HSV-1 vaccine development. For example, if the HSV-1 FcR neutralizes antibodies generated in response to vaccination, then vaccine strategies will need to account for the FcR and may require the targeting of specific domains on gE in order to block the HSV-1 FcR activity.

## ACKNOWLEDGMENT

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