The Herpes Simplex Virus Type 1 US7 Gene Product Is a 66K Glycoprotein and Is a Target for Complement-dependent Virus Neutralization

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SUMMARY

The US7 open reading frame of herpes simplex virus type 1 (HSV-1), previously identified by nucleotide sequencing, has been expressed in a recombinant vaccinia virus (US7-VAC). Antiserum raised against HSV-1 reacted with a 66K glycoprotein in US7-VAC-infected cells and this polypeptide was present on both nuclear and cell surface membranes. In the presence of tunicamycin the protein was reduced in size to 58K showing it contained N-linked sugar residues. Antisera from animals vaccinated with US7-VAC recognized 66K and 58K polypeptides in HSV-1-infected cells and neutralized HSV-1 infectivity in vitro in the presence of complement.

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is a large DNA virus which replicates in the nucleus of infected cells. The 155 kbp genome codes for at least seven membrane-associated glycoproteins (for reviews, see Spear, 1985; Marsden, 1987). These have been intensively studied since they are targets for antibody-mediated neutralization of virion infectivity and hence have potential as anti-HSV vaccines. Three of the glycoprotein genes map within the unique long (UL) region of the genome and are designated gB (Bzik et al., 1984; Pellet et al., 1985), gC (Frink et al., 1983) and gH (Buckmaster et al., 1984; McGeoch & Davison, 1986; Gompels & Minson, 1986). A further five open reading frames with glycoprotein characteristics were identified from the nucleotide sequence of the unique short (Us) region (McGeoch et al., 1985). Open reading frame US6 codes for gD (Spear, 1976), US8 for gE (Baucke & Spear, 1979) and US4 for gG (Ackermann et al., 1986; Richman et al., 1986; Frame et al., 1986; Sullivan & Smith, 1987) which has multiple forms in HSV-1-infected cells and virions. No product has yet been assigned to US5 but very recently US7 was shown to encode a 65K glycoprotein, designated gI (Longnecker et al., 1987). This protein may be the same as gp70 which appears to complex with gE to form the Fc receptor of HSV-1-infected cells (Johnson & Feenstra, 1987).

Our approach to the identification of the US7 gene product was to express this open reading frame in recombinant vaccinia virus (US7-VAC). Serum raised against HSV-1 was used to identify the HSV protein in recombinant vaccinia virus-infected cells and antibody raised against the recombinant vaccinia virus was used to immunoprecipitate the US7 gene product from HSV-1-infected cells. Here we demonstrate that US7 encodes a 66K glycoprotein and show that it is present on the cell surface and nuclear membranes of US7-VAC-infected cells. In addition antibody raised against US7-VAC neutralizes HSV-1 infectivity in vitro in the presence of complement, demonstrating that the US7 gene product, gI, is a virion component.

METHODS

Cells and viruses. BHK-21 cells were passaged in Glasgow-modified Eagle's medium (GMEM) containing 10% tryptose phosphate broth and 10% newborn calf serum. CV-1 cells and human thymidine kinase-negative (TK−) 143 cells were grown in GMEM supplemented with 10% foetal bovine serum (FBS). HeLa S3 cells were
maintained in suspension culture medium (Gibco) containing 5% horse serum. HSV-1 strain SC16 was grown and titrated in BHK cells. Vaccinia virus strain Western Reserve (WR) was grown in HeLa cells and titrated on CV-1 cells (Mackett et al., 1985).

Construction of recombinant plasmid and vaccinia virus. A 1863 bp BamHI–EcoRI DNA fragment containing the HSV-1 strain Patton US7 gene was excised from plasmid pSP64/US7 (Richman et al., 1986) and cloned into BamHI- and EcoRI-digested plasmid insertion vector pGS62 (Smith et al., 1987) downstream of the vaccinia virus promoter taken from a gene encoding a 7.5K protein. Recombinant plasmids were screened for a clone containing the correctly positioned US7 gene by restriction endonuclease digestion and agarose gel electrophoresis of minipreparations of DNA. The plasmid, called pVUS7, was transfected into vaccinia virus-infected CV-1 cells and progeny virus was used to form plaques on TK-143 cells in the presence of 25 μg/ml bromodeoxyuridine. Plaques were screened for the presence of TK- recombinant virus containing the US7 gene inserted within the vaccinia virus TK gene by dot blot hybridization (Mackett et al., 1982) and a positive clone was plaque-purified and then grown and purified as described previously (Mackett et al., 1985). A pure recombinant vaccinia virus containing the US7 gene was called US7-VAC.

Binding of antibody to vaccinia virus plaques. Plaques formed by recombinant US7-VAC or wild-type vaccinia virus (WT-VAC) on CV-1 cell monolayers were fixed with methanol and then incubated with rabbit antibody followed by 125I-labelled affinity-purified staphylococcal Protein A (Amersham) as described previously (Smith et al., 1983).

Radioisotopic labelling and immune precipitation. BHK cells infected at an m.o.i. of 10 with HSV-1 (SC16) were incubated in reduced glucose medium (0.9 mg/ml) from 3.5 to 8 h post-infection and radiolabelled from 4 to 8 h post-infection with 60 μCi/ml D-[1-14C]glucosamine (60 mCi/mmol; Amersham). Where indicated tunicamycin was added at 10 μg/ml 30 min before, and during the labelling period. Lysates were prepared from infected cells and treated with antisera as previously described (Sullivan & Smith, 1987). Precipitated polypeptides were electrophoresed on 10% polyacrylamide gels.

Western blotting. BHK cells infected with WT-VAC or US7-VAC at 30 p.f.u./cell were harvested and lysed 16 h post-infection. Lysates were run on 10% polyacrylamide gels and polypeptides transferred to nitrocellulose membranes as described (Sullivan & Smith, 1987). Membranes were incubated with antisera followed by 125I-labelled affinity-purified Protein A. After washing and drying an autoradiograph was prepared.

Immunofluorescence. CV-1 cells were grown on glass coverslips and infected with WT-VAC or US7-VAC. Sixteen h after infection cells were fixed in 5% paraformaldehyde and, where indicated, permeabilized with 1% Triton X-100 (Randall et al., 1985). Hyperimmune rabbit anti-HSV-1 antibody, which had been preabsorbed on WT-VAC-infected cells for 20 h at 4 °C, was added for 1 h at room temperature, followed by fluorescein isothiocyanate (FITC)-conjugated sheep anti-rabbit immunoglobulin (Wellcome). Photographs were taken under u.v. illumination at ×1000 magnification.

In vitro neutralization of HSV-1 infectivity. Heat-inactivated pooled mouse sera were diluted in GMEM and mixed with 350 p.f.u. of HSV-1 strain SC16 for 30 min at 37 °C. Then 2.5% fresh or heat-inactivated complement was added and the mixture incubated at 37 °C for a further 30 min. Residual infectivity was assayed in duplicate on BHK cell monolayers. Cells were stained with toluidine blue 2 days later and plaques were counted.

RESULTS

The genome of recombinant vaccinia virus US7-VAC was analysed by restriction endonuclease digestion, agarose gel electrophoresis and Southern blotting. A 32P-labelled DNA fragment spanning the HSV-1 US7 open reading frame bound to the HindIII J fragment of recombinant virus US7-VAC but not WT-VAC. The HindIII J fragment of US7-VAC was also increased in size, consistent with the insertion of the vaccinia virus 7.5K promoter and US7 gene (data not shown). These data confirmed that US7-VAC had the predicted genome structure.

Evidence for expression of the US7 gene in cells infected with US7-VAC was obtained by binding anti-HSV-1 antibody to plaques formed by the recombinant virus (Fig. 1). Anti-HSV-1 hyperimmune rabbit sera bound to plaques formed by US7-VAC but not by WT-VAC. Anti-HSV-2 antibody also recognized the US7-VAC but not WT plaques, demonstrating that the HSV-1 US7 gene product and the HSV-2 homologue (Hodgman & Minson, 1986; McGeoch et al., 1987) have type-common antigenic determinants. Both these anti-HSV sera also bound to a lesser extent to uninfected cells. As a control, rabbit anti-vaccinia virus antibody bound to both US7-VAC and WT-VAC plaques.
Identification of HSV-1 US7 gene product

Identification of the US7 gene product

To identify the US7 polypeptide(s) expressed by recombinant vaccinia virus, lysates of cells infected with US7-VAC or WT-VAC were analysed by Western blotting using rabbit anti-HSV-1 antibody (Fig. 2). Polypeptides of 66K and 58K were detected in US7-VAC-infected cells (arrowheads) but not in WT-VAC- or mock-infected cells. Preimmune rabbit sera did not react with these proteins. If the infection was carried out in the presence of tunicamycin, the 66K protein disappeared and the 58K form was the major species, indicating that the 66K form contains N-linked sugar residues. The reduced level of gI-specific polypeptides in the presence of tunicamycin could reflect decreased stability of the underglycosylated form, and/or reduced synthesis after prolonged incubation in the presence of this drug. Preimmune sera did not react with any polypeptides specific to US7-VAC-infected cells in the presence of tunicamycin.

Cellular location of US7 protein

The distribution of US7 proteins in US7-VAC-infected cells was examined by indirect immunofluorescence. Cells infected with US7-VAC or WT-VAC were incubated with rabbit anti-HSV-1 antibody followed by FITC-conjugated sheep anti-rabbit IgG (Fig. 3). If antibodies were reacted with cells with non-permeabilized surface membranes, HSV-1-specific antigen was detected on the surface of US7-VAC- but not WT-VAC-infected cells (panels a and b, respectively). In cells with permeabilized membranes (c and d) HSV-1 antigen was also detected strongly on the nuclear membrane and within the cytoplasm (c). Very little binding was observed on WT-VAC-infected cells (d). The US7-specified polypeptides are therefore transported to both the nuclear and cell surface membranes in the absence of other herpesvirus proteins.

US7 protein in HSV-1-infected cells

To detect the US7-specified polypeptide(s) in HSV-1-infected cells antibody was raised against US7 antigen by immunization of mice with US7-VAC. These sera were then used in immunoprecipitation assays using [14C]glucosamine-labelled HSV-1-infected cell lysates. A
The US7 gene product is a target for antibody neutralization of virus

Pooled antisera from ten mice immunized with recombinant vaccinia virus US7-VAC or YC3-VAC were tested for their ability to neutralize HSV-1 infectivity in the presence of fresh or inactivated complement. Fig. 5 shows that antibody raised against US7-VAC was able to neutralize HSV-1 infectivity in the presence of complement but that sera raised against another TK⁺ vaccinia virus recombinant expressing an unrelated gene were not. In the absence of fresh complement no virus neutralization was observed. The extent of neutralization was indistinguishable from that obtained in parallel with antisera raised against a vaccinia virus recombinant expressing HSV-1 gG (Sullivan & Smith, 1987). We had noted that anti-HSV-2 antibody reacted with plaques formed by US7-VAC and therefore were interested to know whether anti-US7-VAC would neutralize HSV-2 infectivity. No neutralization was observed and we could not detect any HSV-2 polypeptides with anti-US7 serum (data not shown).
Identification of HSV-1 US7 gene product

DISCUSSION

The open reading frame US7 of HSV-1, which was identified by nucleotide sequencing (McGeoch et al., 1985), has been expressed in recombinant vaccinia virus. The product of this gene is a 66K glycoprotein which associates with nuclear and cell surface membranes of infected cells and which contains N-linked carbohydrate. Consistent with this cellular location, the deduced amino acid sequence of the US7 gene contains hydrophobic amino acid sequences at the amino and carboxy termini which could function as membrane signal and anchor sequences, respectively. In addition the protein contains three potential N-linked carbohydrate attachment sites within the primary amino acid chain of Mr 41,366. In the absence of N-linked carbohydrate we find the US7 gene product has an apparent Mr of 58K. Although this is considerably larger than the predicted primary translation product it is consistent with a 55K protein produced by \textit{in vitro} translation of hybrid-selected RNA from the US7 region (Lee et al., 1982) and a 52K protein produced from \textit{in vitro} translation of RNA transcribed from an SP6 vector containing the US7 gene (Richman et al., 1986).

Recently the US7 gene product was identified in another way. Longnecker et al. (1987) constructed a mutant of HSV-1 from which US7 was deleted and identified a monoclonal antibody (Fd69) that reacted only with cells infected with viruses containing the US7 gene. Fd69 reacted with a 65K glycoprotein which was transported to the cell surface. Johnson & Feenstra

Fig. 3. Location of US7 gene product in US7-VAC-infected cells. Sixteen h after infection with WT-VAC (b, d) or US7-VAC (a, c) at 30 p.f.u./cell, CV-1 cells were fixed in paraformaldehyde and then reacted with rabbit anti-HSV-1 serum either directly (a, b) or after permeabilization (c, d) with 1% Triton X-100. Bound antibody was detected with FITC-conjugated sheep anti-rabbit IgG. Photographs were taken using an oil immersion lens. Bar marker represents 10 μm.
(1987) identified a 70K HSV-1 glycoprotein (designated g70) that is distinct from gE, gD and gG and which is probably encoded by US7. The g70 appears to complex with gE and form a receptor able to bind IgG.

The product of the US7 open reading frame is the seventh glycoprotein shown to be encoded by the HSV-1 genome. Consistent with previous nomenclature this is called gI. The previous six virus-coded glycoproteins are all virion components and targets for virus neutralization by antibody (for review, see Marsden, 1987). Since antibody raised against gI neutralizes virus infectivity in vitro, gI must also be a virion component. Despite being virion components the majority of these virus-coded glycoproteins are not essential for virus replication in vitro, since viable mutants with deletions in gC, gE, gG, gI and the putative glycoprotein product of US5 have been constructed (Longnecker & Roizman, 1986, 1987; Weber et al., 1987; Longnecker et al., 1987).

Regarding the functions of these non-essential glycoproteins, it is possible that they broaden cell tropism in vivo. Specifically, gG is reported to affect the virus pathogenesis in the central nervous system of mice (Weber et al., 1987). Glycoprotein E and a 70K glycoprotein which is probably gI interact to form the Fc receptor of HSV-1-infected cells (Johnson & Feenstra, 1987).
Identification of HSV-1 US7 gene product

Although the role in vivo of this complex is unclear it is possible that the Fc binding capacity of virions or infected cells is a mechanism helping evasion of membrane lysis by complement-dependent antibody (Lehner et al., 1975; Frank & Friedman, 1987). If antibody bound to a virus glycoprotein either on the surface of an infected cell or virus particle were also complexed to the Fc receptor via the Fc region of the immunoglobulin, this might prevent complement binding and membrane lysis.

Whatever the precise role of gI is, it is apparent that it has a gene homologue in a colinear position of the HSV-2 genome (Hodgman & Minson, 1986; McGeoch et al., 1987) that is also immunologically cross-reactive. Varicella-zoster virus gpI and pseudorabies virus gp63 also have structural homology with HSV-1 gI (Davison, 1983; Davison et al., 1985, 1986; Davison & McGeoch, 1986; Petrovskis et al., 1986). These data suggest a conservation of function. Although we observed that antisera raised against HSV-2 recognize HSV-1 gI, serum raised against HSV-1 gI does not neutralize HSV-2 infectivity in vitro or react with HSV-2 proteins. This may be due to the relatively low titre or low avidity of antibody to HSV gI compared with the anti-HSV-2 antibody which was derived from a hyperimmunized rabbit. Although the neutralization of HSV-1 infectivity observed with anti-HSV-1 gI antibody is complement-dependent and poor compared with that obtained with anti-gB, -gH or -gD antibodies, gI could have a role as a component of future anti-HSV-1 vaccines. In addition to its use in identifying HSV-1 gI, the recombinant vaccinia virus expressing gI will also be used to determine whether immunity to gI can prevent lethal or latent HSV-1 infections in mice and whether T helper and T cytotoxic cells directed against the protein are produced during HSV-1 infection.

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REFERENCES
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