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The insulin degrading enzyme binding domain of varicella-zoster virus (VZV) glycoprotein E is important for cell-to-cell spread and VZV infectivity, while a glycoprotein I binding domain is essential for infection

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ABSTRACT

Varicella-zoster virus (VZV) glycoprotein E (gE) interacts with glycoprotein I and with insulin degrading enzyme (IDE), which is a receptor for the virus. We found that a VZV gE deletion mutant could only be grown in cells expressing gE. Expression of VZV gE on the surface of cells did not interfere with VZV infection. HSV deleted for gE is impaired for cell-to-cell spread; VZV gE could not complement this activity in an HSV gE null mutant. VZV lacking the IDE binding domain of gE grew to peak titers nearly equivalent to parental virus; however, it was impaired for cell-to-cell spread and for infectivity with cell-free virus. VZV deleted for a region of gE that binds glycoprotein I could not replicate in cell culture unless grown in cells expressing gE. We conclude that the IDE binding domain is important for efficient cell-to-cell spread and infectivity of cell-free virus.

Introduction

Varicella-zoster virus (VZV) causes chickenpox and shingles. The virus expresses at least seven glycoproteins. Glycoprotein B (gB) binds to cellular heparan sulfate proteoglycans (Jacquet et al., 1998), and is likely important for entry of VZV into the cell. gC is not required for growth of virus in vitro (Cohen and Seidel, 1994), but is critical for replication in fetal human skin in the SCID-hu mouse (Moffat et al., 1998). gH facilitates cell-to-cell spread of virus (Rodriquez et al., 1993). gL acts as a chaperone for transport of gH to the cell surface (Duus et al., 1995). gI forms a complex with gE, and while gI is not required for replication of VZV in some cells, gI is required for VZV replication in Vero cells, T cells, and human skin (Cohen and Nguyen, 1997; Moffat et al., 2002). gK is required for replication of VZV and may have a role in syncytia formation (Mo et al., 1999), while gM is important for cell-to-cell spread (Yamagishi et al., 2008).

gE is the most abundant glycoprotein on the surface of VZV-infected cells (Montalvo et al., 1985). gE is also present in the cytoplasm of infected cells and on the surface of virions. gE binds to the Fc portion of human immunoglobulin (Litwin et al., 1992). Epithelial cells expressing gE show increased cell-to-cell contact with enhanced trans-epithelial resistance (Mo et al., 2000). During infection, gE is taken up by endocytosis from the plasma membrane and incorporated into virions (Maresova et al., 2005). The ORF47 protein kinase phosphorylates gE, resulting in transport of endocytosed gE to the trans-Golgi network (Kenyon et al., 2002). gE forms a heterodimer with VZV gI (Kimura et al., 1997) which enhances the ability of gE to serve as an Fc receptor (Litwin et al., 1992). The SP1 transcription factor is important for regulating expression of VZV gE (Berarducci et al., 2007).

gE binds to insulin degrading enzyme (IDE) which serves as a receptor for the virus (Li et al., 2006). IDE is important for infection of cell-free VZV, and for cell-to-cell spread of the virus in vitro. A portion of the amino terminus of gE (amino acids 32–71) is required for binding to IDE and a region within in the first third of gE (amino acids 163–208) is important for its ability to form a complex with gI (Li et al., 2007).

A gE mutant isolated from patients with varicella showed enhanced cell-to-cell spread (Santos et al., 2000). While VZV deleted for gE failed to replicate in vitro, the latter was not complemented in a cell line, and thus it was not formally shown to be essential (Mo et al., 2002). Viable VZV mutants could be constructed in a gE domain (amino acids 568 to 571- AYRV) that directs the protein to the trans-Golgi network, but not in a domain (amino acids 582 to 585, YAGL) that mediates endocytosis of gE (Moffat et al., 2004). Deletion of amino acids 27–51 of gE resulted in a virus that was not impaired for growth in cell culture, deletion of amino acids 51 to 187 of gE yielded a virus that was impaired for replication in vitro, while deletion of

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amino acids 27–187 resulted in a virus that could not replicate in cell culture (Berarducci et al., 2006).

We have constructed deletion mutants of VZV lacking the IDE binding domain of gE, a gI binding domain of gE, and nearly the entire open reading frame of gE; the latter two mutants could only be grown in cells expressing gE. We also determine if VZV gE expressed in mammalian cells can complement the growth of an HSV-1 gE deletion mutant or inhibit the growth of VZV.

Results

VZV gE is required for virus replication

Cosmid VZV MstII A-68D is deleted for amino acids 1 to 469 of the 623 amino acid gE (Fig. 1). Transfection of melanoma cells with plasmid pCMV62 and cosmids NotI A, Not IB, Mst IIA, and Mst IIB resulted in infectious VZV 7 days later, while transfection of cells with plasmid pCMV62 and cosmids NotI A, Not IB, Mst IIB, and Mst IIA-68D failed to yield CPE despite multiple transfections.

Baculovirus expressing gE was constructed so that melanoma cells could be infected and express the glycoprotein to complement a gE deletion mutant virus. Baculo gE-RV contains gE driven by the human CMV IE promoter. Lysates of Sf9 cells infected with Baculo gE-RV and immunoblotted for gE did not show evidence of gE expression (Fig. 2A, lane 2). Lysates of melanoma cells infected with the virus also failed to show expression of gE (Fig. 2A, lane 3). Since sodium butyrate, a histone deacetylase inhibitor, enhances expression of foreign genes expressed by baculoviruses in mammalian cells (Condreay et al., 1999), we treated Baculo gE-RV-infected cells with sodium butyrate, and detected gE (Fig. 2A, lane 4). Similarly, when cells were infected with Baculo gE-RV and then infected with VZV deleted for gE (see below) the glycoprotein was detected (Fig. 2A, lane 7). The size of gE in cells infected with Baculo gE-RV was similar to that of cells infected with VZV (Fig. 2A, lane 8).

gE was present predominantly on the surface of melanoma cells infected with VZV ROka, and on the surface and in the cytoplasm of cells infected with Baculo gE-RV (in the absence of gI) (Fig. 2B). Addition of butyrate to cells infected with Baculo gE-RV resulted in a marked increase in expression of gE. Cells infected with Baculo gE-RV and VZV deleted for gE (see below) showed gE predominantly on the surface with some gE also in the cytoplasm.

Melanoma cells were infected with baculovirus expressing gE 1 h before transfection. Infection of cells with Baculo gE-StuI (which contains VZV gE driven by the baculovirus polyhedron promoter and the CMV IE promoter) followed by transfection with plasmid pCMV62 and cosmids NotI A, Not IB, Mst IIB, and Mst IIA-68D resulted in infectious VZV 11 days later in one experiment, and 20 days later in a second experiment. Infection of melanoma cells with Baculo gE-RV followed by transfection with pCMV62 (which expresses the IE62 protein and enhances the efficiency of the transfection) and the four cosmids (including the ORF68 deletion mutant cosmid) yielded VZV after 11 days in one experiment and 17 days in the second experiment. Virus resulting from the first infection with Baculo gE-RV was termed ROka68D and was chosen for use in subsequent experiments. The virus was passaged in cells that had been infected with Baculo gE-RV one day prior to infection with VZV.

To verify that ROka68D had the expected genome structure, virion DNA was cut with BamHI and Southern blotting was performed using probes for gE. Digestion of ROka68D resulted in two bands of 2.5 and 2.1 kb, while digestion of ROka yielded bands of 4.0 and 2.1 (Fig. 3A). PCR was performed, using primers that span the gE deletion in ROka68D, and sequence analysis showed that ROka68D had the expected deletion in gE.

Immunoblotting of cells infected with Baculo gE-RV and ROka68D using monoclonal antibody to gE yielded bands of 60 to 90 kDa (Fig. 4A). A band of 52 kDa was detected using antibody to VZV IE4. After one passage in melanoma cells without Baculo gE-RV, the level of gE was reduced while the amount of viral IE4 was not diminished (Fig. 4B).

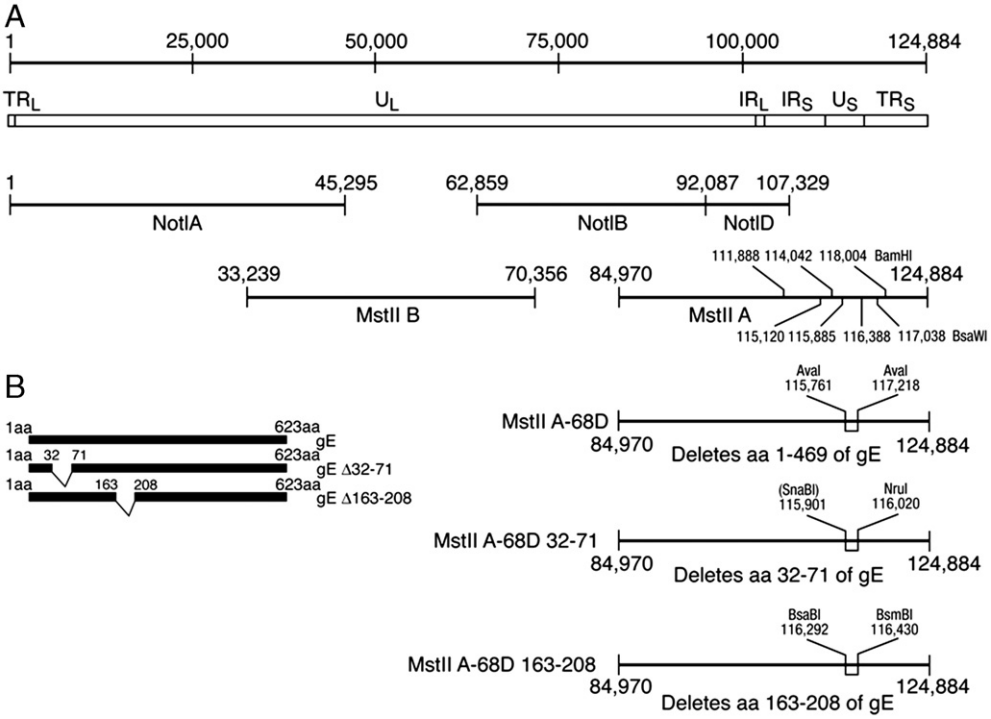

Fig. 1. Construction of recombinant VZV deleted for gE and for the IDE and gI binding domains of gE. (A) The prototype VZV strain Dumas genome is 124,884 bp in length (line 1), with unique long (UL), unique short (US) and internal repeat (IR) and terminal repeat (TR) regions (line 2). Four cosmids encompass the VZV genome (lines 3, 4). Selected BamHI and BsaWI restriction sites used in Southern blots of virion DNA are shown in cosmid MstII A. Cosmid MstII A-68D is deleted for codons 1–469 of ORF68 which encodes glycoprotein E (line 5). Cosmid MstIIA-68D32-71 is deleted for codons 32 to 71 which contain the IDE binding domain of gE (line 6). Cosmid MstIIA-68D163-208 is deleted for codons 163–208 which encode the gI binding domain of gE (line 7). (B) The structures of VZV gE, gEΔ32-71, and gEΔ163-208 are shown.