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The Amino Terminus of Varicella-Zoster Virus (VZV) Glycoprotein E Is Required for Binding to Insulin-Degrading Enzyme, a VZV Receptor▽

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Varicella-zoster virus (VZV) glycoprotein E (gE) is required for VZV infection. Although gE is well conserved among alphaherpesviruses, the amino terminus of VZV gE is unique. Previously, we showed that gE interacts with insulin-degrading enzyme (IDE) and facilitates VZV infection and cell-to-cell spread of the virus. Here we define the region of VZV gE required to bind IDE. Deletion of amino acids 32 to 71 of gE, located immediately after the predicted signal peptide, resulted in loss of the ability of gE to bind IDE. A synthetic peptide corresponding to amino acids 24 to 50 of gE blocked its interaction with IDE in a concentration-dependent manner. However, a chimeric gE in which amino acids 1 to 71 of VZV gE were fused to amino acids 30 to 545 of herpes simplex virus type 2 gE did not show an increased level of binding to IDE compared with that of full-length HSV gE. Thus, amino acids 24 to 71 of gE are required for IDE binding, and the secondary structure of gE is critical for the interaction. VZV gE also forms a heterodimer with glycoprotein gI. Deletion of amino acids 163 to 208 of gE severely reduced its ability to form a complex with gI. The amino portion of IDE, as well an IDE mutant in the catalytic domain of the protein, bound to gE. Therefore, distinct motifs of VZV gE are important for binding to IDE or to gI.

Varicella-zoster virus (VZV) is a member of the alphaherpesvirus subfamily. VZV causes chickenpox during primary infection, in which it disseminates throughout the body. The virus establishes latency in dorsal root and cranial nerve ganglia and can reactivate to cause shingles. During acute infections, VZV is probably transmitted to other persons as a cell-free virus; during viremia, the virus circulates in T cells (1). In cell cultures, VZV is highly cell associated, and cell-to-cell spread is the exclusive route of infection.

The early events of VZV infection are similar to those observed with the other human alphaherpesvirus, herpes simplex virus (HSV). Both viruses attach to cell surface heparan sulfate and engage cellular receptor(s) (44, 49). Mannose 6-phosphate inhibits the infectivity of cell-free VZV, which implicates the cation-independent mannose 6-phosphate receptor (MPR^{ci}) in facilitating entry of cell-free viruses by interacting with viral glycoproteins that contain phosphorylated N-linked complex oligosaccharides (17, 49). A soluble form of mannose 6-phosphate receptor blocks cell-to-cell spread of HSV, and HSV gD colocalizes with the mannose 6-phosphate receptor in endosomes (5, 6). The involvement of MPR^{ci} at an early step of VZV infection is further supported by the finding that stable MPR^{ci}-deficient cell lines are refractory to infection by cell-free VZV (8).

Previously, we showed that insulin-degrading enzyme (IDE) binds VZV glycoprotein E (gE) and serves as a cellular receptor for the virus (24). Down-regulation of IDE by small interfering RNAs or blocking of IDE by antibodies or by bacitracin inhibited VZV infection and impaired the cell-to-cell spread of the virus. Human IDE was overexpressed by transfection into cell lines, resulting in increased entry and enhanced infection with both cell-free and cell-associated virus. Nectin-1, nectin-2, herpes virus entry mediator A, and 3-*O*-sulfated heparan sulfate bind HSV gD and function as receptors for HSV (10, 18, 33, 41, 45).

Several studies showed that gE has a vital role in VZV infectivity. First, gE is required for virus replication (4, 29). Second, syncytium formation, which is characteristic of VZV infection, is caused by virus-induced membrane fusion when infection occurs by cell-to-cell spread. gE is an integral part of the VZV fusion machinery (12), and in HSV, gE is indispensable for cell-to-cell spread of the virus (16, 37). Third, antibodies to VZV gE can neutralize virus infectivity (46).

In addition to binding IDE, VZV gE forms a heterodimer with glycoprotein I (gI) (21, 32, 47). The presence of gI facilitates gE maturation and cycling between the cell surface and endosomes through the *trans*-Golgi network, where envelopment of virions occurs (20, 34). gE labeled with biotin at the cell surface is incorporated into virions as a complex together with gI (27). gI is important for processing of gE to its mature form. Cells infected with VZV deleted for gI show aberrant gE maturation and distribution. Although gI is dispensable for VZV replication in vitro in human melanoma cells, gI is essential for VZV replication in Vero cells and in human skin and thymus implants in SCID mice (11, 26, 29, 31). The gE-gI complex functions as a virus-encoded Fc receptor (25). Although the extracellular domain of gE has been shown to contain a gI binding domain (4, 38), the actual binding site has not been determined.

In this study, we constructed a series of VZV gE deletion mutants to map the region of gE that binds to IDE and to VZV gI. We also used peptides corresponding to portions of gE to determine amino acid sequences that block the interaction of

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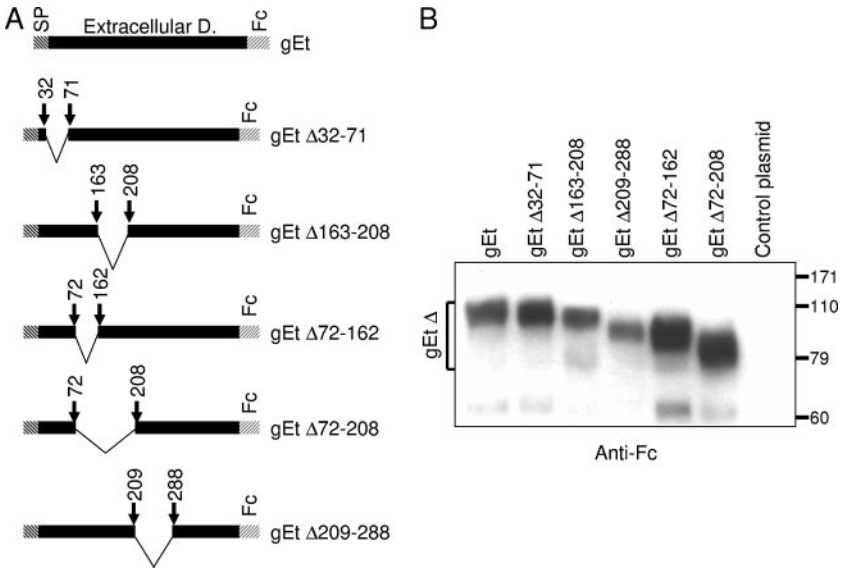

FIG. 1. Expression of VZV gEt and gEt truncation mutants. (A) Map of the extracellular domain of VZV gE fused to immunoglobulin Fc (gEt) and gEt truncation mutants. (B) Immunoblot of VZV gEt and truncation mutants, using anti-human Fc antibody. The numbers to the right of panel B are molecular weights in thousands.

gE with IDE. In addition, we analyzed mutants to identify the region of IDE needed to bind VZV gE. These findings indicate the domains of gE and IDE that are important for the interactions of these proteins so that they can initiate the early steps of VZV infection.

MATERIALS AND METHODS

Cells, plasmids, peptides, and antibodies. MeWo (human melanoma) cells (obtained from C. Grose, University of Iowa) were grown in minimum essential medium with 10% fetal bovine serum (FBS). HeLa and CV-1/EBNA cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's modified eagle medium (Invitrogen, Carlsbad, CA) with 10% FBS. Plasmids encoding His-tagged wild-type human IDE and three IDE mutants, IDE-E111Q, IDE-N, and IDE-C, were described previously (23). Plasmid pDC409-gIt was described previously (24). A plasmid expressing full-length HSV-2 gE (pcDNA3-gE) was provided by Jerry Weir (FDA, Bethesda, MD). Three VZV gE peptides were synthesized by Biosynthesis (Lewisville, TX). Peptide gEP-1 corresponds to gE amino acids 24 to 50, gEP-2 to gE amino acids 51 to 71, and gEP-3 to gE amino acids 39 to 63. All three peptides were unmodified, and the purity of the peptides was over 85%, as analyzed by high-pressure liquid chromatography. Rabbit anti-IDE antibody IDE-1 was directed against amino acids 56 to 68 of human IDE exon 2 (24). Rabbit anti-IDE antibody UCG43 was a gift from Marsha Rosner (University of Chicago) (9). Anti-hemagglutinin (anti-HA) monoclonal antibody HA.11 was obtained from Covance Research Products, Inc. (Berkeley, CA), and anti-HA monoclonal antibody 3F10 was obtained from Roche Diagnostics Corporation (Indianapolis, IN). Rabbit polyclonal anti-gI was a gift from Paul Kinchington (University of Pittsburgh). Bacitracin was obtained from Sigma-Aldrich, Inc. (St. Louis, MO).

Construction of gE deletion mutants. Plasmid pDC409-gE-Fc encodes the full-length extracellular domain of gE (amino acids 1 to 537) with a human immunoglobulin (Ig) Fc tag fused in-frame to its carboxy terminus (24). The protein expressed by this plasmid is referred to as gEt (Fig. 1A). Since the first 22 amino acids of gE are predicted to be a signal peptide, this region was preserved in all of the gE deletion mutants constructed. A plasmid expressing gEtΔ32-71 was generated by introducing a SnaBI site at nucleotide 93 of the gE open reading frame (ORF) in pDC409-gE-Fc by site-directed mutagenesis, using primer 5′-GCGTATAACGAATCCGGTCAGAGCATACGTATTGCGATAC GATG-3′ and a Quick Change kit (Strategene, La Jolla, CA). Codons 32 to 71 of gE were subsequently removed by digesting the plasmid with SnaBI and NruI and ligating the large fragment to itself. In addition to the deletion, amino acid 31 of gE was changed from Ser to Tyr. To produce additional mutants, gEt was removed from pDC409-gE-Fc by digestion with SalI and NotI and inserted into the corresponding sites of pBlueScript SK^+ (Stratagene), resulting in pBlueScript SK^+-gEt. A plasmid expressing gEtΔ163-208 was created by digesting pBlueScript SK^+-gEt with BsmBI, blunting the DNA with the Klenow fragment of DNA polymerase I, digesting it with BsaBI, and ligating the large fragment to itself. This results in loss of codons 163 to 208 of gE. The mutated gEt cassette was then released from the vector by digesting with SalI and NotI and inserted in place of wild-type gEt at the corresponding site of pDC409-gE-Fc. To construct plasmids expressing gEtΔ72-162 and gEtΔ72-208, nucleotide 210 of the gE ORF was deleted upstream of the NruI restriction site by site-directed mutagenesis in cloning vector pBlueScript SK^+-gEt, using mutagenesis primer 5′-GGG TAAATCGGGGAGAGTCTCGCGAAAAGCGTACGATC-3′ to keep gE in-frame after the deletions were made. The resulting plasmid was cut with NruI and BsaBI (which deletes codons 72 to 162) or BsmBI followed by Klenow to blunt the ends and then by NruI (which deletes codons 72 to 208). Each of the large fragments was then ligated to itself. The mutated gEt sequences were excised from the vector by digestion with SalI and NotI and inserted into the corresponding site of pDC409-gE-Fc in place of wild-type gEt. In addition to the deletions in gEtΔ72-162 and gEtΔ72-208, amino acid 71 of gE was changed from Ser to Arg in each protein at the junction of the deletion. To construct a plasmid expressing gEtΔ209-288, the SalI-NotI fragment of gE from plasmid pDC409-gE-Fc was inserted into the corresponding sites of pCMV-Script (Stratagene). The resulting plasmid was cut with BsmBI, blunted with Klenow, and digested with ScaI, and the large fragment was ligated to itself. Nucleotide 867 of gE was deleted from the plasmid by site-directed mutagenesis, using primer 5′-GGGT GTCTTGAAAGTACTCGGACAGAAAAACAATACTTGG-3′ to maintain the gE ORF. In addition to the deletion in gEtΔ209-288, amino acid 119 was changed from Leu to Thr at the junction of the deletion. The gE cassette was then released with SalI and NotI and inserted in place of wild-type gEt at the corresponding site of pDC409-gE-Fc. Each of the mutant constructs was sequenced to verify that it had the expected sequence.

Construction of chimeric VZV-HSV gE. Plasmid pcDNA3-gE expressing full-length HSV-2 gE with an HA tag at its carboxy terminus was digested with BstEII, blunted with Klenow, and digested with KpnI, and the large fragment containing the vector, cytomegalovirus promoter, and HSV-2 gE without the first 29 codons was gel purified. The nucleotide sequence which encodes the first 71 amino acids of VZV gE was amplified by PCR, using primers 5′-GGAAGGTA CCGCCTGTAATATGGG-3′ (which introduces a KpnI site upstream of the ATG) and 5′-GCTTTTCGCGAAGACTCTCC-3′ (which includes an internal NruI site), and inserted into the BluntII-TOPO vector (Invitrogen). The VZV gE fragment was then digested with KpnI and NruI and ligated to the fragment derived from pcDNA3-gE, which contains HSV-2 gE lacking the first 29 amino acids. The resulting plasmid, pcDNA3-gE-chimera, encodes VZV gE amino

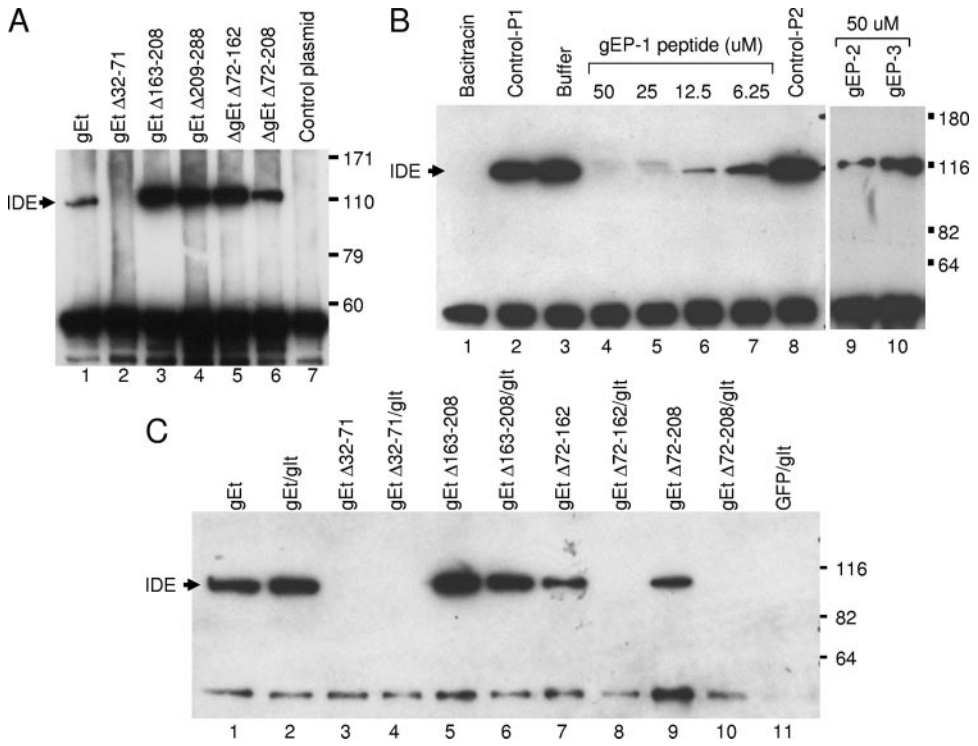

FIG. 2. Deletion of gE amino acids 32 to 71 abolishes binding to IDE. (A) gEt or gEt truncation mutants were immobilized onto protein A-Sepharose beads, human melanoma cell lysates were added, and after washing and boiling, the proteins were immunoblotted with anti-IDE antibody. (B) The pull-down assay described for panel A was performed in the presence or absence of peptides at the indicated concentrations, followed by immunoblotting with anti-IDE antibody. (C) Pull-down assays were performed with cells cotransfected with gEt and gIt and immunoblotted with anti-IDE antibody. The numbers to the right of the panels are molecular weights in thousands.

acids 1 to 71, followed by HSV-2 gE amino acids 30 to 545. The nucleotide sequence of VZV gE, as well as the VZV-HSV gE junction, was confirmed by sequencing.

Expression of gE proteins and pull-down assays. CV1/EBNA cells were transfected with plasmids encoding wild-type gEt or gEt mutants, each of which has an IgG Fc tag, using Lipofectamine 2000 (Invitrogen). At 16 h after transfection, additional medium supplemented with 0.5% FBS with a low level of Ig (HyClone Laboratories Inc, Logan, UT) was added. Five days after transfection, tissue culture supernatants were collected, gE proteins were immunoprecipitated with protein A Sepharose beads (Sigma-Aldrich) at 4°C overnight, and the beads were washed extensively in lysis buffer (25 mM Tris-HCl [pH 7.4], 5 mM EDTA, 15 mM NaCl, and 0.1% NP-40). Melanoma cell lysates were prepared by incubating cells at a concentration of 1×10^7 cells/ml in the lysis buffer. IDE proteins expressed in *Escherichia coli* were prepared as described previously (23). Pull-down assays were performed as previously described (24). Briefly, gE proteins bound to protein A Sepharose were incubated with melanoma cell or *E. coli* lysates at 4°C for 1.5 h, washed, boiled, and resolved by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. The proteins were transferred to nitrocellulose membranes, probed with primary antibodies and horseradish peroxidase-conjugated secondary antibodies, and visualized by enhanced chemiluminescence (SuperSignal; Pierce, Rockford, IL).

Expression and purification of recombinant IDE. Plasmids encoding His-tagged wild-type human IDE and IDE mutants IDE-E111Q, IDE-N, and IDE-C were expressed and purified as described previously (9, 23). Briefly, *E. coli* Rosetta cells (Novagene/EMD Biosciences, San Diego, CA) were transformed with the plasmids, and the cells were cultured at 30°C until the optical density at 600 nm reached 0.6. Isopropylthio-β-galactoside (IPTG) was added to a final concentration of 0.2 mM, and the cells were cultured for an additional 18 h. The cells were lysed in buffer A (50 mM sodium phosphate [pH 8.0], 300 mM NaCl, 5 mM 2-mercaptoethanol) and sonicated. Proteins were bound to a Talon metal affinity column (Clontech Laboratories, Inc., Mountain View, CA), washed with phosphate-buffered saline, and eluted with 0.25 M imidazole diluted in phosphate-buffered saline.

RESULTS

Amino acids 24 to 71 of gE are critical for binding to IDE. Previous studies have shown that the amino terminus of HSV gD (amino acids 7 to 15 and 24 to 32) directly interacts with one of its receptors, HVEM (herpes virus entry mediator), and is important for virus binding and entry (7, 14). Furthermore, the first 53 amino acids of gD are necessary for functional interactions with HVEM, nectin-2, and 3-*O*-sulfated heparan sulfate for both HSV-1 and HSV-2 (48). Amino acids 36, 38, and 39 of HSV gD are important for its interaction with the major virus receptor nectin-1 (13). Since the amino terminus of HSV gD is important for receptor binding, we prepared truncation mutations and synthetic peptides that target the amino portion of VZV gE to investigate the structural requirements for its interaction with IDE. First, we generated a series of plasmids with different deletions in the extracellular domain of gE that were fused to the Fc portion of human IgG (Fig. 1A). Each of the plasmids was transfected into CV1/EBNA cells, and 5 days later, tissue culture supernatants were immunoblotted with antibody to the human Fc portion of IgG. Each of the gE deletion mutants was expressed, and their sizes were similar to those predicted from the amino acid sequence (Fig. 1B).

To determine which portions of gE are important for binding to IDE, pull-down assays were performed using the gE-Fc mutants and cell lysates. Wild-type and mutant gE-Fc proteins

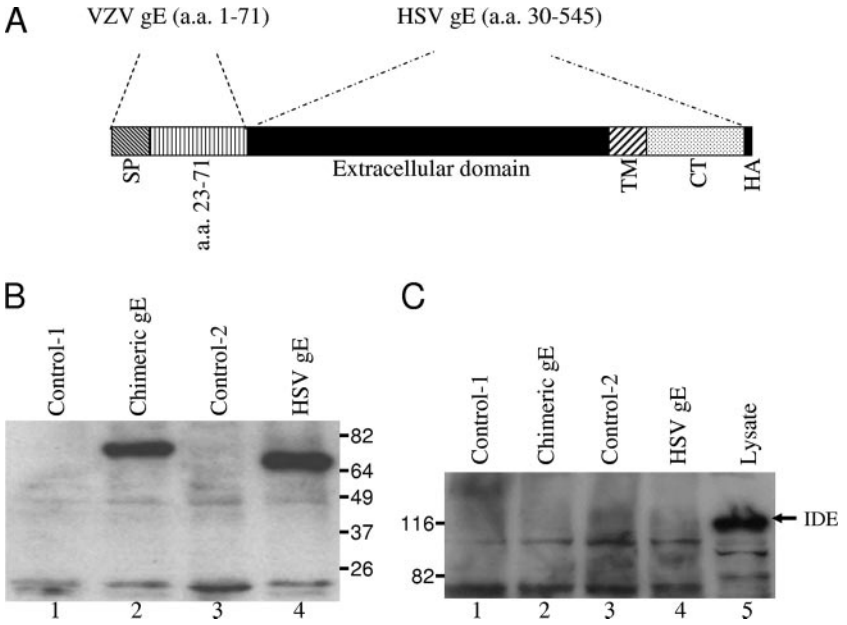

FIG. 3. VZV-HSV chimeric gE protein does not bind to IDE. (A) Structure of VZV-HSV chimeric gE. (B) Expression of VZV-HSV chimeric gE (lane 2) and HSV-2 gE (lane 4) was detected by immunoblotting with antibody to HA. Control plasmid 1 contains the chimeric gE sequence but does not express the protein; control plasmid 2 expresses GFP. (C) Immunoblot of cell lysate with anti-IDE antibody shows IDE in the lysate, but immunoprecipitation of HA-tagged chimeric gE or HSV gE using antibody to HA does not coimmunoprecipitate IDE. Control plasmids 1 and 2 are as described for panel B and do not coimmunoprecipitate IDE. The numbers to the right of panel B and the left of panel C are molecular weights in thousands.

bound to Sepharose beads were incubated with melanoma cell lysates, washed, boiled, and immunoblotted with anti-IDE antibody. While full-length gEt and mutants gEtΔ163-208, gEtΔ72-162, gEtΔ72-208, and gEtΔ209-288 interacted with IDE, gEtΔ32-71 lost the ability to bind to IDE (Fig. 2A). gEtΔ72-208 showed a slightly reduced interaction with IDE compared with those of some of the other mutants, which might be a result of the large deletion, which could alter the folding of the molecule.

To further confirm the gE binding domain for IDE, three overlapping peptides corresponding to the predicted amino terminus of gE (after its signal sequence is cleaved) were synthesized. gEP-1 corresponds to amino acids 24 to 50 of VZV gE. IDE has a preference for basic or hydrophobic amino acids at the carboxyl side of cleavage sites (2); gEP-1 ends with a hydrophobic tyrosine residue. Pull-down assays using gE proteins and cell lysates (containing IDE) were performed in the presence or absence of the peptides. While gEP-2 (corresponding to amino acids 51 to 71 of gE) partially blocked gE-IDE complex formation at a concentration of 50 μM, gEP-1 blocked gE binding to IDE in a concentration-dependent fashion. gEP-3 (corresponding to gE amino acids 39 to 63), which overlaps the gap between gEP-1 and gEP-2, did not show significant blocking activity (Fig. 2B). Bacitracin, which blocks the interaction of gEt with IDE (24), served as a positive control, and P1 (a peptide containing the integrin RGD sequence) and P2 (a peptide containing the integrin $\alpha_2\beta_1$ sequence) were negative controls, each used at a concentration of 50 μM. From these data, taken together with the data from the gE deletion mutants, we concluded that amino acids 24 to 71 of gE contain a binding motif for IDE.

gI forms a complex with gE and functions to assist gE trafficking to the cell surface (26). To investigate the possibility that the interaction of gE with gI affects the binding of gE to IDE, gE-Fc deletion mutants were cotransfected with a plasmid encoding the gI extracellular domain (gIt) which does not have an Fc tag (24). Complexes containing gE-Fc and gIt, but not gIt alone, were immunoprecipitated with protein A beads, washed, incubated with melanoma cell lysates, washed, boiled, and immunoblotted with antibody to IDE. gEtΔ32-71, either expressed alone or in a complex with gIt, failed to interact with IDE (Fig. 2C). In contrast, full-length gEt, gEtΔ163-208, gEtΔ72-162, and gEtΔ72-208 interacted with IDE. Interestingly, the presence of gI in gEtΔ72-162 and gEtΔ72-208 (Fig. 2C, lanes 8 and 10) blocked the IDE binding ability of these two mutants when expressed alone (Fig. 2C, lanes 7 and 9). This is likely due to gI covering the IDE binding domain on gE when the sequence downstream of the IDE binding domain is truncated in the gE deletion mutants.

The secondary structure of the IDE binding domain is likely important for its interaction with IDE. To determine whether amino acids 24 to 71 of gE are sufficient for binding to IDE, we fused the first 71 amino acids of VZV gE to a portion of another herpesvirus glycoprotein to determine if the VZV gE sequence conferred binding to IDE. HSV gE is shorter than VZV gE and has no apparent amino acid sequence homology to the amino terminal region of VZV. HSV gE binds extremely weakly to IDE (24). We constructed a VZV-HSV gE chimeric protein that contains the VZV gE signal peptide and IDE binding domain (amino acids 1 to 71 of VZV gE) fused in-frame with amino acids 30 to 545 of HSV-2 gE with a carboxy terminal HA tag (Fig. 3A). The predicted signal peptide cleav-

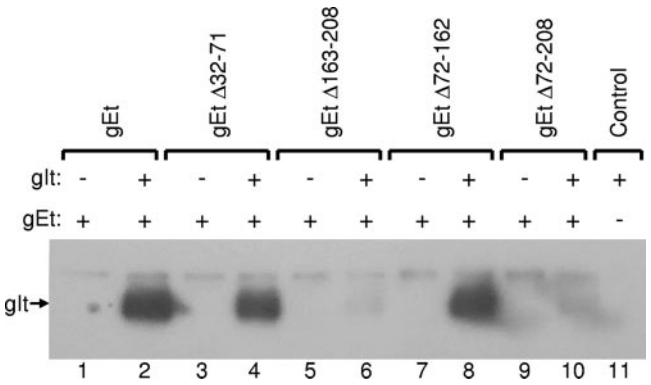

FIG. 4. VZV gE amino acids 163 to 208 are important for interacting with VZV gI. CV1/EBNA cells were transfected with plasmids encoding gEt or gEt mutant proteins in the presence or absence of plasmid gIt, which encodes the extracellular domain of gI without an Fc tag. gEt-gIt complexes were then precipitated with protein A-Sepharose and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. gIt was detected, using antibody to gI. A plasmid expressing GFP was used as a control.

age site for HSV gE is between amino acids 22 and 23 (SignalP-HMM program; Technical University of Denmark). Lysates from HeLa cells transfected with a plasmid expressing the chimeric VZV-HSV gE protein showed a protein of about 80 kDa when immunoblotted with antibody to HA (Fig. 3B). HeLa cell lysates containing the chimeric VZV-HSV gE protein were immunoprecipitated with antibody to HA and immunoblotted with anti-IDE antibody. While the lysates contained IDE (Fig. 3C, lane 5), antibody to the chimeric gE failed to coimmunoprecipitate IDE (Fig. 3C, lane 2). Two control plasmids, one with a chimeric gE sequence that fails to express the protein and one that expresses green fluorescent protein (GFP), served as negative controls in the binding assay. These results suggest that the secondary structure of the IDE binding domain is critical for its interaction with IDE.

VZV gE amino acids 163 to 208 are required for binding to gI. VZV gI forms a complex with gE to chaperon gE through the endoplasmic reticulum and Golgi network to facilitate gE maturation and cycling from the cell surface (34). To determine the gI binding domain of gE, we cotransfected CV1/EBNA cells with plasmids expressing the extracellular domain of gI (gIt) and either the full-length extracellular domain of gE (gEt) or gE mutants, immunoprecipitated gE with protein A beads, washed the immune complexes, and immunoblotted them with antibody to gI. While full-length gEt and mutants gEtΔ32-71 and gEtΔ72-162 interacted with gI, mutants gEtΔ163-208 and gEtΔ72-208 lost nearly all of their ability to bind to gI (Fig. 4).

gE binds predominantly to the amino subunit of IDE. IDE consists of an amino subunit containing domains 1 and 2 and a carboxy subunit containing domains 3 and 4 (40). The crystal structure of IDE indicates that it interacts with insulin, amyloid-β, amylin, and glucagon mainly through domain 1 in the amino subunit and domain 4 in the carboxy subunit. The catalytic domain of IDE is located in the amino subunit (23, 36, 42). A single amino acid change from Glu to Gln in domain 1 at position 111 markedly diminishes the enzymatic activity of IDE, which degrades insulin (35, 36). The IDE-w.t. plasmid, containing domains 1 to 4 (amino acids 42 to 1019), IDE-E111Q corresponding to IDE-w.t. with Glu changed to Gln at position 111, IDE-N containing the amino subunit of IDE (amino acids 42 to 541), and IDE-C containing the carboxy subunit of IDE (amino acids 542 to 1018) were produced in *E. coli*. Immunoblotting with polyclonal antibody to IDE verified that each of the IDE constructs was expressed (Fig. 5A). Using a pull-down assay in which gEt, bound to protein A-Sepharose beads, was incubated with *E. coli* lysates expressing IDE proteins, wild-type IDE, and IDE-E111Q formed complexes with gE (Fig. 5B, lanes 1 and 2). The amino subunit of IDE bound to gE, although less efficiently than the full-length protein (Fig. 5C, lane 3). The carboxy subunit of IDE bound very weakly to gE, and an interaction was detected only when a low-stringency binding buffer was used (Fig. 5C, lanes 5 and 7).

DISCUSSION

VZV gE is essential for VZV infectivity, and the glycoprotein interacts with its cellular receptor, IDE, through its extracellular domain. We used gE deletion mutants and synthetic peptides to determine that amino acids 24 to 71 of gE, located after the signal peptide of the glycoprotein, are required for binding to IDE. Although gE is well conserved among other alphaherpesviruses, the amino terminus of VZV gE is unique (4). VZV is most closely related to simian varicella virus, and while gE of simian varicella virus is similar in length to VZV gE, the amino terminal regions of the two proteins show no apparent homology. HSV gE is shorter than VZV gE and lacks the IDE binding domain of VZV gE; HSV gE binds to IDE very weakly (24). HSV gD is essential for infection and interacts with all the known HSV cellular receptors (43), while HSV gE is required for the virus to spread from cell to cell (15, 37). VZV does not encode a gD homolog, and VZV gE is important for both initial infection and cell-to-cell spread of the virus (4, 24). Thus, VZV gE has both receptor binding and cell-to-cell-spread functions for VZV, while HSV uses separate glycoproteins, gD and gE, to perform these activities. McGeoch proposed that HSV gD may have arisen from duplication of another glycoprotein gene (28). If the HSV gE protein was the progenitor HSV glycoprotein gene, then originally it may have had both receptor binding and cell-to-cell-spread functions (like VZV gE), but after HSV gD arose, the latter protein may have acquired receptor binding activity, while HSV gE lost its receptor binding function (perhaps from the amino terminus of the progenitor protein) but retained its cell-to-cell-spread function.

Our finding that amino acids 24 to 71 of VZV gE are critical for receptor binding is consistent with observations from a previous study of VZV infectivity using gE mutants (4). The authors showed that deletion of amino acids 27 to 51 of gE did not inhibit virus replication, but plaque sizes were reduced, indicative of impaired cell-to-cell spread of VZV. Deletion of gE amino acids 51 to 187 inhibited virus replication, syncytium formation, and plaque size in vitro. Thus, these deletions, both of which overlap the region of gE that is required for binding to IDE (amino acids 24 to 71), impair cell-to-cell spread of the virus. VZV with a linker-insertion mutation at amino acid 27 of gE showed reduced cell-to-cell spread, despite the fact that many virion particles were on the cell surface and that there

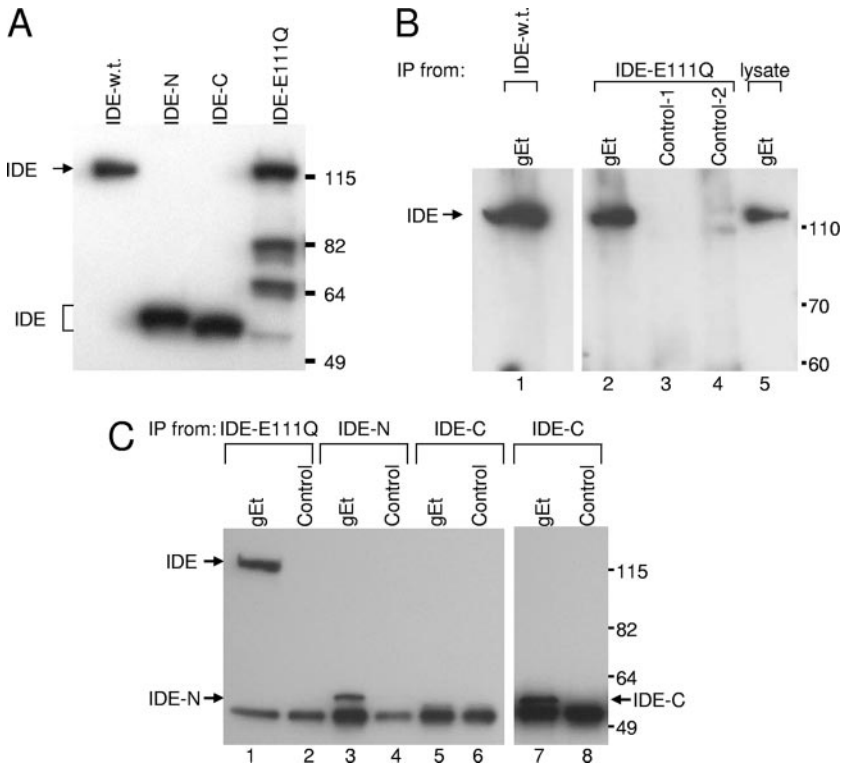

FIG. 5. gE interacts primarily with the amino portion of IDE. (A) Expression of human IDE-w.t., the IDE-N fragment, the IDE-C fragment, and the IDE-E111Q mutant in *E. coli*, detected by immunoblotting with antibody to IDE. The additional bands in IDE-E111Q are presumably due to protein degradation. (B) Binding of IDE-w.t. (lane 1), IDE-E111Q (lane 2), or melanoma cell lysate containing IDE (lane 5) to gEt. gEt was immobilized onto protein A-Sepharose beads, IDE-w.t. or IDE-E11Q was added, and after washing and boiling, proteins were immunoblotted with anti-IDE antibody. Control 1 encodes the vaccinia 7.5 protein fused to Fc, and control 2 encodes soluble Jam fused to Fc. (C) IDE-E111Q, IDE-N, and IDE-C bind to gEt. Pull-down assays were performed as described for panel B, using 10 μg of each purified IDE protein. The controls in lanes 2, 4, and 6 encode vaccinia P7.5 fused to Fc, while the control in lane 8 encodes EBV BZLF2 fused to Fc. Lanes 7 and 8 are from a separate experiment in which a lower-stringency buffer (phosphate-buffered saline) was used for the binding assay than for the experiment shown in lanes 1 to 6. The numbers to the right of the panels are molecular weights in thousands.

was apparently a normal level of gE incorporated into most virions. This is consistent with our earlier observation that treating VZV-infected cells with bacitracin, an IDE inhibitor which blocks the interaction of gE and IDE, results in reduced cell-to-cell spread of the virus even though normal levels of virion particles are present on the cell surface and gE synthesis and the turnover rate of the protein are not altered (24). A single amino acid substitution of alanine for serine at amino acid 31 of gE modestly impaired virus replication but did not reduce plaque size in vitro (4). This amino acid change markedly impaired VZV infection in skin xenografts, leading the authors to speculate that amino acid 31 of gE may be important for interaction with other viral or cellular molecules during infection of skin cells. These results are compatible with our findings that the amino terminal portion of VZV gE is important for binding to IDE.

While we found that amino acids 24 to 71 of VZV gE are critical for binding to IDE and that the latter is important for cell-to-cell spread of the virus, other amino acids of VZV gE are also important for cell-to-cell spread of the virus. A change in amino acid 150 from aspartic acid to asparagine has been found in some clinical isolates of VZV and is responsible for increased cell-to-cell spread of the virus both in vitro and in skin xenografts (19, 39).

IDE binds to a wide range of ligands that do not share a high degree of homology in their amino acid sequences. Three different groups of IDE binding proteins have been shown to have very limited sequence homology (40). Atrial natriuretic, brain natriuretic, and C-type natriuretic peptides form one group; glucagon and glucagon-like peptides form a second group, and insulin, insulin-like growth factors 1 and 2, and proinsulin form a third group. While amino acids 24 to 71 of gE are required for binding to IDE, they do not have amino acid homology with these known substrates of IDE. The secondary structures of some of these proteins have been postulated to be sufficiently similar for their interactions with IDE (22), and this was recently validated by the finding that the crystal structure of IDE bound to four of its ligands (40). Therefore, we postulated that the secondary structure of gE likely has some degree of similarity to other IDE binding proteins. We found that a chimeric protein in which the VZV gE IDE binding domain (amino acids 1 to 71) was fused to a portion of HSV-2 gE (amino acids 30 to 545) did not bind to IDE. This may not be surprising if the secondary structure of VZV gE is important for binding to IDE, since its fusion to another protein (HSV gE) may alter its configuration. The crystal structures of IDE bound to four of its substrates indicate that portions of its substrates fit into a ligand cavity and

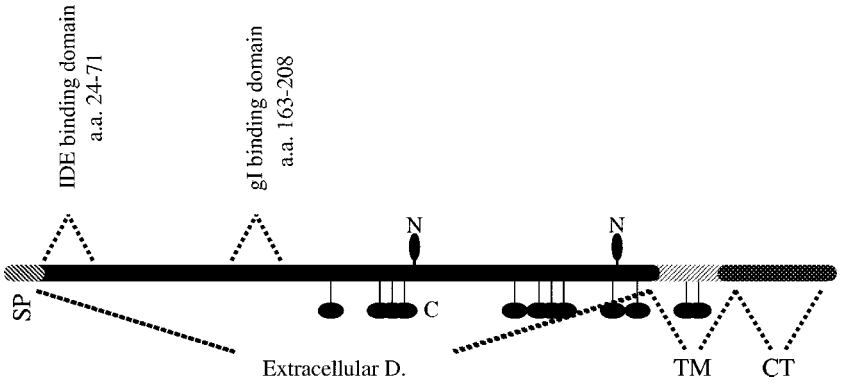

FIG. 6. Map of VZV gE motifs that are important for binding to IDE and gI. N, putative N-linked glycosylation sites; C, cysteine residues; SP, signal peptide; TM, transmembrane domain; CT, cytoplasmic tail.

that IDE forms a cage around its substrates (40). The conformation of chimeric VZV-HSVgE might not be appropriate to present an IDE binding motif that can fit into the ligand cavity of IDE. Prediction of the secondary structure of the chimeric VZV-HSV gE suggests that some of the β sheets in VZV gE are lost and replaced by additional α-helical regions in the middle portion of the extracellular domain of HSV gE (Q. Li and J. I. Cohen, data not shown).

Purified IDE protein from human liver degrades insulin but not VZV gE (24), suggesting that the catalytic domain of IDE might not be important for its interaction with VZV gE. Therefore, it was not surprising that both wild-type IDE and the IDE-E111Q mutant (which is markedly impaired for degradation of insulin) both bound gE to a similar extent. While the amino and carboxy portions of IDE alone bound to gE, the interaction of gE with the IDE C fragment was very weak. The crystal structures of IDE bound to four of its ligands indicate that the IDE-N and IDE-C domains interact with its ligands and form an enclosed cavity to encapsidate its substrates (40), which are short peptides ranging from 21 to 40 amino acids (40). We mapped the IDE binding domain of VZV gE to 48 amino acids (residues 24 to 71) located at the amino terminus of gE after its signal peptide (Fig. 6). Similarly, the crystal structures of IDE bound to its ligands indicate that the amino terminal three to five amino acids of four substrates (insulin, amyloid-β, amylin, and glucagon) are important for forming β sheets with IDE (40).

gE and gI are conserved in each of the alphaherpesviruses. Two linker insertion mutants of HSV-1 gE at amino acids 235 and 264 lost the ability to coimmunoprecipitate with gI, indicating that these amino acids are required for binding to HSV gI (3). The corresponding region of VZV gE (amino acids 350 to 431) shares about 40% similarity with this region of HSV-1. Our data show that amino acids 163 to 208 of gE are critical for its ability to form a heterodimer with gI. gEtΔ163-208 and gEtΔ72-208 were markedly impaired for complex formation with gI, but they did bind to IDE. Amino acids 163 to 208 could be important either for maintaining the secondary structure of VZV gE, allowing gE to bind to gI, or for containing the gI binding domain. Our results indicate that the gE domain required for binding to gI is distinct from the gE domain required for binding to IDE (Fig. 6). These results are consistent with two prior observations. First, Berarducci and colleagues (4) showed that VZV gE amino acids 51 to 187 were not required for gE colocalization with gI in cells infected with gE mutants but did have a role in cell-to-cell spread and virus replication. Second, Mo and colleagues showed that gE is important for cell-to-cell contacts in polarized cells, even in the absence of gI (30).

We have shown that the amino terminal portion of the mature form of VZV gE is required for binding to IDE and that this region of the protein is not required for binding to VZV gI. While these studies identify domains of gE that are important for its interactions in vitro, it will be important to study the role of these domains during VZV infection in cell culture. Future studies using recombinant proteins will address the affinity of the gE-IDE interaction and may lead to a more detailed structural analysis of the interaction. Such studies may aid in the design of small molecules to inhibit VZV infection.

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