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Receptor Properties of Two Varicella-Zoster Virus Glycoproteins, gpI and gpIV, Homologous to Herpes Simplex Virus gE and gI

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The varicella-zoster virus (VZV) genome contains 70 reading frames (ORF), 5 of which encode the glycoproteins gpI, gpII, gpIII, gpIV, and gpV. ORF 67 and 68 lie adjacent to each other in the unique short region of the VZV genome and code for gpIV and gpI, respectively. These two genes, which are contained within the *Hin*dIII C fragment of the VZV genome, were subcloned in the correct orientation downstream from the promoter regions of the eukaryotic expression vectors pCMV5 and pBJ. After transfection, 5 to 20% of the Cos cells bound antibody specific for the given glycoprotein. In this study, it was shown that only the cells transfected with the gpI construct bound to the Fc fragment of human immunoglobulin G. Neither the transfected gpIV gene product nor the vector only bound to the Fc fragment. Thus, VZV gpI is confirmed to be the VZV-encoded Fc-binding glycoprotein. Like the wild-type form of gpI expressed in VZV-infected cells, gpI precipitated from transfected cells contained both N-linked and O-linked glycans and was heavily sialated. In addition, the transfected gpI gene product was phosphorylated both in cell culture and in protein kinase assays by mammalian casein kinases I and II. Extensive computer-assisted analyses of the VZV gpI sequence, as well as those of alphaherpesviral homolog glycoproteins, disclosed properties similar to those of other cell surface receptors; these included (i) exocytoplasmic regions rich in cysteine residues, (ii) membrane-proximal regions with potential O-linked glycosylation sites, and (iii) cytoplasmic domains with consensus phosphorylation sites.

Varicella-zoster virus (VZV) is one of the human herpesviruses. It is the etiologic agent of chicken pox in children and herpes zoster in adults. VZV is the first herpesvirus to have been attenuated in cell culture for the purpose of human immunization. Based on genomic organization and biologic behavior, this herpesvirus is further classified along with herpes simplex virus (HSV) types 1 and 2 as an alphaherpesvirus (32). The genomes of the *Alphaherpesvirinae* are composed of two covalently linked segments, the unique long (U_L) and the unique short (U_S). The organization and the structure of the HSV and VZV genomes are quite similar, with some notable exceptions, e.g., the VZV genome is smaller than that of HSV, 125 kbp verses 160 kbp, and codes for fewer proteins. In regard to the number of glycoproteins, VZV specifies only five glycoproteins, while HSV codes for a greater number. At least four of the HSV glycoproteins are located in the U_S region of the genome: gG, gD, gI, and gE; in contrast, only two glycoproteins are encoded within the U_S genome of VZV. These two glycoproteins, gpIV and gpI, are the homologs of HSV gI and gE, respectively.

Although it is now known that each of the five glycoproteins encoded in the VZV genome has an HSV homolog (8), only the VZV gB homolog called gpII approaches 50% identity (Table 1). The other four glycoproteins share considerably less sequence identity with their HSV homologs (Table 1). Thus, it is unclear whether a functional relationship exists between homologous glycoproteins of these viruses (8, 15). HSV-1 and HSV-2 and, more recently, VZV have been shown to induce receptors for the Fc portion of immunoglobulin G (IgG) on the surface of the infected cell (6, 7, 20, 24, 26, 29, 41). In the HSV system, the U_S glycoprotein gE mediates the Fc-binding activity and a gE-gI complex is thought to result in an Fc receptor (FcR) of higher affinity (2, 10, 18, 21, 22). In VZV, however, the Fc-binding activity has not yet been ascribed to a given viral glycoprotein. To address this issue, we transfected the VZV homologs of HSV gE and gI into mammalian cells and examined their products for FcR activity. This study identifies a functional relationship between the VZV U_S glycoproteins and their HSV counterparts and also further outlines cell surface receptor properties shared by the VZV gpI-gpIV complex and their alphaherpesviral homologs, including pseudorabies virus (PRV) (3).

MATERIALS AND METHODS

Cell culture. Simian cell lines derived from kidney fibroblasts, Cos-1 and Cos-7, were maintained in tissue culture in Dulbecco minimal essential medium high glucose, supplemented with 10% Nu-serum (Collaborative Research Inc., Bedford, Mass.), 2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 1% nonessential amino acids (GIBCO, Grand Island, N.Y.), and 1 mM Na pyruvate (Sigma). The murine L-cell fibroblast cell line DAP.3 was maintained in Eagle minimal essential medium supplemented with 10% fetal calf serum (Irvine Scientific, Irvine, Calif.), L-glutamine, penicillin, and streptomycin.

Antisera and MAb. Murine monoclonal antibodies (MAb) 3B3 and 6B5 specific for the VZV glycoproteins gpI and gpIV, respectively, have been described in detail elsewhere (15, 28). Procedures for purification and biotinylation of MAb have also been published previously (24).

Description of eukaryotic expression vectors. The *Hin*dIII library of the VZV genome was prepared and cloned into pBR322 by Ecker and Hyman (11). VZV genes 67 and 68 which code for glycoproteins gpIV and gpI, respectively, are

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TABLE 1. Amino acid sequence comparison of VZV glycoproteins with HSV homologs[a]

VZV	HSV	Length[b]	% Identity	% Similarity
gpI	gE	632	27	47
gpII	gB	913	49	67
gpIII	gH	683	26	48
gpIV	gI	358	24	43
gpV	gC	559	24	42

[a] Data were generated by using the GCG BestFit program (9, 35). Sequences for VZV glycoproteins gpI, gpII, gpIII, and gpIV are from SwissProt AC: P09259, P09257, P09260, and P09258, respectively. Sequences for HSV glycoproteins gE, gB, gH, gI, and gC are from SwissProt AC: P04488, P10211, P06477, P06487, and P10228, respectively. Sequence for VZV gpV is from GenBank AC: X04370 (residues 21113 to 19434).

[b] Length refers to the number of amino acids over which the homology spans.

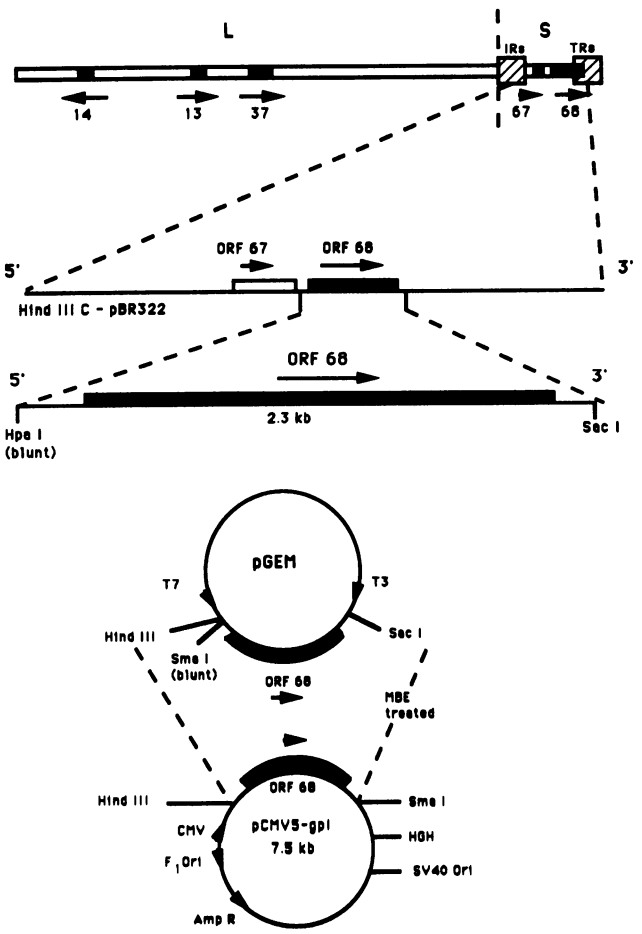

FIG. 1. Cloning strategy for VZV ORF 68 (gpI). Gene 68 was excised from the *Hin*dIII-C–pBR322 construct and inserted into pGEM. The gene was then shuttled into the eukaryotic expression vector pCMV5. Details are given in the text. SV40 Ori, simian virus 40 origin; IRs, internal repeat sequences; TRs, terminal repeat sequences; MBE, mung bean exonuclease; CMV, cytomegalovirus.

located in the *Hin*dIII C fragment (8). Gene 68 was first excised from the *Hin*dIII-C–pBR322 construct and ligated into the Riboprobe Gemini pGEM-blue vector (Promega, Madison, Wis.) and then shuttled into the plasmid pCMV5 (1), which was supplied by Mark Stinski (University of Iowa). The pGEM-ORF 68 construct was linearized at the *Sac*I site of the plasmid which is located downstream from the insert. This enzyme produced a 3′ overhang which was removed by digestion with mung bean nuclease to create a blunt end. The *Hin*dIII site of pGEM-blue located 5′ to the insert was then cleaved. The *Hin*dIII-*Sac*I fragment containing open reading frame (ORF) 68 was isolated on an agarose gel and purified with Geneclean (Bio 101, La Jolla, Calif.). This fragment was ligated in the correct orientation into pCMV5 which had been cut with *Hin*dIII (5′) and *Sma*I (blunt, 3′). Positive clones containing ORF 68 were verified by minipreps and restriction enzyme digestion with *Bcl*I, which cuts once within the insert but does not cut pCMV5. A schematic representation of cloning strategy is depicted in Fig. 1.

Gene 67 was likewise excised from the *Hin*dIII C fragment of pBR322 by digestion with *Acc*I. The 1,256-bp band corresponding to the *Acc*I 114469 to *Acc*I 115725 fragment was isolated and purified by Geneclean. The 5′ overhang of this fragment was blunt ended by filling in with Klenow fragment of DNA polymerase. Blunt-end ligation was then used to attach *Xba*I phosphorylated linkers (Promega). After the fragment was digested with *Xba*I and gel purified, it was inserted with T4 DNA ligase into the plasmid pBJ, which had previously been linearized with *Xba*I, gel purified, and treated with calf intestinal phosphatase. Positive colonies were selected by digestion with *Mlu*I, which cuts once within the insert but not within the vector. The orientation of the insert was determined by double digestion with *Pml*I and *Hin*dIII. The plasmid pBJ (37) was supplied by M. Davis (Stanford, Calif.). The cloning strategy is depicted in Fig. 2. Plasmid constructs were amplified in the competent HB101 strain of *Escherichia coli* cells and purified by standard methods using double CsCl gradients, or by Circleprep Large (Bio 101). DNA-modifying enzymes and restriction enzymes were purchased from New England Biolabs, Inc. (Beverly, Mass.), Bethesda Research Laboratories, Inc. (Gaithersburg, Md.), or Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and used with the manufacturers' buffers according to the given specifications.

Transfection conditions. Transfection was done by three means: electroporation, DEAE-dextran, and calcium phosphate coprecipitation. In the first method, Cos-1 or Cos-7 cells (10×10^6/ml) in a volume of 0.5 ml of Dulbecco's minimal essential medium (without serum) were transfected with 10 μg of plasmid DNA. The cell porator (Bethesda Research Laboratories) was set at 240 V, 1600 μF, and low resistance. Cells were seeded into 75-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.) in complete tissue culture medium and harvested at 48 to 72 h by treatment with 10 mM EDTA (Sigma Chemical Co., St. Louis, Mo.). In the DEAE-dextran procedure, 10^6 Cos-7 cells were seeded into tissue culture dishes (100 by 20 mm; Corning) 1 day before transfection. From 4 to 8 μg of DNA were transfected per dish, according to the method described by Seed and Aruffo (33). Transfectants were analyzed at 48 to 72 h posttransfection.

For calcium phosphate coprecipitation, 20 μg of the pCMV5-gpI construct was cotransfected into the murine L-cell line DAP.3, along with 1 μg of the pCB6 plasmid which bears the neomycin resistance selection marker. Transfections were performed by using the Transfinity calcium phosphate transfection system kit (Bethesda Research Laboratories) according to the manufacturer's specifications. Transfectants were cultured for 48 h in medium which

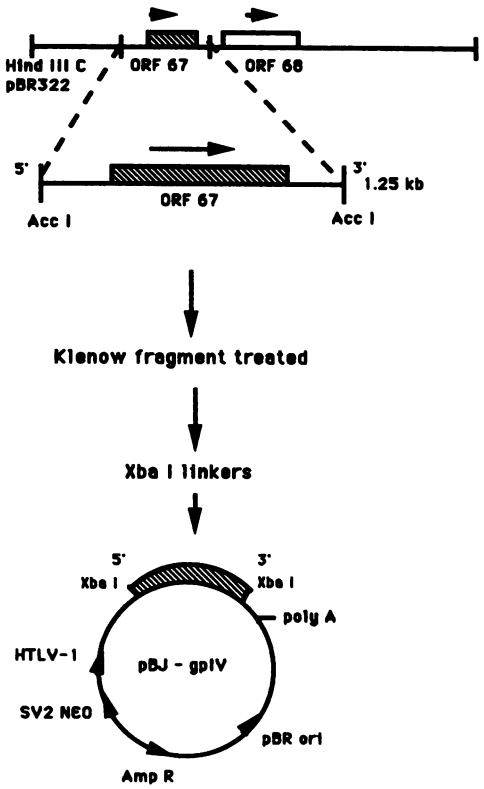

FIG. 2. Cloning strategy for VZV ORF 67 (gpIV). *Xba*I linkers were added to the *Acc*I fragment containing gene 67 which had been excised from the *Hin*dIII-C–pBR322 construct. Next, gene 67 was ligated into pBJ at the *Xba*I site. Details are given in the text. HTLV-1, human T-cell leukemia virus 1; SV2 NEO, simian virus 2 neomycin resistance marker.

did not contain the selection agent. After 48 h, culture medium was replaced with medium containing 1 mg of Geneticin (G418 sulfate) (GIBCO) per ml for 2 to 3 days, at which time the concentration of G418 was reduced to 250 μg/ml. About 2.5 weeks posttransfection, cells were analyzed for surface expression of gpI by flow cytometry.

Immunofluorescence and flow cytometry. Transfected Cos cells were centrifuged onto microscope slides in a Cytospin centrifuge (Shandon Inc., Pittsburg, Pa.) for 10 min with slow acceleration at 1,000 rpm. The cytoslides were then dried, fixed in acetone for 10 min, and analyzed by immunofluorescence, as previously reported (24). Methods for flow cytometric analysis of VZV-infected cells also have been reported by this laboratory (24). For the FcR binding assay, 0.5×10^6 transfected cells were incubated with human IgG Fc fragment (Calbiochem, La Jolla, Calif.) in phosphate-buffered saline containing 0.1% NaN_3 for 30 min; this was followed by a 30-min incubation with $F(ab')_2$ fluorescein isothiocyanate-conjugated goat anti-human Ig (Tago). Cells were analyzed by fluorescence microscopy.

In vitro phosphorylation and isotopic labeling. In vitro phosphorylation of VZV glycoproteins with purified preparations of mammalian casein kinase I and casein kinase II have been described in detail previously (17, 38). The kinases were provided by Jolinda Traugh (University of California Riverside). For isotopic labeling, Cos-1 cells were transfected by electroporation and plated in a 75-cm^2 tissue culture flask. At 24 h posttransfection, culture medium was replaced with medium containing 2.5 mCi of $^{32}P_i$ (Amersham). After 48 h of incubation in the presence of isotope, cell lysates were suspended in 1.8 ml of radioimmunoprecipitation assay (RIPA) buffer and immunoprecipitated by published methods (16, 27). For methionine labeling, culture medium was replaced with methionine-deficient minimal essential medium (Sigma) supplemented with L-[^{35}S]methionine (Amersham, Arlington Heights, Ill.) 1 day posttransfection. After an additional 2 days of incubation, the monolayers were harvested, and solubilized infected cell antigen was prepared (27, 28). For glycosylation analyses, the glycoproteins were treated with neuraminidase, *O*-glycanase, and *N*-glycanase, according to established procedures (15, 28) (Genzyme, Cambridge, Mass.).

RESULTS

Expression of gpI and gpIV in transfected cells. The pCMV5-gpI construct and the pCMV5 vector were transfected into Cos cells by electroporation or by the DEAE-dextran method as detailed in Materials and Methods. After fixation and immunofluorescence staining with the MAb 3B3, gpI antigen was easily detected throughout the cytoplasm of Cos-1 cells transfected with pCMV5-gpI (Fig. 3A). This preponderant viral glycoprotein is present throughout the cytoplasm of VZV-infected cells (28). To verify that gpI was being transported to the outer membrane of the transfectants, unfixed cells were similarly analyzed. Figure 3B shows that pCMV5-gpI-transfected cells exhibited bright membrane fluorescence after staining with MAb 3B3; however, no fluorescence was observed after staining with a control MAb, 251D9, directed against a nonstructural VZV protein (data not shown). Generally, the shutter interval for photographing the positive cells was 12 to 20 s. The percentage of cells displaying positive membrane staining ranged from 5 to 20% in a series of 10 experiments. When control cells transfected with pCMV5 were photographed for a similar interval, no image was detectable in the photograph (Fig. 3D). If the camera was placed on automatic exposure, dull orange cells without specific staining were visible on film after 120 s (±30 s).

Subsequently, we attempted to establish stable cell lines expressing gpI. The pCMV-gpI construct was cotransfected along with the vector pCB6, which contains the neomycin resistance gene, into the murine L-cell line DAP.3, by the Ca^{2+} phosphate coprecipitation method. After transfection, cells were propagated for 2.5 weeks in the presence of G418, a neomycin analog. The positively selected cells were then analyzed for the surface expression of VZV gpI, but four rounds of this procedure failed to yield cell lines which stably expressed gpI (data not shown). The failure of gpI to be synthesized in the stable cell lines may indicate that this gene is toxic to mammalian cells. This phenomenon has been reported for other herpesviral glycoproteins (13, 38).

The surface expression of gpIV was also assessed by indirect immunofluorescence. This glycoprotein gpIV was easily visualized in the pBJ-gpIV transfected cells, as detected with the MAb 6B5 (Fig. 3C). Similar to transfected gpI cultures, the staining pattern observed in the unfixed cells represented that of a membrane glycoprotein. Cells transfected with pBJ alone were not similarly stained by the anti-gpIV MAb nor were pBJ-gpIV transfectants recognized by an irrelevant VZV MAb (data not shown, but similar to Fig. 3D). Under these transfection conditions, with the

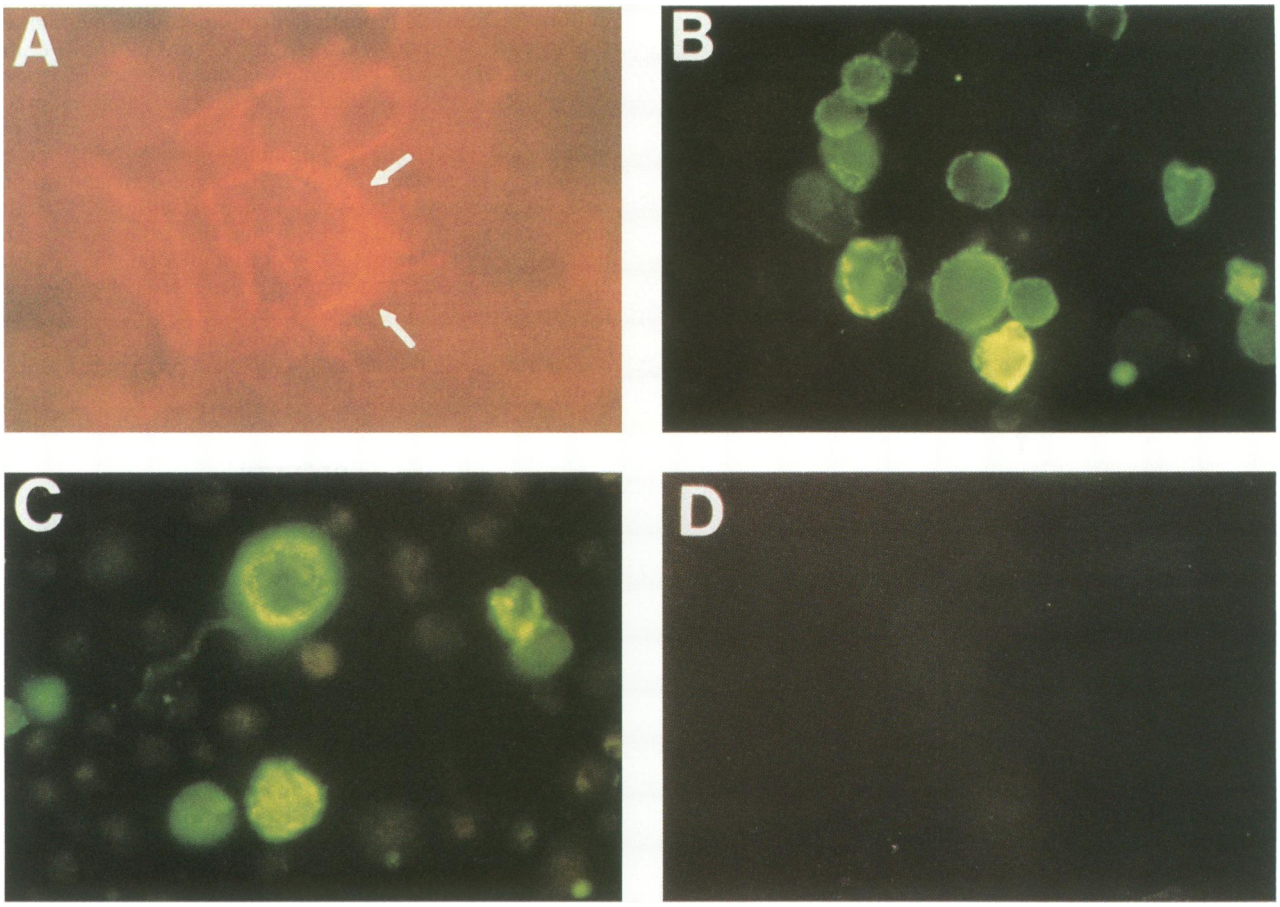

FIG. 3. Expression of VZV glycoproteins gpI and gpIV in transfected cell cultures. Indirect immunofluorescence was used to demonstrate the expression of VZV gpI and gpIV gene products in transfected cell cultures. (A) Cytoslide preparations of Cos-1 cells transfected with pCMV5-gpI were fixed with acetone and stained with gpI-specific MAb 3B3-biotin and then treated with streptavidin-phycoerythrin. Arrows designate the VZV-specific staining. (B) Cos-7 cells transfected with pCMV5-gpI were stained without fixation using MAb 3B3 and then $F(ab')_2$ fluorescein isothiocyanate-conjugated goat anti-mouse Ig. (C) Live Cos-7 cells transfected with pBJ-gpIV and stained with gpIV-specific MAb 6B5 and then fluorescein isothiocyanate-conjugated goat anti-mouse Ig. (D) Control pCMV5 transfectants were incubated with MAb 3B3 to evaluate specificity of immunostaining. Exposure times to Kodak 400 ASA color film ranged from 12 to 20 s.

pBJ-gpIV construct, up to 20% of the Cos cells expressed gpIV on their outer cell membrane.

Characterization of gpI from transfected cells. One day after transfection, cells in 100-mm tissue culture monolayer dishes were labeled with [^{35}S]methionine, and cell lysates were prepared 2 days later. Both pCMV5- and pCMV5-gpI transfected cultures were precipitated with 3B3 antibody. A protein of M_r 98,000 was clearly visible in the pCMV5-gpI precipitations; this protein was not visible in the control lanes (Fig. 4). Because the transfected gpI gene product was about the same size as the gpI precipitated from VZV-infected cells, it appeared that the glycosylation and processing events of the transfected gpI were similar to those of the wild-type glycoprotein. In support of this observation, we examined the susceptibility of gpI from transfected cultures to cleavage with neuraminidase and *O*-glycanase (Fig. 4). In each instance, a decrease in M_r indicated that the gpI product synthesized under transfection conditions contained both O-linked and sialic acid residues, similar to the mature wild-type product (Fig. 4). These analyses with *O*-glycanase confirmed our earlier observation that the forms of gpI synthesized in the presence of tunicamycin must contain O-linked glycans (28). Studies of N-linked glycosylation of the transfected gpI product are described in the penultimate section of the Results.

Characterization of gpIV from transfected cells. As shown in Fig. 3C, VZV gpIV was easily detected on the surface of the live cells transfected with pBJ-gpIV. The 6B5 antibody reagent recognizes a conformational epitope on the extracytoplasmic portion of gpIV; this epitope can be detected on solubilized gpIV but is destroyed by immunoblotting procedures (24). Therefore, to further characterize this glycoprotein, cultures transfected with pBJ alone and pBJ-gpIV were incubated in the presence of [^{35}S]methionine for 48 h and then harvested as described in Materials and Methods. After immunoprecipitation with MAb 6B5, a protein of M_r 55,000 was detected in the pBJ-gpIV lysate but not in the pBJ lysate (Fig. 5). This product comigrated with authentic gpIV isolated from [^{35}S]methionine-labeled VZV-infected cells by immunoprecipitation with the same antibody reagent (Fig. 5). In contrast to MAb 3B3, which recognizes a very stable epitope on all forms of gpI (27, 28), the antigenic site recognized by 6B5 did not appear to be present on intermediary forms of the glycoprotein.

IgG FcR activity in transfected cells. VZV-infected cells have been previously shown to express an Fc-binding activ-

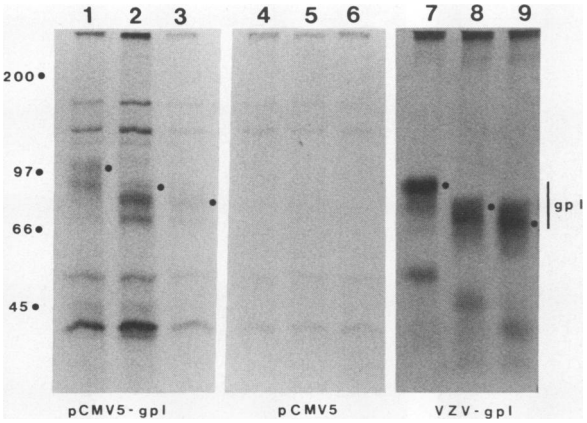

FIG. 4. Analysis of the transfected VZV gpI gene product. Cos-1 cells were transfected with plasmid DNA alone (lanes 4 to 6) or with the plasmid into which had been cloned the ORF coding for gpI (lanes 1 to 3). At 24 h posttransfection, the medium was replaced with methionine-deficient medium containing [^{35}S]methionine (100 μCi/ml). The cells were harvested at 72 h posttransfection and immunoprecipitated with anti-gpI MAb 3B3 and protein A-Sepharose beads. Untreated precipitates are shown in lanes 1 and 4, neuraminidase-treated precipitates are shown in lanes 2 and 5, and *O*-glycanase-treated precipitates are shown in lanes 3 and 6. In a similar fashion, VZV gpI was precipitated from [^{35}S]methionine-labeled VZV-infected cells and analyzed untreated (lane 7) or treated with neuraminidase (lane 8) or *O*-glycanase (lane 9). Molecular mass marker proteins (in kilodaltons) are designated on the left side of this and subsequent figures; these include myosin, phosphorylase *b*, bovine serum albumin, ovalbumin, and carbonic anhydrase.

ity not detectable on the surface of the mock-infected cells (24). VZV gpI shares 27% overall amino acid sequence identity with gE, the Fc-binding glycoprotein encoded by the HSV genome (Table 1). Therefore, gpI-transfected cells were tested for their ability to bind the Fc fragment of human IgG. Transfected cells were first incubated with human IgG Fc fragment, at final concentrations of 0.1, 0.5, and 1.0 mg/ml. Cells transfected with pCMV5-gpI bound to the IgG Fc fragment when present at the two higher concentrations (Fig. 6A); however, no binding was observed in cells transfected with the pCMV5 vector alone (data not shown, but similar to Fig. 3D). Within each of four transfection experiments, we consistently observed that approximately the same percentage of cells which stained positively with the MAb 3B3 also bound to the Fc fragment of human IgG. Furthermore, staining with the Fc fragment (Fig. 6A) displayed an immunofluorescence pattern typical of a membrane glycoprotein, as shown earlier in Fig. 3B.

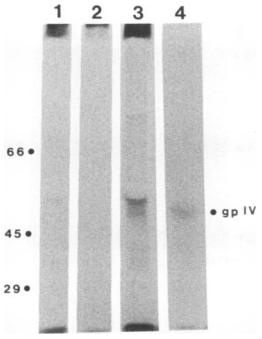

FIG. 5. Identification of the transfected gpIV gene product. Cos-1 cells were transfected with plasmid DNA alone (lane 1) or with pBJ-gpIV (lanes 2 and 3). Cultures were labeled and harvested as described in the legend to Fig. 4. Lanes 1 and 3 show lysates after precipitation with anti-gpIV antibody 6B5, while lane 2 shows a lysate precipitated with protein A beads alone but without specific antibody. Lane 4 includes authentic gpIV precipitated from [^{35}S]methionine-labeled VZV-infected cells. The location of gpIV is indicated by a closed circle in the right margin. Numbers on left show size in kilodaltons.

The pCMV-gpI-transfected cells were also tested for the ability to bind to purified preparations of human IgM and IgA myeloma proteins. As with IgG, the final concentrations of IgM and IgA were 0.1, 0.5, and 1.0 mg/ml. Neither of these two isotypes bound to the transfected cells (data not shown). Thus, the specificity of the VZV FcR is confined to the IgG isotype. When cells transfected with pBJ-gpIV were tested for the ability to bind human IgG Fc fragment, no binding was observed (Fig. 6B); likewise, cells transfected with pBJ vector alone did not bind Fc fragment (data not shown, but similar to Fig. 6B). Taken together, these data indicated that the VZV glycoprotein gpI, like its HSV homolog gE, contained the virally encoded Fc-binding activity. VZV gpIV by itself did not bind IgG Fc fragment. This latter finding is consistent with reports that HSV gI alone does not act as an FcR.

Phosphorylation of transfected viral products. Previously, gpI precipitated from infected cells has been shown to be modified by mammalian phosphotransferases (27). The ability of the transfected gpI product to be similarly modified in a system free of other viral constituents was investigated. One day after transfection, Cos-7 cells were labeled with $^{32}P_i$ and cultured for an additional 2 days. Figure 7A shows that a phosphorylated form of gpI was precipitated from the pCMV5-gpI transfectants, but no phosphoprotein corresponding to VZV gpI was observed in the Cos cells transfected with the vector only. Examination of the amino acid sequence of gpI reveals that the viral glycoprotein contains putative phosphorylation sites for both casein kinase I and casein kinase II in its cytoplasmic tail (15, 17). When the transfected gpI gene product was similarly analyzed, it was also found to be modified by the same two kinases in vitro (Fig. 7A). As a control experiment, gpI precipitated from VZV-infected cells was phosphorylated in a protein kinase assay with casein kinase II and included in lane 7. Because ^{32}P-labeled glycoproteins are easily detectable on radiographic film, we investigated whether the radiolabeled proteins shown in Fig. 7A could be used as substrates in further oligosaccharide studies. To establish that the transfected gene product retained its N-linked glycans, we did analyses with N-glycanase on the phosphorylated molecule isolated from infected and transfected cell cultures. As shown in Fig. 7B, both gpI products were equally sensitive to digestion with this endoglycosidase, which cleaves high-mannose and complex-type oligosaccharide moieties. The electrophoretic pattern in Fig. 7B also documented the similar migrations of transfected gpI and wild-type gpI, after phosphorylation catalyzed by casein kinase I.

Sequence comparisons of VZV gpI and herpesvirus homologs. In 1986, Petrovskis et al. (31) identified two glycoproteins in the U_S genome of the porcine alphaherpesvirus, PRV. The PRV glycoprotein designated gI was reported to share regions of homology with HSV gE and VZV gpI. Likewise, the PRV glycoprotein designated gp63 was homologous to HSV gI and VZV gpIV. Comparisons of the amino acid sequences revealed that the highest homology existed in

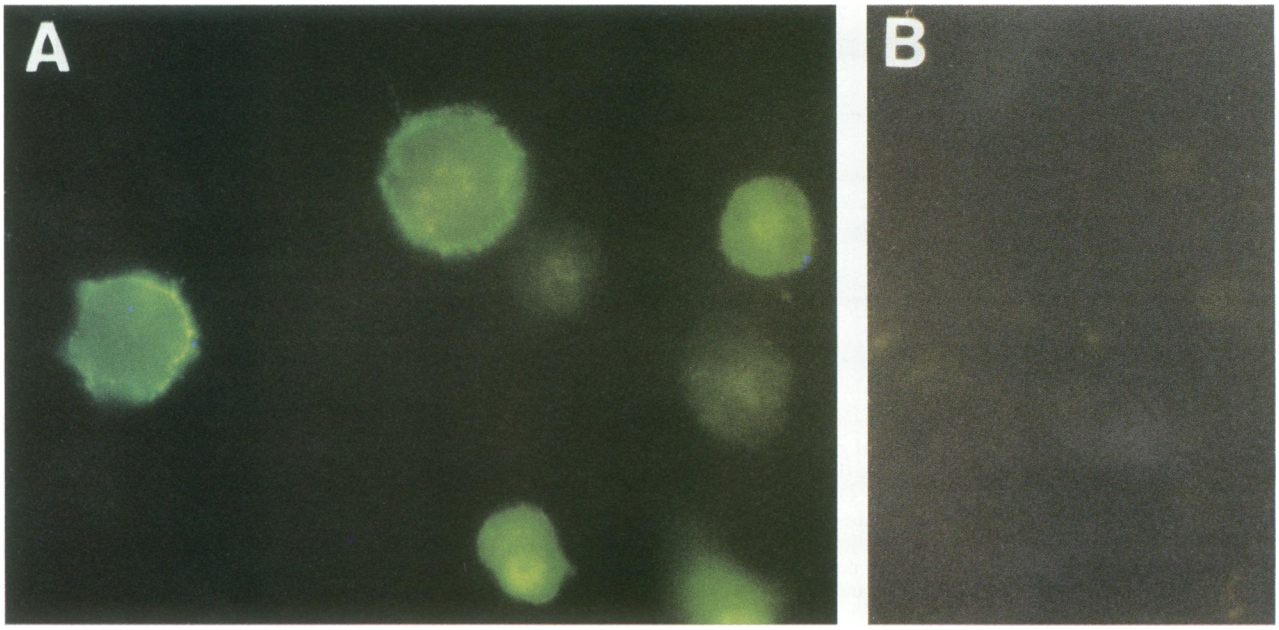

FIG. 6. Expression of an FcR in transfected cell cultures. Human IgG Fc fragment was incubated with live pCMV5-gpI-transfected cells and then with $F(ab')_2$ fluorescein isothiocyanate-conjugated goat anti-human Ig. Several exemplary cells in panel A display typical membrane staining. In panel B, human IgG fragment was incubated with live pBJ-gpIV-transfected cells. About 10 dull orange cells with no specific immunostaining are faintly detectable in panel B.

the regions of HSV gE amino acids 211 to 381, VZV gpI amino acids 328 to 500, and PRV gI amino acids 213-376. Six of the 10 extracellular cysteine residues of VZV gpI are located in the region between residues 328 and 500. The Petrovskis analysis revealed that these six cysteine residues were conserved across the three viruses.

In Table 2, we show the results of further examination of the sequences of these three viral glycoprotein homologs, in particular, the regions downstream from those analyzed by Petrovskis et al. (31). VZV gpI shares an overall amino acid sequence identity of 27% with HSV gE and 30% with PRV gI. In the downstream regions, the amino acid sequence identity was only mildly reduced to 24% for HSV gE and to 27% for PRV gI. Similar motifs among the three viral glycoproteins are detailed in Fig. 8. For example, a serine-threonine-proline-rich region 5′ proximal to the transmembrane domain of a receptor is indicative of potential O-linked glycosylation sites (34). Examination of similar regions in the three alphaherpesviruses yielded values of 43% serines-threonines-prolines in VZV gI, 32% in HSV gE, and 50% in PRV gI. Another shared motif relates to phosphorylation. VZV gpI is known to contain consensus sequences for mammalian protein kinases near its carboxy terminus (15, 17); likewise, the cytoplasmic region of HSV gE had nearly identical sites for phosphorylation by both enzymes. Although not as apparent, a consensus sequence was also

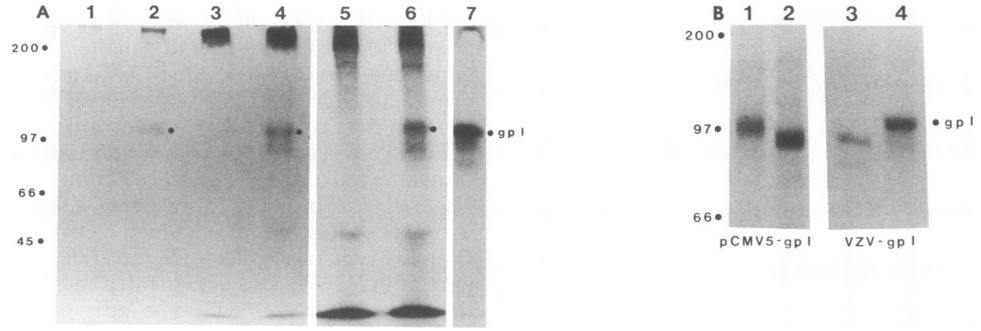

FIG. 7. Phosphorylation of the transfected gpI gene product. (A) Cells were transfected with either pCMV5 (lanes 1 and 3) or pCMV5-gpI (lanes 2 and 4). Three days after transfection, gpI was precipitated from cell lysates with MAb 3B3 and used as a substrate in protein kinase assays with either casein kinase I (lanes 1 and 2) or casein kinase II (lanes 3 and 4). Alternatively, cells transfected with pCMV5 (lane 5) or pCMV5-gpI (lane 6) were labeled 24 h posttransfection with $^{32}P_i$ and harvested on day 3, when immunoprecipitations with MAb 3B3 were performed. Lane 7 contains authentic gpI precipitated from VZV-infected cells and phosphorylated in a protein kinase assay with purified casein kinase II, as described in Materials and Methods. The location of gpI is indicated by closed circles. (B) Cells transfected with pCMV5-gpI were harvested, and gpI was used as a substrate for casein kinase 1 (see panel A, lane 2). The phosphorylated gpI was treated with *N*-glycanase (lane 2) or left untreated (lane 1). In a similar fashion, VZV gpI from infected cells was phosphorylated with casein kinase I, then either left untreated (lane 4) or treated with *N*-glycanase before electrophoresis (lane 3). Numbers on left show size in kilodaltons.

TABLE 2. Amino acid sequence comparison of alphaherpesviral glycoproteins[a]

Residue no.	Residue no.	% Identity	% Similarity
VZV gpI[b]	HSV gE[c]		
1–623	1–550	27	47
328–500	211–381	34	54
491–623	371–550	24	45
VZV gpI	PRV gI[d]		
1–623	1–577	30	49
328–500	213–376	38	57
491–623	389–577	27	49

[a] Data were generated by using the GCG BestFit program (9, 35).
[b] SwissProt accession number: P09259.
[c] SwissProt accession number: P04488.
[d] GenBank accession number: M14336 (residues 1229 to 2963).

found in the cytoplasmic region of PRV gI. The recognition site for casein kinase II is S/T followed by D/E in the N + 3 position. This site is made more favorable by the presence of acidic residues in the vicinity. A casein kinase I recognition sequence, although less well defined, may include D/E/Q followed by S one or two amino acids downstream (39). Another recognition determinant for casein kinase I is a phosphoserine in the −3 position preceding the serine to be modified (40).

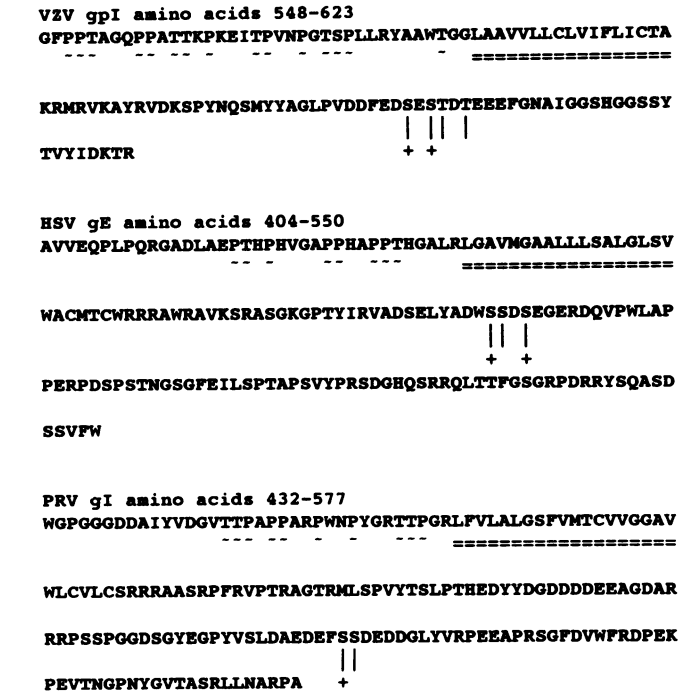

FIG. 8. Comparison of the C-terminal regions of homologous glycoproteins encoded by three alphaherpesviruses. The amino acid sequences of the 5′ membrane-proximal domain, the transmembrane domain, and the complete cytoplasmic region of VZV gpI, HSV gE, and PRV gI are shown. Key features common to all three sequences are recognition sites for casein kinase I (+) and casein kinase II (|) and proposed signals for O-linked glycosylation (~). The transmembrane domain is denoted by underlining (=).

DISCUSSION

HSV types 1 and 2 are known to specify glycoproteins which have IgG FcR activity (6, 7, 20, 26, 29, 41). Recently, we demonstrated that VZV-infected cells expressed a similar activity not present in the mock-infected host cell (24). This receptor was associated with a viral glycoprotein, in that surface expression of the VZV glycoproteins was required to detect FcR activity. However, the question regarding which of the five VZV-encoded glycoproteins was responsible for this activity was not addressed. The VZV homolog of the HSV-encoded FcR was the most likely candidate. This possibility was confirmed in the current study by using VZV gpI transfection studies. Cos cells transfected with a plasmid containing VZV gene 68 (gpI) were found to bind human IgG Fc fragment, while cells transfected with plasmids containing VZV gene 67 or no viral insert did not react with the Fc fragment. These data confirm the hypothesis that gpI is indeed the VZV Fc-binding glycoprotein, whereas the second VZV U_S glycoprotein does not possess Fc-binding properties. In addition, this result demonstrates that a functional relationship exists between VZV gpI and its HSV homolog gE.

The VZV-encoded FcR has been more difficult to detect than that of HSV and appears to be a receptor of lower affinity. In the FcR binding assay described by Litwin et al. (24), concentrations of human IgG or IgG Fc fragment of 0.5 to 1.0 mg/ml were required to detect binding to the VZV-infected cells. By contrast, 10- to 20-fold-lower concentrations of antibody were sufficient to demonstrate Fc binding in the HSV system (6, 7, 26). In earlier investigations of the VZV FcR, conditions which were favorable for detection of FcR activity in HSV-infected cells failed to uncover FcR activity in VZV-infected cells (12, 14, 19). This discrepancy is most likely due to the lower affinity of the VZV receptor for Ig. The differences in the properties of the VZV and HSV receptors may be reflective of the fact that the two viral glycoproteins share only 27% amino acid sequence identity (Table 1). There remain intriguing disparities between the homologs: whereas HSV-1 gE is a minor viral component with easily detectable FcR activity, VZV gpI is the major structural glycoprotein with only weakly detectable Fc binding properties (15, 36). The FcR activity of HSV-2 appears to be less than that of HSV-1 but greater than that of VZV (30, 36).

An extensive computer-assisted comparison of VZV gpI and gpIV with the three classes of human cellular IgG FcR failed to show significant amino acid sequence homology (23). Likewise, when these two viral glycoproteins were further analyzed for homology to other members of the Ig gene superfamily, no relationship was found. The members of the Ig gene superfamily are noted for their ability to interact with each other through their Ig-like domains. Although neither VZV gpI nor HSV gE displays the characteristic Ig fold, it is intriguing that they are still able to interact with at least one member of the Ig gene superfamily. In the HSV system, the association of gE and gI may enhance the IgG-binding properties of the viral receptor (2, 10, 18). As VZV gpIV bears a degree of amino acid sequence homology to HSV gI, it is possible that gpI and gpIV may also associate to form a receptor of higher affinity than VZV gpI alone. This question was not addressed in the present study as it was not possible to quantify the amount of Fc fragment bound to the VZV transfectants by immunofluorescence microscopy.

Recently, Smith et al. (34) described the receptor for

tumor necrosis factor and defined an unusual family of cellular and viral proteins. Two salient features of this family of receptors are O-linked glycosylation sites immediately upstream from the transmembrane region and an exocytoplasmic region rich in cysteine residues which also contains N-linked glycosylation sites. Our examination of the predicted amino acid sequence of VZV gpI revealed two cysteine-rich regions, as well as a 35-amino-acid membrane-proximal potential O-glycosylation domain. Consideration of these features, together with the consensus sequences for casein kinases I and II in the cytoplasmic tail of gpI, illustrates that this viral glycoprotein displays features commonly seen in many cell surface receptors. The HSV homolog of VZV gpI is also phosphorylated within the infected cell (12), but the specific kinases were not investigated. Based on the potential sequence recognition sites shown in Fig. 8, both casein kinases I and II may be involved. Although not noted by Smith et al. (34) in their analysis of the tumor necrosis factor receptor, our reexamination of their sequence data reveals potential recognition sequences for protein kinases in the cytoplasmic tail of the tumor necrosis factor receptor.

Thus, the data presented herein demonstrate that VZV gpI displays motifs similar to many receptors and, in addition, is able to functionally act as a receptor. Whether the Fc portion of human IgG is the true or sole biologic ligand for this viral cell surface receptor remains to be proved. That this glycoprotein may play a previously unsuspected but important role in the pathogenesis of VZV infection is suggested by recent PRV studies. Glycoprotein gI of PRV has been shown to modulate neurovirulence of the virus and thus may have adhesion molecule-like properties (4, 5, 25). Based on the homology among HSV gE, VZV gpI, and PRV gI, this receptor glycoprotein may contribute to the pronounced neurotropism of *Alphaherpesvirinae*. Such a possibility supports an earlier hypothesis that U_S genes influence the biologic niche of an alphaherpesvirus within its natural host (32).

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