Arch Virol (2002) 147: 335–348

Archives of Virology

Expression of the simian varicella virus glycoprotein L and H

C. V. Ashburn and **W. L. Gray**

Department of Microbiology and Immunology,
University of Arkansas for Medical Science, Little Rock, Arkansas, U.S.A.

Accepted October 2, 2001

Summary. Simian varicella is used as a model to investigate varicella-zoster virus pathogenesis and to evaluate antiviral therapies. In this study, the simian varicella virus (SVV) glycoprotein L (gL) was characterized along with its association with glycoprotein H (gH). The SVV gL gene encodes a predicted 175 amino acid polypeptide that shares 43.5% and 27.9% amino acid identity with the VZV gL and HSV-1 gL, respectively. The SVV gL polypeptide sequence lacks a consensus glycosylation site and a typical signal sequence, but does possess an endoplasmic reticulum targeting sequence found commonly in chaperone proteins. Transcriptional analysis indicated that the SVV gL and the uracil DNA glycosylase (UDG) genes share a common 5′ RNA start site and are co-expressed on a 2.0 kb transcript. gL and gH expression in SVV-infected Vero cells was demonstrated by immunofluorescence and immunoprecipitation analyses using specific antisera generated against gL and gH peptides. Similar to other herpesvirus gH and gL homologs, the SVV gL and gH form a complex within infected cells. gL and gH transcripts and antigens were detected in tissues of monkeys with acute simian varicella. The simian varicella model offers an opportunity to investigate the role of the gL and gH in viral pathogenesis.

Introduction

Varicella zoster virus (VZV), an alphaherpesvirus, is the etiologic agent responsible for two distinct human diseases; varicella (chickenpox) and herpes zoster (shingles). Following the primary varicella infection, VZV establishes a latent infection within the dorsal root ganglia of the host. Herpes zoster occurs following the reactivation of VZV from latent infection. Studies of VZV pathogenesis and development of antiviral therapies are hampered by the lack of a suitable animal model.

Simian varicella virus (SVV) is responsible for a natural varicella-like disease in non-human primates. The clinical and pathogenic aspects of SVV and VZV

infections are similar [23]. SVV and VZV are antigenically related and the viral genomes are similar in size and structure, colinear in gene organization, and share 70–75% DNA homology [3, 9, 13, 25]. Based on the clinical similarities between simian and human varicella and the relatedness of SVV and VZV, simian varicella is used as a model for investigating the molecular mechanisms involved in VZV pathogenesis and for evaluation of antiviral agents and vaccines [29].

Herpesvirus glycoproteins, which are incorporated within the viral envelope and cellular membranes, are involved in various aspects of replication, including viral attachment, entry, and cell-to-cell spread. In addition, herpesvirus glycoproteins are potent inducers of the host immune response. Several SVV and VZV glycoprotein genes have been identified including gB, gC, gE, gH, gI, gK, gL, gM and gN [5, 14].

The gH and gL are highly conserved among members of the herpesvirus family [4]. These two herpesvirus glycoproteins are closely associated with each other and generally form a complex within infected cells [7, 17]. The VZV and herpes simplex virus type-1 (HSV-1) gH play an essential role in cell membrane fusion during viral entry and egress [27, 30]. The VZV and HSV-1 gL serve as chaperones for transport of the gH from the endoplasmic reticulum to the Golgi complex and are required for the proper maturation and distribution of the gH on the cell surface [7, 10, 17, 20].

This study characterizes the SVV gL and its association with the SVV gH. The SVV gH encodes a 852 amino acid glycoprotein that shares 55% and 25% identity with the VZV and HSV-1 gH, respectively [26]. The results of this study demonstrate expression of gL and gH within SVV-infected Vero cells and tissues derived from monkeys with acute simian varicella. The study provides a foundation for investigating the role of the SVV gL/gH in viral replication, pathogenesis, and immunity and for development of diagnostic assays for simian varicella.

Materials and methods

Virus and cell culture

The Delta herpesvirus strain of SVV was propagated in African green monkey kidney cells (Vero). SVV-infected Vero cells were passaged by mixing infected and uninfected cells at a ratio of 1:4, respectively. Vero cells were cultured in Eagle's minimal essential media (EMEM) supplemented with penicillin (5,000 U/ml), streptomycin (5,000 U/ml) and 5% newborn calf serum.

Sequencing of the SVV gL

Isolation and cloning of the SVV genome has been previously described [13]. Dideoxy chain termination sequencing was used to determine the sequence of the SVV gL [28]. Sequencing products were separated on 6% polyacrylamide gels containing 7 M urea, 90 mM Tris base, 90 nM boric acid, and 2 mM EDTA, and visualized by exposure to Kodak X-OMAT film.

The DNA sequence of the SVV gL was assembled and analyzed using the Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin [6]. DNA (FastA) and protein (TFastA) homology searches against the GenBank database was performed using the methods of Pearson and Lipman [24]. Employing the algorithm of Needleman and Wunsch [22], the GCG Pileup program was used to align and

compare multiple sequences. The predicted molecular weights of the SVV gL and gH were determined using the GCG PeptideSort program. The DNA sequence of the SVV gL is available in the GenBank library under the accession numbers AF108378 and AF275348.

Mapping of the SVV gL and uracil DNA glycosylase (UDG) transcripts

The transcriptional start sites for the SVV gL and UDG were determined using the GeneRacer Kit (Invitrogen Corp.). Briefly, 1 μg of total RNA isolated from SVV-infected Vero cells was dephosphorylated with calf intestinal phosphatase (CIP) and treated with 0.5 units of tobacco acid phosphatase (TAP) resulting in decapped mRNAs. The GeneRacer 5′-primer RNA oligonucleotide was ligated onto the decapped mRNA, followed by reverse transcription using avian myeloblastosis virus reverse transcriptase (AMV-RT) and an oligo dT primer to create the first strand cDNA. The 5′ ends of the SVV ORF gL and uracil DNA glycosylase (UDG) were determined by PCR using a gene specific primer (gL: 5′-TCCATGTGTTTTATACAGCC-3′; UDG: 5′-TGCAATGCTGTGGATAATCG-3′) and a 5′-primer complementing the ligated RNA oligonucleotide. The PCR products were gel purified and sequenced to identify the precise 5′ start site of the SVV gL and UDG transcripts.

Generation of SVV gL and gH antipeptide sera

Two synthetic peptides, SVVgL47 and SVVgH760 were selected based on antigenic potential and synthesized by Alpha Diagnostic International (San Antonio, TX). The gL peptide (NH_2-CISFQENLWKYSAPSVPNLKED-COOH) included gL amino acids 47–68 and the gH peptide (NH_2-CKDTERQLAALENSTVTAFNPDVHG-COOH) included gH amino acids 758–783. Rabbits were immunized three times with 10 mg of each peptide conjugated to a carrier protein. Rabbits were bled and antibody titers determined by enzyme linked immunoassay.

In vitro translation of SVV gL and gH

The entire SVV gL and gH ORFs were cloned into the pCITE expression vector (Novagen Inc., Milwaukee, WI), which contains a Cap Independent Translation Enhancer (CITE) that allows the DNA of interest to be transcribed by the bacteriophage T7 RNA polymerase and then in vitro translated. The gL and gH ORFs were PCR amplified from SVV genomic DNA using gL (5′-ATTATATGGAAGGACATAGG-3′ and 5′-TTACTCCAGGAGGAATACCG-3′), and gH (5′-GTACGCACCGCGGCTAC-3′, and 5′-CGTGACTACAGAAACTGTTG-3′) specific primers.

In vitro transcription and translation were performed with the gL/pCITE and gH/pCITE using the STP-3 In Vitro Transcription/Translation System (Novagen Inc.). Briefly, transcription reactions were conducted at 30 °C for 15 minutes and included 0.5 μg of plasmid DNA (RNAase free), T7 RNA polymerase, and STP3 transcription buffer. In vitro translation was performed at 30 °C for 60 min in a 50 μl reaction containing the transcription product, 40 μCi [^{35}S] methionine ($>$ 800 Ci/mmol), and the STP3 translation mix including rabbit reticulocyte lysate. Final products were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and visualized by autoradiography.

Analysis of SVV gL and gH expression by immunoprecipitation and immunofluorescence

SVV-infected and uninfected Vero cells proteins were labeled with [^{35}S]-methionine and immunoprecipitation analysis was conducted as previously described [9]. Briefly, 2×10^6 counts per minute (cpm) of either the in vitro translated gL, gH, and pCITE, or SVV-infected

and uninfected Vero cell lysates were precleared overnight at 4 °C with normal rabbit serum. The precleared antigen was precipitated at 4 °C with *Staphylococcus aureus* protein A (Staph A, Pansorbin, CalBiochem). Supernatants were reacted with 25 μl of the anti-gL or anti-gH antibody, and incubated at 4 °C for 3 h. Reactions were again precipitated with Staph A and the pellets washed 3 × with solubilization buffer (0.025 M Tris-HCl, 0.25 M NaCl, 0.005 M EDTA, 0.5% deoxycholate, and 1.0% Triton X-100), and once with Tris-EDTA pH 7.5. The bound antigen-antibody complexes were eluted in SDS-PAGE buffer (0.002% bromphenol blue, 5% 2-mercaptoethanol, 10% glycerol, 10% SDS, and 0.1 M Tris-HCl pH. 6.8) and analyzed by SDS-PAGE and autoradiography.

For immunofluorescence analysis, SVV-infected and uninfected Vero cells were fixed to slides (5×10^4 cells per slide) and gL and gH expression was examined as previously described [8]. Cell surface expression was detected by blocking with 5% normal goat serum for 1 h, followed by washing and reaction with primary antibody (anti-gL or anti-gH) for 1 h at 25 °C. After washing, cells were reacted with the secondary fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit lgG antibody for 1 h at 25 °C. Intracellular expression was determined as above except the cells were treated with 0.01% Triton X-100 for 1 h prior to blocking to permeabilize the cells, and then incubated overnight with the primary antibody. Fluorescence was observed by microscopy.

Experimental infection of nonhuman primates

Studies involving nonhuman primates were conducted at the Tulane Regional Primate Center. St. Kitts vervet monkeys were inoculated by intratracheal inoculation with 3×10^4 pfu of SVV-infected Vero cells. The clinical features of the infection have been previously reported [15]. Monkeys were sacrificed on day 10 postinfection during the acute stage of simian varicella and tissues were collected. Liver, lung, and pooled thoracic dorsal root ganglia tissues designated for RNA isolation were quick-frozen and processed as described below. Liver and skin tissues designated for immunohistochemistry were fixed in Streck's Tissue Fixative (S.T.F., Streck Laboratories Inc.), and paraffin embedded.

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from SVV-infected and uninfected Vero cells or monkey tissues using the guanidinium isothiocyanate-cesium chloride method as previously described [11]. RNA samples were treated with 1 U DNAse/μg RNA for 1 h at 37 °C. RT-PCR was performed using the ACCESS RT-PCR SYSTEM (Promega Corp.). Reactions (50 μl) included 0.2 mM dNTP mix, 1 μM each of downstream and upstream primers (gL fwd 5′-AATACTCAGC GCCTTCGGTA-3′, gL rev 5′-TGCAATGCTGTGGATAATCTG-3′; gH fwd 5′-TCAGATTC AACAGCTACGGC-3′ and gH rev 5′-TATCGCGGCAAGGTTAACTG-3′), 1 U/μl AMV RT, 1 U/μl *Tfl* DNA Polymerase, and 0.5 μg RNA sample. First strand synthesis was carried out at 48 °C for 45 min. AMV RT inactivation and RNA/cDNA/primer denaturation were performed at 94 °C for 2 min. Second strand synthesis then proceeded for 40 cycles at 94 °C for 30 sec, 60 °C for 1 min, and 68 °C for 2 min followed by a final extension step at 68 °C for 7 min. PCR products were visualized by agarose gel electrophoresis and ethidium bromide staining.

Detection of gH and gL in SVV-infected tissues by immunohistochemistry

Immunoperoxidase staining was used to determine SVV gL and gH expression within the skin and liver tissues of acutely infected St. Kitts vervet monkeys. Serial sections of paraffin-embedded tissues were cut and mounted. Tissues were blocked with 5% normal goat sera, washed, and incubated with a 1:1 mixture of rabbit anti-gL and anti-gH antisera (1:2000 dilution) at 25 °C. After washing, the tissues were incubated with biotinylated goat

anti-rabbit lgG (1:5000) antibody conjugate. The tissues were washed and developed using a strepavidin peroxidase complex and diaminobenzidine substrate (Vector Laboratories Inc.).

Results

SVV gL sequence and transcriptional analysis

DNA sequence analysis of the SVV BamHl D restriction fragment (map units 0.8 to 0.825) revealed a 525 bp ORF encoding a predicted 175 amino acid gL homolog that is slightly larger than the 159 amino acid VZV gL (Fig. 1). The

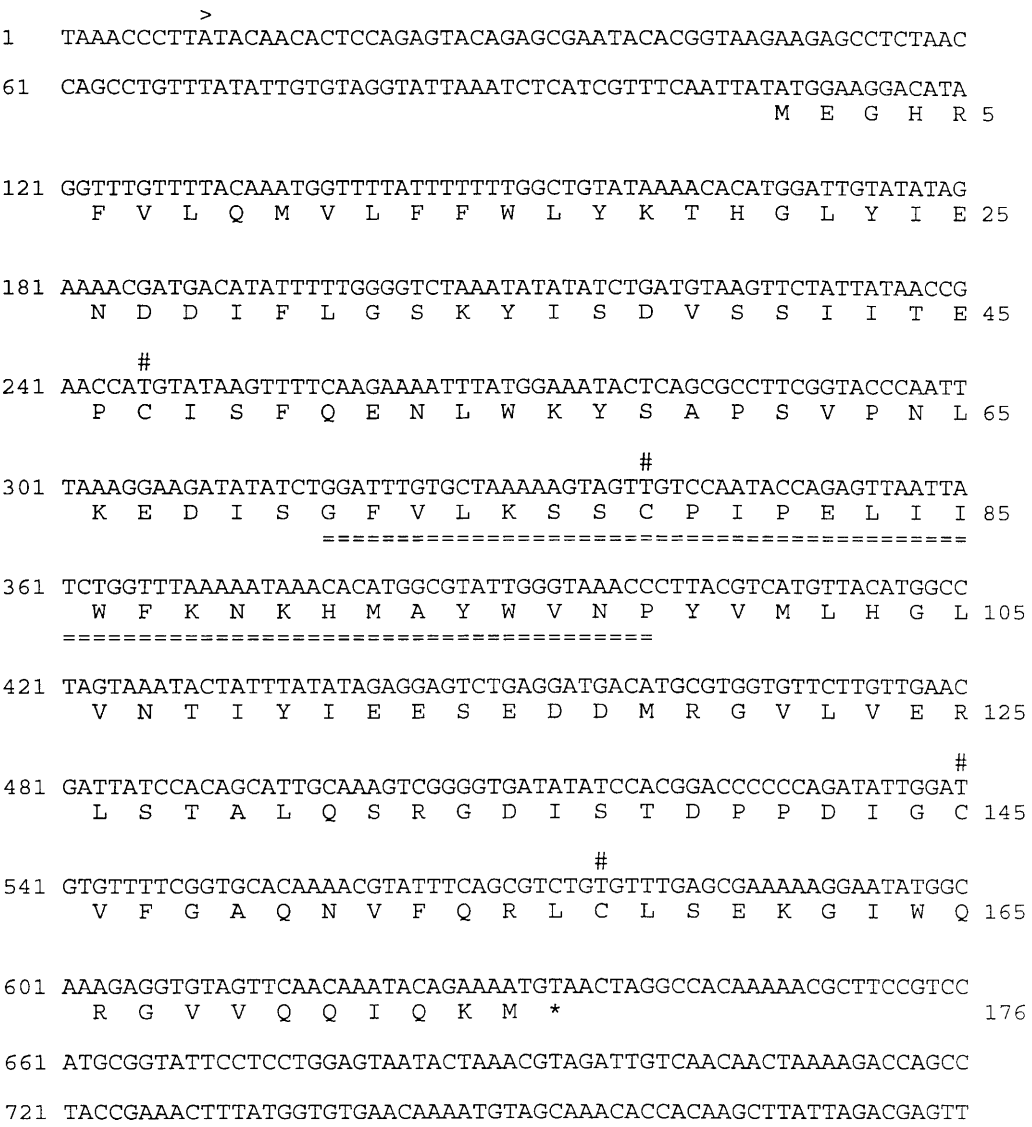

Fig. 1. The SVV gL ORF and predicted polypeptide sequence. The transcriptional start site is indicated with an arrow at the adenine at position −98 relative to the ATG initiation codon. The ER targeting sequence (amino acids 71–98) is designated by the double dashed line. Cysteine residues (amino acid residues 47, 78, 145, 157) are indicated by #

DNA sequence environment of the proposed ATG initiation codon is in partial agreement with Kozak's rule for mRNA translation initiation [18]. The SVV gL shares 43.5% and 27.9% amino acid identity with the VZV and HSV-1 gL, respectively. The alignment of the SVV gL with other herpesvirus gL homologs is shown in Fig. 2. The SVV gL, like the VZV gL, but unlike HSV-1 gL, does not include a predicted cleavable amino terminal signal sequence. However, the SVV gL includes the hydrophobic 28 amino acid (residues 71–98) endoplasmic reticulum (ER) targeting/binding sequence that is highly conserved among other herpesvirus gL homologs as well as cellular ER targeting proteins [7, 31]. The SVV gL includes 4 conserved cysteines (residues 47, 78, 145, 157) which may be important for intramolecular disulfide bonding and appropriate conformation. Indeed, the four homologous cysteines in the VZV gL are required for proper chaperone function and interaction of the gH [7]. Unlike the VZV gL, the SVV gL does not have a consensus glycosylation site (Asn-X-Ser or Asn-X-Thr).

A previous study employing Northern blot hybridization analysis suggested that the SVV gL (ORF 60), UDG (ORF 59), and possibly the ORF 58 genes are co-expressed as a single 2.0 kb transcript [2]. This possibility was further explored by determining whether the SVV gL and UDG share the same 5′ RNA start site. The gL and UDG transcriptional start sites were confirmed by PCR amplification of the 5′ terminus and sequence analysis using gL and UDG specific primers. Agarose gel analysis of the two PCR products resulted in 175 bp (gL) and 500 bp (UDG) fragments corresponding to the predicted size for the two genes to have the same transcriptional start site (Fig. 3). The PCR products were purified and sequenced to determine the mRNA start site of the SVV gL and UDG. The adenine at position −98 relative to the gL ATG initiation codon (Fig. 1) was determined to be the transcriptional start site for both the gL and UDG. Combined with the previous Northern blot hybridization data, the results indicate that the gL and UDG genes are co-expressed on the same 2.0 kb transcript. The lack of a polyadenlyation sequence immediately downstream of the gL stop codon is consistent with this finding.

Generation of specific anti-gL and gH antisera

Antisera to the SVV gL and gH was generated by immunizing rabbits with synthetic peptides generated from the SVV gL and gH amino acid sequence (gL: residues 47–68; gH: residues 758–783). The specificity of the anti-gL and anti-gH antisera was determined by immunoprecipitation of SVV gL and gH in vitro translation products. In vitro translation of the gL generated a 20 kDa protein (Fig. 4A, lane 1), similar to the predicted 20.2 kDa size of the gL based on amino acid sequence analysis. In vitro translation of the gH resulted in a 97 kDa product (Fig. 4A, lane 2), comparable to the predicted 96.7 kDa size based on amino acid sequence analysis. A 43 kDa protein was generated by in vitro translation of the pCITE vector alone (Fig. 4A, lane 3).

The anti-gL antiserum immunoprecipitated the 20 kDa gL in vitro translation product (Fig. 4B, lane 1), but not the in vitro translation products of the gH (Fig. 4B, lane 2) or the pCITE (Fig. 4B, lane 3). The anti-gH antiserum

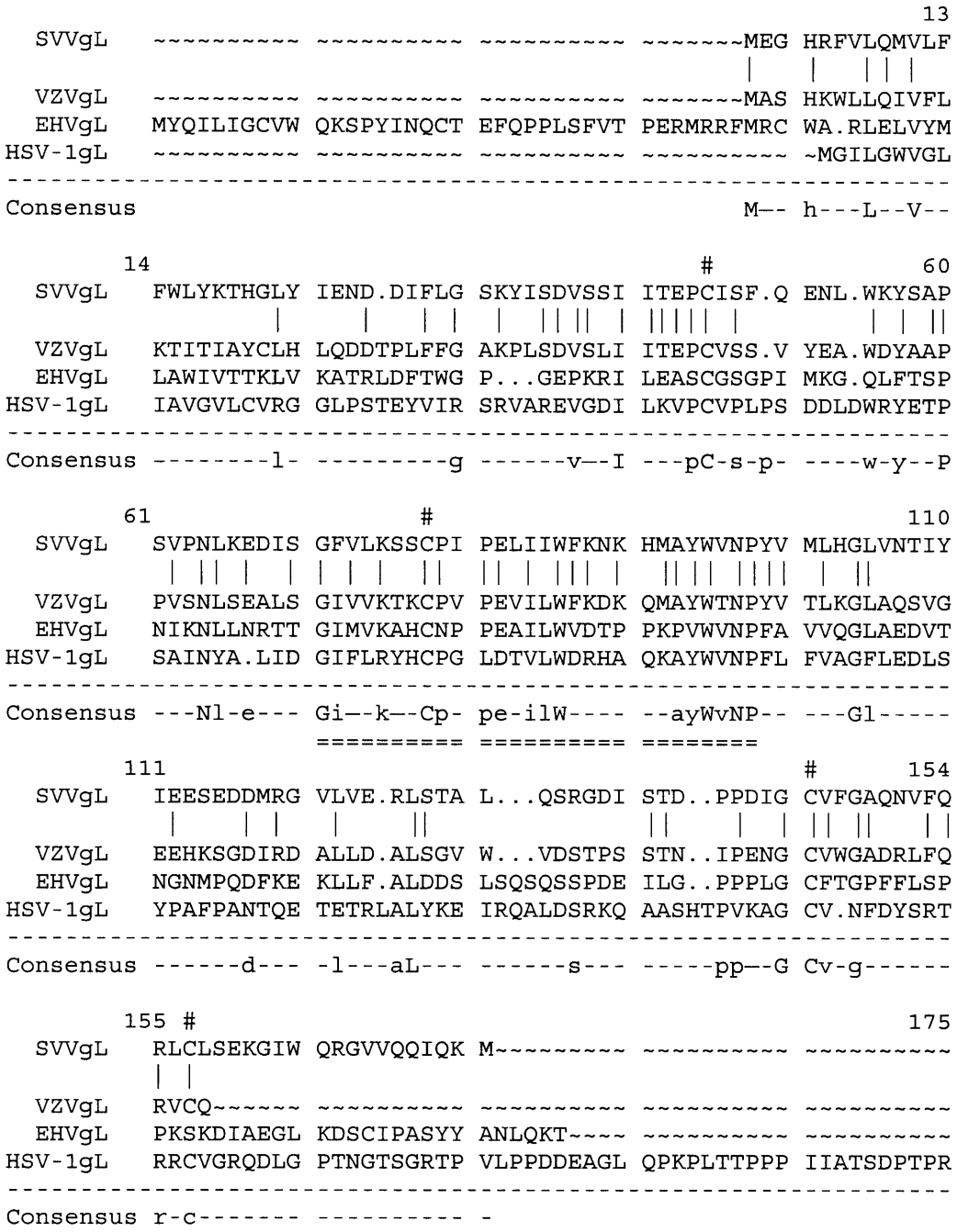

Fig. 2. Alignment of the predicted SVV gL polypeptide sequence with other herpesvirus gL homologs. The algorithm of Needleman and Wunsch [22] was utilized to align the predicted polypeptide sequences of the SVV, VZV, equine herpesvirus type 1 (EHV-1), and HSV-1 gL. The double-dashed lines indicate the ER targeting sequence. Conserved cysteine residues are designated by #. Conserved amino acids between the predicted SVV gL and VZV gL are indicated by vertical lines. Complete and partial conservation among the gL homologs is indicated by capital and small letters, respectively, on the consensus line

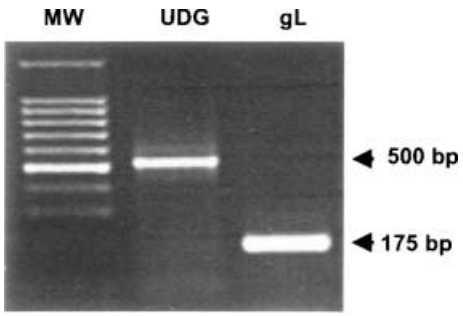

Fig. 3. Analysis of the SVV gL and UDG transcriptional start sites. Total cell RNA (1 μg) derived from SVV-infected Vero cells was treated using the GeneRacer (Invitrogen Corp.) protocol. cDNA generated using a oligo dT primer was PCR amplified with the GeneRacer 5′-primer and a gL or UDG reverse primer. Reaction products were analyzed by agarose (1%) gel electrophoresis and ethidium bromide staining. *MW* 100 bp molecular weight marker

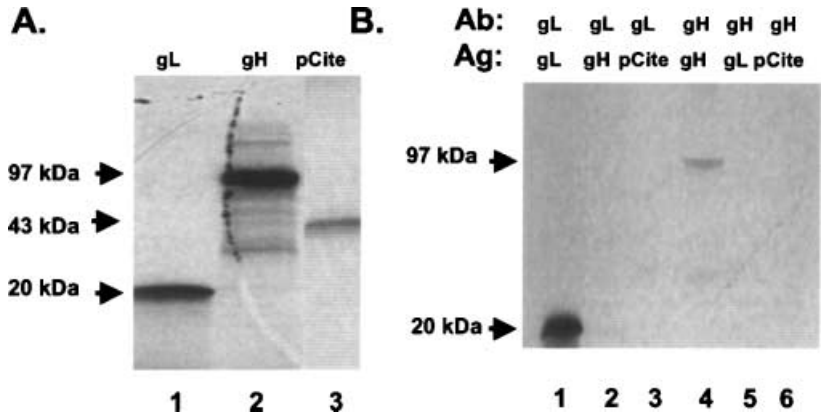

Fig. 4. Analysis of SVV gL and gH antisera specificity by immunoprecipitation of gL and gH in vitro translation products. **A** SDS-PAGE analysis of in vitro translated gL (*1*) and gH (*2*), and the pCITE vector alone (*3*). **B** SDS-PAGE analysis of gL (*1* and *5*), gH (*2* and *4*), and pCITE (*3* and *6*) in vitro translation product antigens (Ag) immunoprecipitated with antibody (Ab) against the SVV gL (*1–3*) and gH (*4–6*). The protein sizes based on molecular weight protein standards are indicated

immunoprecipitated the 97 kDa gH in vitro translation product (Fig. 4B, lane 4), but not the in vitro translated gL (Fig. 4B, lane 5) or pCITE (Fig. 4B, lane 6) products. These data confirm the specificity of the anti-gL and anti-gH antisera.

gL and gH antigen expression in SVV-infected Vero cells

Expression of gL and gH in SVV-infected and uninfected Vero cells was analyzed by immunofluorescence employing the specific anti-gL and gH rabbit antiserum as the primary antibody followed by goat anti-rabbit lgG FITC conjugated secondary antibody. Intracellular and surface expression of the gL within SVV-infected Vero cells (Fig. 5A), but not in uninfected Vero cells (Fig. 5B), was revealed

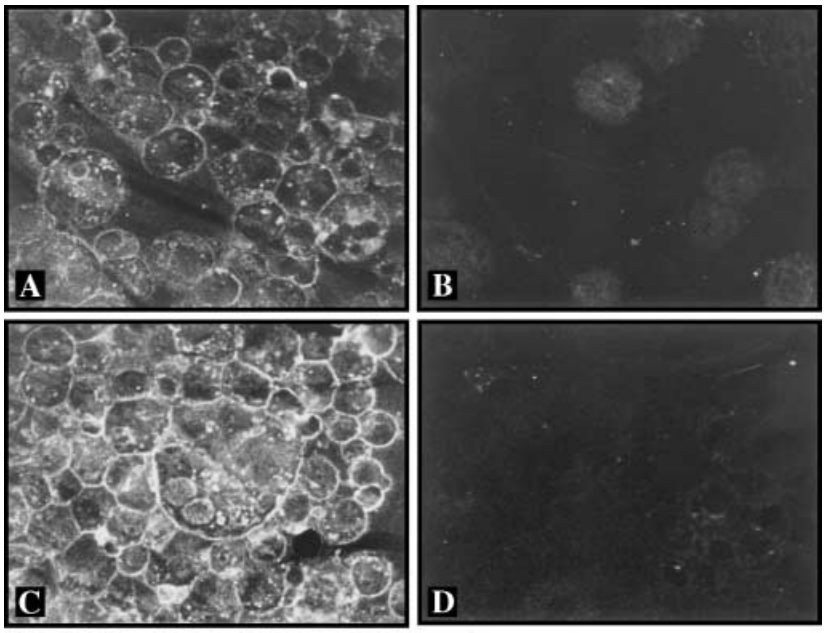

Fig. 5. Detection of SVV gL and gH antigens in SVV-infected Vero cells by immunofluorescence. SVV-infected Vero cells (**A** and **C**) and uninfected Vero cells (**B** and **D**) were reacted with gL antiserum (**A** and **B**) or SVV gH antiserum (**C** and **D**) followed by FITC-labeled goat anti-rabbit lgG antiserum. Cells were permeabilized by treatment with 0.01% Triton X-100 prior to blocking and addition of primary antiserum

by immunofluorescence using anti-gL antisera. SVV gH expression was also detected by immunofluorescence on the surface of and within SVV-infected Vero cells (Fig. 5C), but not in uninfected Vero cells (Fig. 5D) using anti-gH antisera. Normal rabbit serum did not detect SVV antigens in infected cells (data not shown).

Immunoprecipitation analysis using [^{35}S]-methionine labeled lysates derived from SVV-infected and uninfected Vero cells was used to confirm gL and gH expression in infected cells. The anti-gL antisera immunoprecipitated the 20 kDa gL, and also immunoprecipitated two high molecular weight species, $\approx$115 and 97 kDa, corresponding in size to the mature and immature forms of the gH, respectively [26] (Fig. 6, lane 1). The anti-gH antisera immunoprecipitated from infected cells the 97 kDa gH protein in addition to the 20 kDa gL protein (Fig. 6, lane 3). The anti-gL and anti-gH antisera did not immunoprecipitate products from uninfected Vero cells (Fig. 6, lanes 2 and 4, respectively). Normal rabbit serum did not immunoprecipitate the 20 or 97 kDa products from SVV-infected cells (data not shown). These results confirm that the gL and gH proteins are expressed in infected Vero cells. In addition, the findings indicate that the SVV gL and gH form a complex in infected cells as indicated by the anti-gL antibody co-precipitating the 97 kDa gH protein and the anti-gH antibody co-precipitating the 20 kDa gL protein.

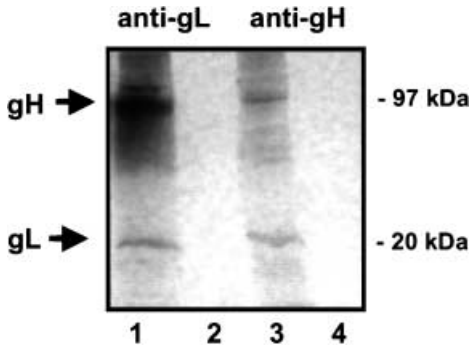

Fig. 6. Detection of SVV gL and gH antigens in SVV-infected Vero cells by immunoprecipitation. SDS-PAGE analysis of radiolabeled SVV-infected (*1* and *3*) and uninfected (*2* and *4*) Vero cell lysates immunoprecipitated with gL antiserum (*1* and *2*) or gH antiserum (*3* and *4*). The protein sizes based on molecular weight protein standards are indicated

Detection of SVV gL and gH transcripts and antigens in tissues of acutely infected monkeys

St. Kitts vervet monkeys were experimentally infected with 3×10^4 SVV-infected Vero cells by intratrachael inoculation. As previously reported [15], the monkeys exhibited clinical signs of simian varicella, including vescicular skin rash and hepatitis, by day seven postinfection. Tissue specimens were collected from acutely infected monkeys on day 10 postinfection.

RT-PCR analysis was used to examine gL and gH transcription in tissues during acute SVV infection. Total cell RNA was isolated from the liver, lung, and pooled thoracic dorsal root ganglia of an acutely infected monkey. RT-PCR was conducted employing SVV gL and gH specific primers and amplified products of the expected size were detected by agarose gel electrophoresis (Fig. 7). The results demonstrated that SVV gL and gH genes are transcribed within the liver, lung, and ganglia during acute infection.

Immunohistochemistry was employed to examine gL and gH antigen expression in tissues of monkeys with acute simian varicella. gL and gH antigen expression could be detected in infected tissues by immunohistochemistry using the

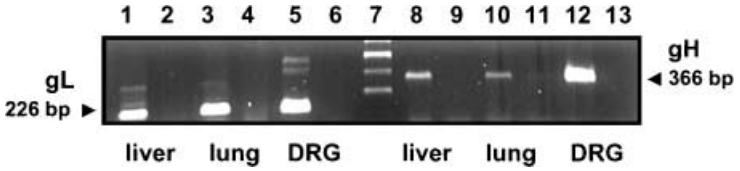

Fig. 7. Transcriptional analysis of the SVV gL and gH in tissues of an acutely infected monkey. The SVV gL (*1–6*) and gH (*8–13*) were PCR amplified from RNA isolated from the liver (*1*, *2*, *8*, and *9*), lung (*3*, *4*, *10*, and *11*), and pooled thoracic dorsal root ganglia (DRG, *5*, *6*, *12*, and *13*). Reactions were conducted with (*1*, *3*, *5*, *8*, *10*, and *12*) or without (*2*, *4*, *6*, *9*, *11*, and *13*) RT. *7* 100 bp DNA molecular weight standards. The expected size of the gL and gH PCR products are indicated

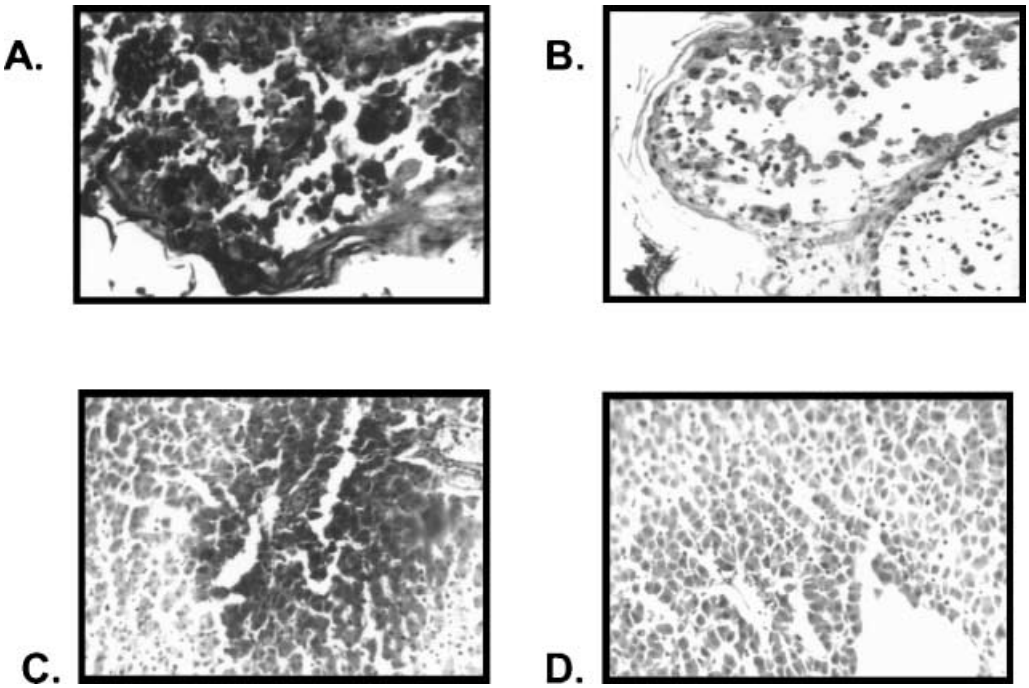

Fig. 8. Immunohistochemical detection of SVV gL and gH antigens in tissues of an acutely infected monkey. Skin vesicle tissue derived from an SVV-infected monkey reacted with gL/gH antiserum (**A**) or with normal rabbit serum (**B**). Liver tissue derived from an SVV-infected monkey reacted with gL/gH antiserum (**C**) or with normal rabbit serum (**D**)

individual anti-gL or anti-gH antisera, respectively (data not shown). However, more intense immunostaining was detected when the diluted primary antibody utilized a 1:1 mixture of the anti-gL and anti-gH antisera. The dark brown staining within infected cells of a skin vesicle indicates expression of the gL/gH (Fig. 8A). No immunostaining was observed when infected skin vesicle specimens were reacted with normal rabbit serum (Fig. 8B) or when skin derived from an uninfected monkey was reacted with the anti-gL/gH antisera (data not shown).

Immunohistochemical examination of liver tissue derived from a SVV-infected monkey revealed gL/gH antigen expression within multifocal necrotic areas of the liver (Fig. 8C). No immunostaining was observed when infected liver specimens were reacted with normal rabbit serum (Fig. 8D) or when liver tissue derived from an uninfected monkey was reacted with the anti-gL/gH antisera (data not shown). The data demonstrate that SVV gL and gH antigens can be detected within the skin and the liver tissues during acute simian varicella.

Discussion

The VZV gL plays an essential role in viral replication by serving as a chaparone for gH transport within infected cells [7]. The current model indicates the gL forms a complex with the precursor form of gH within the ER and then escorts the

immature gH to the Golgi complex. The gH is processed to its mature form during transport within the Golgi network. The VZV gL/gH complex then dissociates and the mature gH migrates to the cell membrane while the gL is presumably degraded within the cell cytoplasm.

This study identifies and characterizes the SVV gL and its association with gH. The 20 kDa SVV gL polypeptide is similar in size, and shares significant homology (43.5% amino acid identity) with the VZV gL. Like VZV and other gL homologs, the SVV gL has a consensus ER targeting/binding sequence. The results indicate that the SVV gL associates with the gH within infected cells. These findings suggest that the SVV gL, like the VZV gL, functions as a chaperone for gH transport within SVV-infected cells. Immunofluorescence results suggest the SVV gL is transported to the cell surface, similar to the HSV-1 gL and other herpesvirus gLs, but in contrast to the findings for the VZV gL [7, 8]. In addition, unlike the VZV gL, the SVV gL amino acid sequence lacks a consensus glycosylation site, suggesting that glycosylation is not essential for a functional gL in SVV-infected cells.

The conservation of the gL and gH within all classes of herpesviruses indicates an important role in viral replication and pathogenesis [4]. The VZV gH/gL antigens are targets for the immune response and may play a role in the induction of immunity following natural varicella or immunization with the VZV vaccine [1, 19]. The simian varicella model provides an opportunity to investigate the role that gL and gH play in viral pathogenesis and immunity. In this study, SVV gL and gH transcripts and antigens were detected in tissues of acutely infected monkeys. The finding of gL and gH RNA in the neural ganglia at 10 days postinfection suggests that, during acute infection, active viral replication occurs in the ganglia, the tissue in which viral latency is ultimately established [21].

Simian varicella epizootics occur sporadically in research facilities housing nonhuman primates [29]. These outbreaks are often associated with high morbidity and mortality. Rapid diagnosis of the disease is essential to prevent and control simian varicella outbreaks. A polymerase-chain reaction (PCR) assay has been developed for rapid detection of SVV DNA in tissues of infected monkeys [16]. However, detection of SVV antigens within infected tissues has been limited due to the lack of SVV specific antisera. In this study, immunohistochemical analysis using anti-gH and gL antisera detected SVV antigens in the skin and liver from acutely infected monkeys. SVV antigen detection employing the gL and gH antisera, as well antisera previously generated against the SVV gE [12], provides a valuable tool for rapid diagnosis of simian varicella.

Acknowledgements

We would like to thank Dr. Charleata Carter for assistance with the fluorescent microscopy and Ms. Jennifer Jones for assistance with the immunohistochemistry. We thank Dr. Ken Soike of the Tulane Regional Primate Center for his assistance with the nonhuman primate infections. This study was supported by Public Health Service Grants Al37571, RR11713 from the National Institutes of Health, and by the UAMS Graduate Student Research Fund.

References

1. Arvin AM, Kinney-Thomas E, Shriver K, Grose C, Koropchak CM, Scranton E, Wittek AE, Diaz PS (1986) Immunity to varicella-zoster viral glycoproteins, gp I (gp 90/58) and gp III (gp118), and to a nonglycosylated protein, p 170. J Immunol 137: 1346–1351
2. Ashburn CV, Gray WL (1999) Identification and characterization of the simian varicella virus uracil DNA glycosylase. Arch Virol 144: 2161–2172
3. Clarke P, Rabkin SD, Inman MV, Mahalingam R, Cohrs R, Wellish M, Gilden DH (1992) Molecular analysis of simian varicella virus DNA. Virology 190: 597–605
4. Cranage MP, Smith GL, Bell SE, Brown C, Bankier AT, Tomlinson P, Barrell BG, Öberg B (1988) Identification and expression of a human cytomegalovirus glycoprotein with homology to the Epstein-Barr virus BXLF2 product, varicella-zoster virus gpIII, and herpes simplex virus type 1 glycoprotein H. J Virol 62: 1416–1422
5. Davison AJ, Scott JE (1986) The complete DNA sequence of varicella-zoster virus. J Gen Virol 67: 1759–1816
6. Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12: 387–395
7. Duus KM, Grose C (1996) Multiple regulatory effects of varicella-zoster virus (VZV) gL on trafficking patterns and fusogenic properties of VZV gH. J Virol 70: 8961–8971
8. Duus KM, Hatfield C, Grose C (1995) Cell surface expression and fusion by the varicella-zoster virus gH:gL glycoprotein complex: analysis by laser scanning confocal microscopy. Virology 210: 429–440
9. Fletcher TM, III, Gray WL (1992) Simian varicella virus: characterization of virion and infected cell polypeptides and the antigenic cross-reactivity with varicella-zoster virus. J Gen Virol 73: 1209–1215
10. Forghani B, Ni L, Grose C (1994) Neutralization epitope of the varicella-zoster virus gH: gL glycoprotein complex. Virology 199: 458–462
11. Gray WL, Baumann RP, Robertson AT, O'Callaghan DJ, Staczek J (1987) Characterization and mapping of equine herpesvirus type 1 immediate early, early, and late transcripts. Virus Res 8: 233–244
12. Gray WL, Mullis LB, Soike KF (2001) Expression of the simian varicella virus glycoprotein E. Virus Res 79: 27–37
13. Gray WL, Pumphrey CY, Ruyechan WT, Fletcher TM (1992) The simian varicella virus and varicella zoster virus genomes are similar in size and structure. Virology 186: 562–572
14. Gray WL, Starnes B, White MW, Mahalingam R (2001) The DNA sequence of the simian varicella virus genome. Virology 284: 123–130
15. Gray WL, Williams RJ, Chang R, Soike KF (1998) Experimental simian varicella virus infection of St. Kitts vervet monkeys. J Med Primatol 27: 177–183
16. Gray WL, Williams RJ, Soike KF (1998) Rapid diagnosis of simian varicella using the polymerase chain reaction. Lab Anim Sci 48: 45–49
17. Hutchinson L, Browne H, Wargent V, Davis-Poynter N, Primorac S, Goldsmith K, Minson AC, Johnson DC (1992) A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. J Virol 66: 2240–2250
18. Kozak M (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44: 283–292
19. LaRussa PS, Gershon AA, Steinberg SP, Chartrand SA (1990) Antibodies to varicella-zoster virus glycoproteins I, II, and III in leukemic and healthy children. J Infect Dis 162: 627–633

20. Li Q, Buranathai C, Grose C, Hutt-Fletcher LM (1997) Chaperone functions common to nonhomologous Epstein-Barr virus gL and varicella-zoster virus gL proteins. J Virol 71: 1667–1670
21. Mahalingam R, Smith D, Wellish M, Wolf W, Dueland AN, Cohrs R, Soike K, Gilden D (1991) Simian varicella virus DNA in dorsal root ganglia. Proc Natl Acad Sci USA 88: 2750–2752
22. Needleman SB, Wunsch CD (1970) A general method applicable to the search for similarities in the amino acid sequence of two proteins. Proc Natl Acad Sci USA 48: 443–453
23. Oakes JE, d'Offay JM (1988) Simian varicella virus. In: Darai G (ed) Virus diseases in laboratory and captive animals. Martinus Nijhoff, Boston, pp 163–174
24. Pearson WR, Lipman DJ (1988) Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85: 2444–2448
25. Pumphrey CY, Gray WL (1992) The genomes of simian varicella virus and varicella zoster virus are colinear. Virus Res 26: 255–266
26. Pumphrey CY, Gray WL (1995) DNA sequence of the simian varicella virus (SVV) glycoprotein H (gH) gene and analysis of the SVV and varicella-zoster virus (VZV) gH transcripts. Virus Res 38: 55–70
27. Rodriguez JE, Moninger T, Grose C (1993) Entry and egress of varicella virus blocked by same anti-gH monoclonal antibody. Virology 196: 840–844
28. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467
29. Soike KF (1992) Simian varicella virus infection in African and Asian monkeys. The potential for development of antivirals for animal diseases. Ann NY Acad Sci 653: 323–333
30. Spear PG (1993) Membrane fusion induced by herpes simplex virus. In: Bentz J (ed) Viral fusion mechanisms. CRC Press, Boca Raton, pp 201–232
31. Yoshida S, Lee LF, Yanagida N, Nazerian K (1994) Identification and characterization of a Marek's disease virus gene homologous to glycoprotein L of herpes simplex virus. Virology 204: 414–419

Author's address: Dr. W. L. Gray, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 511, Little Rock, AR 72205, U.S.A.; e-mail: graywaynel@uams.edu

Received August 20, 2001