JOURNAL OF VIROLOGY, Aug. 1987, p. 2454–2461
0022-538X/87/082454-08$02.00/0

Vol. 61, No. 8

Use of λgt11 and Monoclonal Antibodies To Map the Genes for the Six Major Glycoproteins of Equine Herpesvirus 1†

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Received 22 January 1987/Accepted 22 April 1987

To localize the genes for the major glycoproteins of equine herpesvirus 1 (EHV-1), a library of the EHV-1 genome was constructed in the λgt11 expression vector. Recombinant bacteriophage expressing EHV-1 glycoprotein epitopes as fusion products with β-galactosidase were detected by immunoscreening with monoclonal antibodies specific for each of six EHV-1 glycoproteins. Seventy-four recombinant λgt11 clones reactive with EHV-1 monoclonal antibodies were detected among 4×10^5 phage screened. Phage expressing determinants on each of the six EHV-1 glycoproteins were represented in the library. Herpesviral DNA sequences contained in λgt11 recombinants expressing epitopes of EHV-1 glycoproteins were used as hybridization probes for mapping insert sequences on the viral genome. Genes for five EHV-1 glycoproteins (gp2, gp10, gp13, gp14, and gp21/22a) mapped to the genome L component; only one EHV-1 glycoprotein (gp17/18) was expressed from the unique S region of the genome where genes of several major glycoproteins of other herpesviruses have been located. Two glycoproteins of EHV-1, gp13 and gp14, mapped to positions colinear with genes of major glycoproteins identified in several other alphaherpesviruses (gC- and gB-like glycoproteins, respectively). The genomic locations of other EHV-1 glycoproteins indicated the existence of major glycoproteins of EHV-1 (gp2, gp10, and gp21/22a) for which no genetic homologs have yet been detected in other herpesviruses. The results confirm the general utility of the λgt11 expression system for localizing herpesvirus genes and suggest that the genomic positioning of several high-abundance glycoproteins of EHV-1 may be different from that of the prototype alphaherpesvirus, herpes simplex virus.

Equine herpesvirus 1 (EHV-1), a member of the alphaherpesvirus group, is a significant viral pathogen of horses (2, 13, 36). Because of its association with abortion, respiratory tract infections, and neurological disease, the virus is a major economic problem of the equine industry (2, 8, 10, 13). Current efforts to control the losses resulting from EHV-1 infections are based on antigen-induced stimulation of the antiviral immune response of the horse with either inactivated or live attenuated viral vaccines (7, 9, 10, 40, 58).

The envelope glycoproteins are the principal immunogens of herpesviruses involved in eliciting both humoral and cellular host immune responses (reviewed in references 14 and 48). A thorough characterization of EHV-1 glycoprotein antigens is therefore essential for understanding the mechanisms of host immunity to the virus and for development of more effective vaccines for control of EHV-1 disease. In addition to several minor glycoproteins, eight high-abundance glycoproteins have been identified in the envelope of purified EHV-1 virions. These glycoproteins correspond to EHV-1 structural proteins 2, 10, 13, 14, 17, 18, 21, and 22a with respective molecular masses of 200, 125, 95, 90, 68, 63, 45, and 41 kilodaltons (37, 52; L. W. Turtinen, Ph.D. thesis, University of Kentucky, Lexington, 1983). Although the glycoproteins of several other herpesviruses have been well characterized (15, 23, 49, 54), little is known about the antigenic or molecular structure of the glycoproteins of EHV-1 or their relative importance in the protective antiviral immune response of the horse. Likewise, no information is available on the genomic localization of the coding sequences for EHV-1 glycoproteins.

Because of the putative importance of the envelope glycoproteins in immunity to EHV-1 disease, we have sought to identify and characterize the significant antigenic determinants of the major glycoproteins of EHV-1. Recent work has focused on isolation and mapping of the viral genes encoding EHV-1 glycoproteins. Young and Davis (61, 62) have developed a simple and effective strategy for genomic mapping of the coding sequences of proteins for which specific antibodies are available. The strategy is based on construction of a library of genomic DNA fragments in the vector λgt11 and subsequent screening of the recombinant bacteriophage library with specific antibody for expression of the protein of interest. This technique has been successfully used for precise localization of genes encoding proteins of several other organisms, including the herpesviruses human cytomegalovirus and pseudorabies virus (33, 39, 60, 63).

This paper describes the production and use of EHV-1-specific monoclonal antibodies and the λgt11 expression vector system of Young and Davis to map the genes for the six major glycoprotein antigens of EHV-1.

MATERIALS AND METHODS

Virus and cell culture. The plaque-purified subtype 1 Army-183 strain of EHV-1 was propagated in equine dermal cells (KyED) as previously described (53). Extracellular virus was concentrated from EHV-1-infected cell cultures and purified by isopycnic banding in potassium tartrate gradients (53). Murine myeloma (NS-1) and hybridoma cells were propagated as described previously (59).

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† Paper no. 87-4-20 of the Kentucky Agricultural Experiment Station with approval by the director.

Production of monoclonal antibodies. BALB/c mice were given two intraperitoneal injections (14 days apart) of 300 μg of purified virus in OW3 adjuvant (Fort Dodge Laboratories, Fort Dodge, Iowa). Ten days after the last immunization, the mice were again injected intraperitoneally with 700 μg of virus in phosphate-buffered saline, and their spleens were harvested 3 days later.

Fusion of mouse spleen cells and NS-1 myeloma cells was performed as described by Nowinski et al. (34). Hybridoma culture supernatants were screened for viral antibody by an enzyme-linked immunosorbent assay with purified virus as antigen adsorbed onto the surface of 96-well plates. Hybridomas secreting EHV-1-specific monoclonal antibodies were cloned twice by limiting dilution and then inoculated into pristane-primed mice for preparation of antibody-containing ascites fluid.

Western blot (immunoblot) analysis. Purified virions of EHV-1 were dissociated by boiling for 2 min in a mixture containing 50 mM Tris (pH 7.5), 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 5% glycerol, and 0.001% bromophenol blue (dissociation buffer). After separation by electrophoresis in 7.5% polyacrylamide–0.1% SDS slab gels (53), the EHV-1 polypeptides were electrophoretically transferred to nitrocellulose membranes (51). Strips representing one lane of approximately 20 μg of blotted protein were cut from the nitrocellulose and then longitudinally sliced in half. After blocking of protein-binding sites with 10% normal goat serum–0.05% Tween 20, one half of each strip was incubated with a 1:1,000 dilution of rabbit antiserum against EHV-1 virions and the other half was incubated with a 1:500 dilution of monoclonal-antibody-containing mouse ascites fluid. The strips were washed and incubated with a 1:100 dilution of either biotinylated goat anti-rabbit or anti-mouse immunoglobulin G (Cooper Biomedical, Inc., West Chester, Pa.). Detection of EHV-1 polypeptides on the nitrocellulose reacting with either the polyclonal rabbit antiserum or individual monoclonal antibodies was then achieved by incubating the strips with an avidin-biotinylated peroxidase complex (Vectastain ABC reagent; Vector Laboratories, Inc., Burlingame, Calif.) and then with a solution of 0.05% 4-chloro-1-naphthol–0.01% H_2O_2.

Immunoprecipitation. Immunoprecipitation of radiolabeled polypeptide antigens by monoclonal antibodies was based on the method described by Pereira et al. (38). EHV-1 virions were purified from the culture fluids of infected cells incubated from 4 to 24 h after infection in medium containing 5 μCi of [^{3}H]glucosamine per ml (30 Ci/mmol; New England Nuclear Corp., Boston, Mass.). The envelope proteins were solubilized from purified virions by incubation for 1 h at 37°C in a buffer containing phosphate-buffered saline, 1% (vol/vol) Nonidet P-40, 1% sodium Desoxycholate (BBL Microbiology Systems, Cockeysville, Md.), and 4 mM phenylmethylsulfonyl fluoride. The mixture was centrifuged for 1 h at 30,000 × *g*, and the supernatant fluids (100 μl) were then preadsorbed with 5 mg of protein A-Sepharose (Pharmacia, Inc., Piscataway, N.J.) for 1 h on ice.

After removal of the protein A-Sepharose beads by centrifugation, the preadsorbed antigen was shaken overnight at 4°C with a monoclonal antibody-protein A-Sepharose complex formed by incubating 50 μl of antibody with 5 mg of protein A-Sepharose for 1 h on ice. The antigen-antibody-protein A-Sepharose bead complex was then washed four times with phosphate-buffered saline containing 0.1% Nonidet P-40 and 0.1% sodium Desoxycholate. Proteins were removed from the complex by the addition of dissociation buffer. The solubilized material was boiled for 2 min, electrophoresed in SDS-polyacrylamide gels, and prepared for fluorography (6, 53).

Construction and screening of λgt11 expression library. The methods detailed by Young et al. (60–62) were followed for construction of an expression library of EHV-1 DNA fragments in λgt11. All enzymes and oligonucleotide linkers were obtained from New England BioLabs, Inc., Beverly, Mass. *Escherichia coli* Y1090, λgt11 arms, and in vitro lambda-packaging extracts were products of Promega Biotech, Madison, Wis. EHV-1 DNA was isolated from purified virions as described previously (3) and sheared by sonication to produce fragments 200 to 500 base pairs in length as determined by electrophoresis in 1.5% agarose gels along with a *Hin*cII digest of ϕX174 DNA as size markers. After methylation of internal *Eco*RI sites with *Eco*RI methylase, the DNA fragments were made blunt ended with DNA polymerase I (Klenow fragment) and ligated to phosphorylated *Eco*RI linkers. After cleavage with *Eco*RI, excess linkers were removed by chromatography on Sephacryl-1000 (Pharmacia), and the EHV-1 DNA fragments were ligated to *Eco*RI-cleaved, dephosphorylated λgt11 DNA. The ligated recombinant DNA was packaged into phage heads and amplified by infection of *E. coli* Y1090. The percentage of phage with EHV-1 DNA inserts was determined by plating the library on Y1090 cells in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactoside and isopropyl-β-D-thiogalactoside (IPTG).

Screening of the λgt11 recombinant library with monoclonal antibody probes for EHV-1 antigen expression was performed as described by Young et al. (60). Briefly, nitrocellulose filter disks soaked in 10 mM IPTG were dropped onto 3.5-h phage plaques growing at 42°C on a lawn of Y1090 cells. After further incubation for 3.5 h at 37°C, the disks were removed, blocked by soaking in 10% goat serum–0.05% Tween 20 in phosphate-buffered saline, and then incubated in succession with monoclonal antibody, biotinylated goat anti-mouse immunoglobulin G, and Vectastain ABC reagent. The disks were then developed by being soaked in a solution of 4-chloro-1-naphthol–H_2O_2.

Southern blot analysis. EHV-1 DNA was isolated from purified virions by SDS-proteinase K digestion and phenol extraction and was then digested with *Bam*HI, *Eco*RI, and *Bgl*II restriction endonucleases as described previously (3, 4, 53). The growth and purification of λgt11 phage and the preparation of phage DNA were carried out as described by Helms et al. (24).

Restriction endonuclease fragments of EHV-1 virion DNA were separated by electrophoresis in 0.6% agarose gels and transferred to nitrocellulose by the method of Southern (47). Hybridization probes were prepared from EHV-1 DNA sequences represented in λgt11 recombinant phage expressing epitopes of EHV-1 glycoproteins. Each EHV-1 insert DNA was radiolabeled with [α-^{32}P]dCTP (600 Ci/mmol) and DNA polymerase I (Klenow fragment) after heat denaturation of the recombinant phage DNA and annealing to a λgt11 15-nucleotide primer complementary to the β-galactosidase gene 10 nucleotides upstream from the *Eco*RI site (New England BioLabs). The specific activity of the DNA probes was 1×10^8 to 5×10^8 cpm/μg.

The conditions for prehybridization, hybridization, washing, and autoradiography of Southern blots were those described by Meinkoth and Wahl (31). Briefly, annealing reactions were carried out for 20 h at 42°C in the presence of 50% formamide, 7.5% dextran sulfate, 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% SDS, 100 μg of denatured salmon sperm DNA per ml, and 1× Denhardt

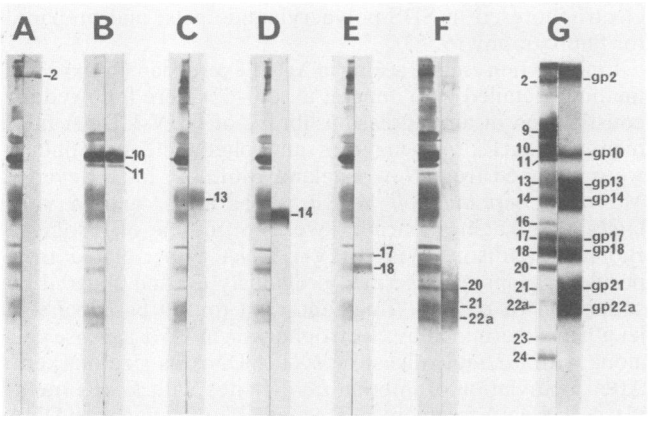

FIG. 1. Identification of the target polypeptide antigens of EHV-1-specific monoclonal antibodies by Western blot analysis. EHV-1 virion polypeptides were separated by electrophoresis in a 7.5% polyacrylamide gel (approximately 20 μg of protein per lane) and then electrophoretically transferred to nitrocellulose. In lanes A through F, the nitrocellulose blots were sliced longitudinally. The left half of each sliced blot was incubated with a polyclonal rabbit antiserum to EHV-1 virion proteins, and the right half was incubated with a monoclonal antibody to EHV-1 (1G12, 13A9, 14H7, 3F6, 5H6, or 13B2 for blots A through F, respectively). The patterns of polypeptide binding are shown for the six monoclonal antibodies selected for screening the λgt11 recombinant library. Lane G contains polypeptides of EHV-1 virions purified from cells infected in the presence of [^{3}H]glucosamine. The left half of the longitudinally sliced polyacrylamide gel was stained with Coomassie brilliant blue, and the right half was dried and subjected to fluorography (6).

solution (17). After a series of successive 30-min rinses at 65°C in 2× SSC, 1× SSC, 0.5× SSC, and 0.1× SSC (all containing 0.1% SDS) followed by 0.1× SSC without SDS, the nitrocellulose strips were dried and placed in contact with X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.) without intensifying screens for 2 to 4 h.

RESULTS

Preparation and characterization of monoclonal antibodies to EHV-1 glycoproteins. Twenty-four mouse hybridomas that stably produced monoclonal antibodies to EHV-1 (as detected by a microplate enzyme-linked immunosorbent assay) after single-cell cloning were expanded and used to prepare antibody-containing mouse ascites fluid. All 24 monoclonal antibodies reacted specifically with methanol-fixed EHV-1-infected cells in an immunofluorescence assay and with the surface of unfixed infected cells as detected by reactivity with anti-mouse immunoglobulin G coupled to horseradish peroxidase. Only one of the monoclonal antibodies exhibited virus-neutralizing activity, which was complement dependent. The immunoreactivity of three of the antibodies was virus subtype specific.

Identification of the target antigen specificity of the monoclonal antibodies was accomplished by incubating the antibodies with reduced and denatured EHV-1 virion polypeptides that had been transferred to nitrocellulose after electrophoresis in SDS-polyacrylamide gels. Only 5 of the 24 anti-EHV-1 monoclonal antibodies failed to react in Western blot analysis, which presumably indicated the existence of discontinuous, conformation-dependent target epitopes (5). Of the remaining 19 antibodies to EHV-1, 2 reacted with EHV-1 glycoprotein 2 (gp2), 2 with gp10, 3 with gp13, 2 with gp14, 8 with both gp17 and gp18, and 2 with a family of EHV-1 glycoproteins that included, in addition to the major glycoproteins gp21 and gp22a, a protein comigrating with EHV-1 capsid protein 20 (Fig. 1). The reactivity of a single monoclonal antibody with a family of immunologically related glycopolypeptides in the virions of herpesviruses has been noted for other herpesviruses (18, 23, 26, 54).

A pool of six monoclonal antibodies (1G12, 13A9, 14H7, 3F6, 5H6, and 13B2) was assembled for screening the λgt11–EHV-1 recombinant library for expression of EHV-1 glycoprotein epitopes. The antibody pool was composed of one monoclonal antibody directed against each of the six high-abundance glycoproteins of EHV-1. The individual antibodies selected for the immunoscreening pool were those exhibiting the strongest reactivity in Western blot analysis (Fig. 1).

To verify that the anti-EHV-1 monoclonal antibodies in the pool were directed against authentic viral glycoproteins rather than against other virion polypeptides that comigrate in SDS-polyacrylamide gels with EHV-1 glycoproteins, the six antibodies were examined for their ability to immunoprecipitate [^{3}H]glucosamine-labeled polypeptides from detergent extracts of purified virions. Anti-EHV-1 monoclonal antibodies 1G12, 13A9, 14H7, 3F6, 5H6, and 13B2 precipitated different glycosylated virion polypeptides that corresponded to each of the six major glycoproteins of the EHV-1 virion (Fig. 2).

Construction of λgt11 library expressing EHV-1 glycoprotein determinants. The *Eco*RI site in the β-galactosidase gene of λgt11 was used to construct an expression library of randomly sheared EHV-1 DNA fragments. *Eco*RI linkers were added to the ends of the DNA fragments to allow insertion into the unique *Eco*RI site of λgt11 and subsequent expression of the insert DNA as a β-galactosidase fusion protein (61).

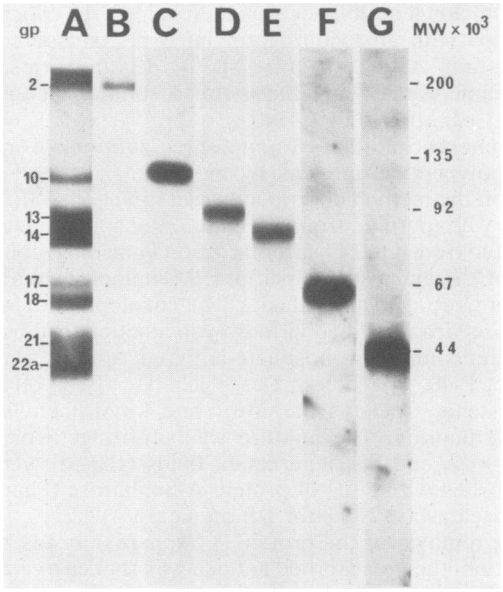

FIG. 2. Autoradiographic images of electrophoretically separated [^{3}H]glucosamine-labeled virion polypeptides of EHV-1 immunoprecipitated by monoclonal antibodies 1G12, 13A9, 14H7, 3F6, 5H6, and 13B2 (lanes B through G, respectively). In lane A, the [^{3}H]glucosamine-labeled polypeptides were electrophoresed without immunoprecipitation. The glycoproteins of the virion are designated on the left. The positions of molecular weight (MW) markers in the gel are shown at the right.

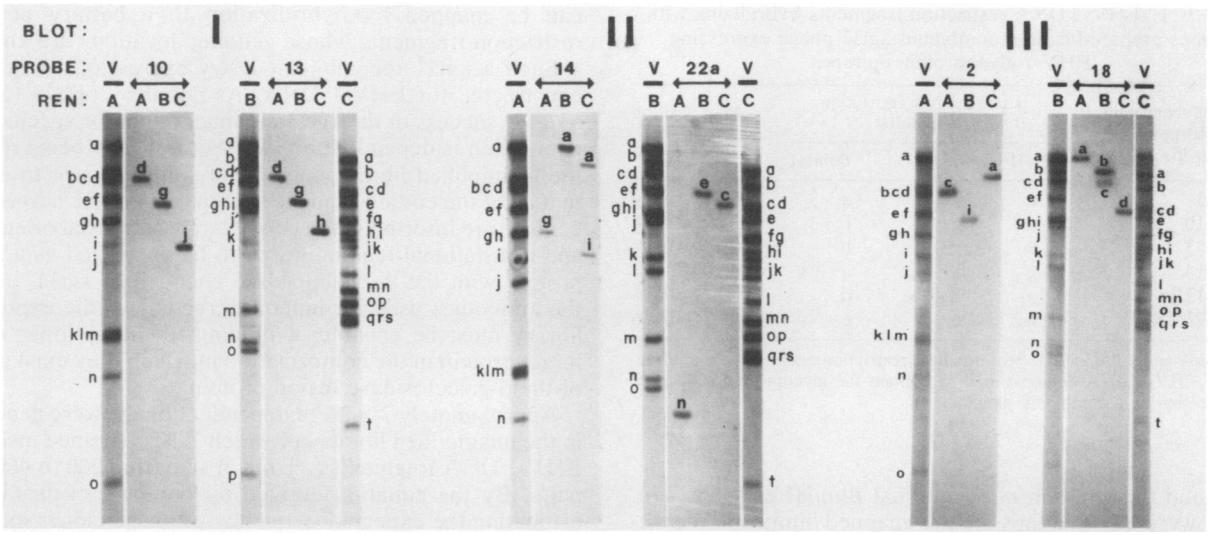

FIG. 3. Autoradiograms resulting from hybridization of restriction fragments of EHV-1 DNA with radiolabeled probes prepared from recombinant λgt11 phage expressing epitopes on EHV-1 glycoproteins. EHV-1 virion DNA was electrophoresed in 0.6% agarose slab gels and then blotted to nitrocellulose after digestion with either *Bgl*II (lanes A), *Eco*RI (lanes B), or *Bam*HI (lanes C). The nitrocellulose was cut into strips and incubated under stringent hybridization conditions with ^{32}P-labeled probe DNA prepared from either EHV-1 virions (V) or recombinant λgt11 phage containing insert DNA sequences encoding epitopes on EHV-1 gp2, gp10, gp13, gp14, gp17/18, or gp21/22a (labeled as probes 2, 10, 13, 14, 18, and 22a, respectively). After being washed and dried, the nitrocellulose strips were realigned and exposed to X-ray film for visualization of the hybridizing restriction fragments. a to s, DNA fragments.

The EHV-1 DNA fragment library constructed in this manner contained 7×10^5 individual phage before amplification. Of these phage, 70% were recombinants containing EHV-1 DNA inserts between 200 and 400 base pairs in length as determined by *Eco*RI digestion and agarose gel electrophoresis of DNA prepared from 12 independent recombinant phage clones. For immunoscreening, the library was amplified in *E. coli* Y1090 by producing a plate stock (61) whose titer was 10^{10} PFU/ml.

Immunoscreening of λgt11 library with monoclonal antibody probes. For primary screening, a pool of six monoclonal antibodies reacting with individual EHV-1 glycoproteins was used to probe 20 85-mm nitrocellulose filters, each containing antigen blotted from approximately 2×10^4 individual phage plaques induced with IPTG to express the β-galactosidase gene. Seventy-four clones expressing antigen reactive with the antibody pool were identified. All 74 phage were isolated and successfully carried through two successive screens with the antibody pool with approximately 100 phage per plate. No positive signals were detected when the same antibody pool was used to screen 10^5 λgt11 phage without inserts.

Recombinant phage isolated in this manner and expressing antigenic determinants of EHV-1 glycoproteins were then arrayed as 1-μl drops onto lawns of *E. coli* Y1090, induced to express β-galactosidase, and probed individually with each of the six monoclonal antibodies that made up the original screening pool. Of the immunoreactive phage clones, 10 were recognized by anti-gp2 (1G12), 14 by anti-gp10 (13A9), 16 by anti-gp13 (14H7), 18 by anti-gp14 (3F6), 10 by anti-gp17/18 (5H6), and 4 by anti-gp21/22a (13B2) monoclonal antibody. Positive signals were obtained for each of the 74 phage clones and for each of the six monoclonal antibodies to EHV-1 glycoproteins. No phage clone reacted with more than one monoclonal antibody in the pool.

Mapping the location of the genes for EHV-1 glycoproteins with immunoreactive recombinant phage DNA as hybridization probes. Two recombinant phage expressing epitopes present on each of the six major EHV-1 glycoproteins (i.e., gp2, gp10, gp13, gp14, gp17/18, and gp21/22a) were selected for preparation of hybridization probes. The EHV-1 insert DNA in each of the 12 recombinant phage was radiolabeled with [α-^{32}P]dCTP and Klenow enzyme after the phage DNA was denatured and annealed to a λgt11-specific primer complementary to the β-galactosidase gene.

The ^{32}P-labeled probes were used for hybridization to restriction endonuclease-generated, Southern-blotted fragments of EHV-1 virion DNA. The restriction enzymes chosen for the hybridization analysis (*Bam*HI, *Eco*RI, and *Bgl*II) were those for which cleavage maps have been determined and published (57). The Army-183 strain of EHV-1 used in this investigation possesses restriction endonuclease cleavage profiles with *Bam*HI, *Eco*RI, and *Bgl*II enzymes that are identical to those of the EHV-1 strain (HVS-25) for which cleavage maps are available (4, 57).

Figure 3 illustrates the EHV-1 DNA restriction fragments yielding positive hybridization signals with the probes used. Probes prepared from each clone of a pair of recombinant phage expressing determinants on a given EHV-1 glycoprotein gave identical hybridization results. With the exception of gp14 probes (whose sequences span the junction between *Bam*HI fragments a and i) and gp17/18 probes (complementary to *Eco*RI fragments containing repeat sequences), none of the probes hybridized to more than one restriction fragment generated by a single endonuclease. Table 1 summarizes the results of hybridization of 12 λgt11–EHV-1 probes to nitrocellulose blots of 36 restriction endonuclease digests of EHV-1 virion DNA. The map positions given are those for the smallest restriction endonuclease subfragment of EHV-1 DNA demonstrated to encompass the glycoprotein sequences expressed by the recombinant phage. For example, the maximal left and right map limits of the EHV-1 insert sequences expressing gp10 epitopes are defined by the *Eco*RI o-g junction and the *Bam*HI j-h junction, respectively. The left limit of expressed gp14 sequences was positioned from a knowledge of the size of the expressed

TABLE 1. EHV-1 DNA restriction fragments hybridizing with probes prepared from recombinant λgt11 phage expressing EHV-1 glycoprotein epitopes

EHV-1 glycoprotein expressed by phage probe	EHV-1 DNA restriction fragment(s)			Map position of insert sequences[a]
	*Bgl*II	*Eco*RI	*Bam*HI	
gp2	c	i	a	0.279–0.344
gp10	d	g	j	0.093–0.114
gp13	d	g	h	0.114–0.148
gp14	g	a	a + i	0.403–0.413
gp17/18	a	b + c	d	0.841–0.874
gp21/22a	n	e	c	0.615–0.636

[a] Expressed as the limits of the smallest restriction endonuclease subfragment of EHV-1 DNA demonstrated to contain the glycoprotein sequences expressed by the recombinant phage.

insert and the location of its internal *Bam*HI cleavage site. There were no overlaps in the mapped limits of the sequences expressed from each of the six EHV-1 glycoproteins.

The map locations of the restriction subfragments hybridizing to probes prepared from the recombinant phage expressing the six major glycoproteins of EHV-1 are shown in Fig. 4. Epitopes of five of the six EHV-1 glycoproteins (gp2, gp10, gp13, gp14, and gp21/22a) mapped to the L component of the viral genome. Only one glycoprotein (gp17/18) mapped to the unique S component. If it is assumed that the EHV-1 genome L component (as depicted in the maps of Fig. 4) is genetically colinear with that of the I_L isomer of herpes simplex virus (HSV) DNA, as indicated by the studies reported by Davison and Wilke (16), then two EHV-1 glycoproteins (gp14 and gp13) mapped to genomic positions colinear with those encoding gB and gC, respectively, of HSV (12, 21, 49).

DISCUSSION

Previous analyses of purified EHV-1 virions revealed the presence of at least 11 glycoproteins (37, 52), but no data have been reported which localize the regions of EHV-1 DNA that encode these glycoproteins. Results of recent studies indicate that the six glycoproteins of EHV-1 focused on in the present study (gp2, gp10, gp13, gp14, gp17/18, and gp21/22a) are important antigenic components of the virus (reviewed in reference 2). They represent the six most abundant glycosylated polypeptides of the EHV-1 virion. Their importance in stimulating an immune response in the natural host for EHV-1 is supported by the demonstration of immunoprecipitating antibodies directed against all six glycoproteins in convalescent equine serum. Furthermore, most of the EHV-1-specific polyclonal antibody activity present in rabbit and mouse sera after immunization with purified virions is directed against these same six glycoproteins of EHV-1 (Turtinen, Ph.D. thesis). In light of these facts, it seemed important to focus on localizing the genetic information for these major EHV-1 glycoproteins.

Direct expression of DNA fragments cloned into the unique *Eco*RI site of the β-galactosidase gene of λgt11 was developed by Young and Davis (61) as a powerful means for localizing genes that encode proteins for which specific antibodies are available. Recombinant phage plaques synthesizing fusion proteins are detected by their reactivity with antibody to the desired protein, and the foreign insert DNA can be mapped by hybridization to a battery of DNA restriction fragments whose genomic locations are known.

Such a λgt11 recombinant DNA expression library was constructed for EHV-1 DNA. As noted by others (50, 60, 63), the success of the λgt11 cloning system for specific gene expression is dependent on two requirements being met: (i) the unamplified library must be of sufficient size to ensure that all of the coding sequences of interest in the herpesvirus genome are inserted in the correct transcriptional orientation and translational reading frame to be expressed as a fusion protein with the β-galactosidase encoded in λgt11, and (ii) the antibodies used for immunoscreening of the expression library must be capable of binding to the epitopes of the target protein in the conformation in which they exist as part of the β-galactosidase fusion protein.

Approximately 7×10^5 independent phage were generated in the unamplified library, of which 70% contained inserts of EHV-1 DNA fragments ranging in size from 200 to 400 base pairs. By the equation derived by Nunberg et al. (35) for estimating the expected frequency of phage clones expressing any specific determinant, the number of phage from the expression library screened in this study (approximately 4×10^5) would be expected to yield 40 independent phage expressing epitopes on each of the six EHV-1 glycoproteins. From the 4×10^5 phage screened, the number of phage reactive with monoclonal antibodies to each of the six major glycoproteins of EHV-1 ranged from 4 to 18.

In regard to meeting the tactical requirement for appropriate antibody quality (39, 50), the monoclonal antibodies used for immunoscreening were selected on the basis of their strong reactivity with EHV-1 glycoproteins in the denaturing environment of the Western blotting assay. It appears likely that the target epitopes of such monoclonal antibodies are continuous epitopes and are therefore recognized by the screening antibodies even in the unnatural conformation imposed by their linkage to the λgt11 β-galactosidase protein (39).

The genetic information for the six glycoprotein determinants expressed in λgt11 was unambiguously localized to the EHV-1 genome by using hybridization analysis and available restriction maps of EHV-1 DNA. The EHV-1 genome has two covalently linked components, L and S, of which only the S component is bounded by inverted repeat sequences

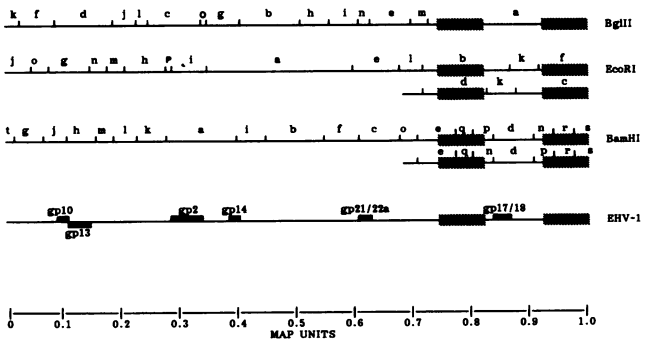

FIG. 4. Physical map of the EHV-1 genome showing the locations of genes encoding the major viral glycoproteins. The structural organization of the genome and the locations of cleavage sites for *Bgl*II, *Eco*RI, and *Bam*HI are those reported by Whalley and co-workers (57). ▨, Proposed repetitive sequences, with the two orientations of this end of the genome shown; ▬, smallest regions of the genome demonstrated by the current studies to contain the EHV-1 glycoprotein sequences expressed by λgt11 recombinants.

(25, 57). Restriction endonuclease linkage maps have been reported for the enzymes *Bam*HI, *Eco*RI, and *Bgl*II (57). The coding sequences for the epitopes recognized by the six monoclonal antibodies were positioned within genomic regions ranging in size from 0.01 to 0.06 map units (1.5 to 9.0 kilobases). Sequences expressing each of the six EHV-1 glycoprotein determinants were localized, without overlap, to separate loci on the viral genome. Inserts expressing gp10 and gp13 epitopes were mapped to contiguous *Bam*HI restriction fragments (j and h). The coding sequences mapped in this study appear to represent genes for six primary translation products that account for 8 of the 11 glycopolypeptides that have been identified in the virion of EHV-1.

Comparison of the map positions determined in this study for the six major glycoproteins of EHV-1 with those reported for the glycoproteins of several other alphaherpesviruses (e.g., HSV, pseudorabies virus, varicella-zoster virus, and bovine herpesvirus 1) revealed both similarities and differences (1, 11, 12, 20, 22, 26, 28, 29, 39, 41, 42, 44, 55, 56). The general pattern of glycoprotein mapping exhibited by other alphaherpesviruses is one with two major glycoproteins (analogs of HSV gB and gC) mapping to the L component of the genome with the remainder encoded from genes clustered in the unique S region. Genes for two major EHV-1 glycoproteins, gp14 and gp13, were located to the same positions within the L component of the genome to which gB and gC analogs, respectively, of other alphaherpesviruses have been mapped. The gene for gp14 was located near the middle and the gene for gp13 was located approximately 0.1 map unit from one end of the genome L component (12, 21, 26–28, 32, 43). The genetic colinearity of sequences encoding EHV-1 gp14 and gB analogs of other herpesviruses was expected on the basis of previous reports of serologic cross-reactivity among these herpesvirus glycoproteins (19, 45, 46). However, confirmation that EHV-1 gp13 is structurally homologous to the gC analogs of other alphaherpesviruses must await the results of DNA sequence analysis. To that end, determination of the nucleotide sequence of the restriction fragment encompassing the EHV-1 gp13 gene is under way.

EHV-1 appears unique among the alphaherpesviruses whose glycoprotein genes have been mapped in that five of its six major glycoproteins are encoded from sequences within the genome L component, while only one (gp17/18) mapped to the U_S region. Likewise, none of the six EHV-1 glycoproteins examined mapped to the L component region corresponding to the coding sequences of the minor HSV glycoprotein gH, i.e., to 0.51 to 0.55 map units on the I_L isomer (11, 22). Two predictions are suggested by this observation: (i) that some of the low-abundance glycoproteins identified in EHV-1 virions as well as EHV-1 glycoproteins not yet identified might map to the S component of the genome and (ii) that additional unidentified glycoproteins from the L component of other alphaherpesviruses might exist. Relevant to this point is the conclusion by McGeoch et al. (30) that, on the basis of DNA sequence data, there are additional HSV glycoproteins to be characterized.

Although a major glycoprotein of EHV-1 (gp17/18) mapped to the U_S region of the genome, it is not possible to predict from information currently available what its structural counterpart might be among the several glycoproteins of other alphaherpesviruses encoded from the S region.

Because the structural relationships of EHV-1 glycoproteins with the glycoproteins of other alphaherpesviruses have not yet been completely determined, no attempt has been made in this report to devise a systematic nomenclature for the glycoproteins of EHV-1 (e.g., gA to gF or gI to gVI) beyond their association with previously identified structural polypeptides of the EHV-1 virion (37, 52).

The results reported in this paper provide new information on the glycoproteins of EHV-1 which may be applied toward the molecular characterization of the important protective antigens of this herpesviral pathogen.

ACKNOWLEDGMENTS

These investigations were supported by grants from The Grayson Foundation, Inc., and from the U.S. Department of Agriculture.

We thank Gregory Stratton for excellent technical assistance and Larry Arthur for providing the NS-1 mouse myeloma cell line.

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