Nucleotide sequence of the genes encoding the canine herpesvirus gB, gC and gD homologues

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The nucleotide sequence of the genes encoding the canine herpesvirus (CHV) gB, gC and gD homologues was determined. These genes are predicted to encode polypeptides of 879, 459 and 345 amino acids, respectively. Comparison of the predicted amino acid sequences of CHV gB, gC and gD with the homologous sequences from other herpesviruses indicates that CHV is an alphaherpesvirus, a conclusion that is consistent with the previous classification of this virus according to biological properties. Alignment of the homologous gB, gC and gD amino acid sequences indicates that most of the cysteine residues are conserved, suggesting that these glycoproteins possess similar tertiary structures. The nucleotide sequence of the open reading frame downstream from the CHV gC gene was also determined. The predicted amino acid sequence of this putative polypeptide appears to be homologous to a family of proteins encoded downstream from the gC gene in most, although not all, alphaherpesviruses.

Introduction

Canine herpesvirus (CHV) causes a fatal haemorrhagic disease in neonatal puppies and a self-limiting, usually subclinical, upper respiratory tract infection in adult dogs (Appel, 1987). Little is known about the genomic structure of CHV. Restriction enzyme maps of the CHV genome and nucleotide sequence of CHV genes have not been published. In particular, genes encoding immunologically pertinent proteins have not been characterized.

During a herpesvirus infection, the majority of the immune response is directed against the viral envelope glycoproteins. These antigens have been shown to elicit both humoral and cellular immune responses. The contribution of the individual glycoproteins in eliciting a protective immune response, however, is not completely understood. Several reports have indicated that immunization with the herpesvirus gB, gC and/or gD glycoproteins can induce a protective immune response. For example, immunization with purified bovine herpesvirus type 1 (BHV-1) gI (gB), gIII (gC) and/or gIV (gD) has been shown to protect cattle against a BHV-1/Pasteurella haemolytica challenge (Babiuk et al., 1987). In addition, immunization with a fowlpox virus recombinant expressing Marek's disease virus (MDV) gB has been shown to protect chickens against a virulent MDV challenge (Nazarian et al., 1992). Immunization with NYVAC (highly attenuated vaccinia virus) recombinants expressing pseudorabies virus (PRV) gII (gB) or gp50 (gD) have also been shown to protect swine against a virulent PRV challenge (Brockmeier et al., 1993). Furthermore, vaccinia virus recombinants expressing PRV gII and gp50, or gII, gIII (gC) and gp50 have been shown to elicit a higher level of protection than recombinants expressing gII or gp50 alone, suggesting a potential synergistic effect with these glycoproteins (Riviere et al., 1992). The results of these studies indicate that an immune response against gB, gC and/or gD can protect target species animals against a herpesvirus challenge.

In this communication, we report the nucleotide sequence of the genes encoding the CHV gB, gC and gD homologues. This information will form a database upon which potential CHV vaccine candidates can be developed.

Methods

Preparation of genomic CHV DNA. CHV (obtained from L. Carmichael, Cornell University, Ithaca, N.Y., U.S.A.) was propagated on Madin-Darby canine kidney (MDCK) cells (ATCC CCL34). Viral DNA was isolated using standard methods (Tartaglia et al., 1990).

DNA hybridization. CHV genomic DNA was digested with restriction enzymes, run on agarose gels and transferred to Gene-Screen membranes (New England Nuclear) under conditions recommended by the manufacturers. Hybridizations were performed at 44 °C, 53 °C or 59 °C in 1M-NaCl, 1% SDS and 10% dextran sulphate. The hybridization probe included a 1800bp BamHI-XbaI fragment, containing an internal segment of the feline herpesvirus (FHV) gB gene (Maeda et al., 1992), a 950 bp BamHI–EcoRI fragment containing the 3’ end of the FHV gC gene (J.-C. Audonnet, unpublished results), and
Cloning and DNA sequencing. CHV genomic fragments were subcloned into pBluescript SK (Stratagene). Plasmid DNA was prepared and manipulated using standard techniques (Maniatis et al., 1982). Nucleotide sequencing was performed on both strands with double-stranded plasmid templates, using the modified T7 DNA polymerase, Sequenase Version 2.0 (U.S. Biochemical Corporation), and standard protocols recommended by the manufacturer. M13 forward and reverse primers were used to obtain initial sequence; and custom primers, prepared with a Biosearch 8700 or an Applied Biosystems 380B oligonucleotide synthesizer, were used for subsequent reactions.

DNA and amino acid sequence analyses. DNA and amino acid sequence analyses were performed with PC/GENE (IntelliGenetics), ALIGN Plus (Scientific and Educational Software) and IBI-Pustell (International Biotechnologies) software packages. Homology searches were conducted on the SWISS-PROT (Release 20 and 23) (IntelliGenetics) databases, using the FASTA program (Pearson & Lipman, 1988).

Results
Identification and sequencing of the CHV gB gene

Hybridization of restriction enzyme-digested CHV genomic DNA with a radiolabelled probe containing the FHV gB, gC and gD genes (Maeda et al., 1992; J.-C. Audonnet, unpublished results) identified one complementary sequence (data not shown). A 6 kb XbaI fragment containing this sequence was cloned into pBluescript SK and the nucleotide sequence of the hybridizing region was determined (Fig. 1).

An open reading frame (ORF) starting at position 201 and ending at position 2840 was identified. The predicted translation product of this ORF is 879 amino acids long. Comparison of this amino acid sequence with the gB glycoprotein of numerous herpesviruses. Additional analyses revealed that the predicted CHV gB gene product was more similar to the gB glycoprotein of alphaherpesviruses, such as herpes simplex virus type 1 (HSV-1), than those of beta- or gammaherpesviruses, such as human cytomegalovirus (HCMV) or Epstein–Barr virus (EBV) (Table 1). These results indicate that CHV should be classified as an alphaherpesvirus, a conclusion that is consistent with the previous classification of this virus according to biological properties (Carmichael et al., 1965; Roizman, 1982).

Analysis of the CHV gB nucleotide sequence

The 5' and 3' non-coding regions of the CHV gB gene contain numerous RNA polymerase II regulatory sequence motifs, such as TATA box, CAAT box and polyadenylation signal sequences (Corden et al., 1980; Proudfoot & Brownlee, 1976) (Fig. 1).

The nucleotide sequence surrounding the initiation codon has been shown to affect the efficiency of translation initiation (Kozak, 1986). In particular, the sequence (A/G)NNATGG has been found to be most efficient. Therefore, according to Kozak's rules, the nucleotide sequence surrounding the CHV gB initiation codon (AGTATGT) is favourable at position –3, but not at position +4 (Fig. 1). The fact that the CHV gB gene does not follow Kozak's rules is not unusual. The FHV (Maeda et al., 1992), PRV (Robbins et al., 1987), varicella-zoster virus (VZV) (Keller et al., 1986), MDV (Ross et al., 1989) and HSV-1 (Bzik et al., 1984) gB genes also contain a pyrimidine at position +4.

Analysis of the predicted CHV gB amino acid sequence

The deduced amino acid sequence of the CHV gB homologue is presented in Fig. 1. Hydropathicity analysis of this amino acid sequence revealed the presence of two prominent hydrophobic peaks (data not shown). The first peak, located at the N terminus, may represent a potential signal sequence. N-terminal signal sequences initiate transport across the endoplasmic reticulum membrane and are critical for the proper post-translational modification and targeting of glycoproteins (Blobel, 1980). Signal sequences vary in length from about 15 to 30 residues and usually consist of a basic N-terminal region, a central hydrophobic region and a short, relatively polar C-terminal region. In addition, the cleavage site usually conforms to the −3, −1 rule, where the residue at position −1 is small (Ala, Ser, Gly, Cys, Thr or Gin) and the residue at position −3 is not aromatic (Phe, His, Tyr or Trp), charged (Asp, Glu, Lys or Arg) or large and polar (Asn or Gln), and residues −3 to +1 are not Pro residues (von Heijne, 1986). Although analysis with PSIGNAL, a PC/GENE program designed to detect eukaryotic signal sequences, did not identify the N terminus of CHV gB as a potential signal sequence, this region does have elements consistent with typical signal sequences, namely a hydrophobic core (residues 2 to 17) and a relatively polar C-terminal region (Fig. 1). That PSIGNAL does not detect a signal sequence in the N-terminal region of CHV gB is not unique. This algorithm also fails to detect a signal sequence in the N-terminal region of the VZV gB homologue.

The second, very broad, hydrophobic peak is located near the C terminus, with predicted membrane-spanning segments between amino acid residues 725 and 741, and 747 and 771 (using the method of Klein et al., 1985), and probably functions as a membrane anchor region. It has been hypothesized that the transmembrane domain of HSV-1 gB, as well as other gB homologues, traverses the
Fig. 1. Nucleotide sequence and predicted amino acid sequence of the CHV gB homologue. The putative cleavage site, transmembrane regions, potential glycosylation sites, TATA box, CAAT box and polyadenylation signal sequences are underlined. Nucleotides and predicted amino acid residues are numbered to the right of the sequence.
Table 1. Homology between the predicted amino acid sequences of 10 herpesvirus gB glycoproteins

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* Values were obtained using the ALIGN Plus program and are expressed as percentage identity. The entire gB amino acid sequence was used. The alignment parameters were: mismatch penalty = 2, open gap penalty = 4, extended gap penalty = 1. References: FHV (Maeda et al., 1992), EHV-1 (Whalley et al., 1989), PRV (Robbins et al., 1987), BHV-1 (Whitbeck et al., 1988), VZV (Keller et al., 1986), MDV (Ross et al., 1989), HSV-1 (Bzik et al., 1984), HCMV (Kouzarides et al., 1987) and EBV (Pellett et al., 1985).

membrane three times (Pellett et al., 1985). As hydrophobicity analysis of the C-terminal region of CHV gB reveals the presence of at least two membrane-spanning regions, it is possible that CHV gB and HSV-1 gB have similar transmembrane structures.

Alignment of the CHV gB amino acid sequence with similar sequences from other herpesviruses revealed extensive homology throughout the entire sequence, with the exception of the N terminus, a region surrounding the putative cleavage site (see below) and a region near the C terminus (data not shown). This alignment also revealed that most of the cysteine residues are conserved. For example, CHV gB contains 11 cysteine residues, 10 of which are conserved in all alpha-, beta- and gammaherpesviruses. In fact, the only cysteine residue in CHV gB that is not conserved is found near the N terminus and may be located in the putative signal sequence. These results suggest that the gB glycoproteins may have similar tertiary structures.

N-linked oligosaccharides can be added to Asn residues which have the sequence Asn-X-Ser/Thr, where X is not a Pro residue (Bause, 1983). Alignment of the CHV gB amino acid sequence with other gB sequences revealed that potential N-linked glycosylation sites are relatively well conserved (data not shown). It is not known whether glycosylation at these sites is structurally or functionally significant.

The gB glycoprotein of most herpesviruses is cleaved internally during maturation, with the subsequent peptides being held together by disulphide bonds. The VZV gB homologue (gpII), for example, is cleaved between Arg and Ser residues, resulting in two glycoproteins of approximately 60K (Keller et al., 1986). The gB glycoproteins of FHV (Maeda et al., 1992), equine herpesvirus type 1 (EHV-1) (Whalley et al., 1989), PRV (Robbins et al., 1987), BHV-1 (Whitbeck et al., 1988), MDV (Ross et al., 1989) and HCMV (Kouzarides et al., 1987) are also cleaved. Furthermore, a sequence, Arg-X-Arg-Arg/Lys--Ser/Ala, similar to the sequence at the VZV cleavage site, Arg-Thr-Arg-Arg--Ser, is present at virtually the same location in each of these gB glycoproteins. Conversely, this sequence is not found in the HSV-1 (Bzik et al., 1984) and EBV (Pellett et al., 1985) gB glycoproteins, which are not cleaved. The significance of this cleavage event is unknown. It does not appear, however, to be essential for replication in vitro, as strains of BHV-1 (Blewett & Misra, 1991) and HCMV (Spaete et al., 1990) that have been mutated at the cleavage site, and therefore encode an uncleavable gB glycoprotein, are still infectious. It is not known whether CHV gB is cleaved internally. However, the sequence Arg-Lys-Arg-Arg--Ser is present at approximately the same location in CHV as in VZV, FHV, EHV-1, PRV, BHV-1, MDV and HCMV (data not shown). Therefore, it is probable that CHV gB is proteolytically cleaved.

Identification and sequencing of the CHV gC gene

A radiolabelled probe containing the 3' end of the FHV gC (and gD) gene(s) did not hybridize to restriction enzyme fragments of CHV genomic DNA (see above). Therefore another method of analysis was used to identify the CHV gC (and gD) gene(s). CHV genomic fragments were randomly cloned into pBluescript SK. The nucleotide sequences of the termini of these fragments were determined and the predicted amino acid sequences of potential ORFs were analysed for homology against the SWISS-PROT (Release 20) amino acid database. Using this method, a 12 kb XbaI fragment encoding an ORF with homology to herpesvirus gC glycoproteins was identified. The nucleotide sequence of this ORF is presented in Fig. 2. The putative CHV gC...
Sequence of CHV gB, gC and gD homologues

The deduced amino acid sequence of the CHV gC homologue is presented in Fig. 2. Hydropathicity gene starts at position 201 and ends at position 1580. The predicted translation product is 459 amino acids long. Comparison of this amino acid sequence with that of gC glycoproteins from other herpesviruses revealed extensive homology, indicating that this ORF encodes the CHV gC homologue (Table 2).

Analysis of the CHV gC nucleotide sequence

Numerous potential TATA box, CAAT box and polyadenylation signal sequences are found in the 5' and 3' non-coding regions of the CHV gC gene (Fig. 2). The nucleotide sequence surrounding the CHV gC initiation codon (AAAATGA) is favourable with respect to Kozak's rules at position -3, but not at position +4. That the CHV gC gene does not follow Kozak's rules is not unusual, as the CHV gB gene (see above), as well as the gC genes of FHV (J.-C. Audonnet, unpublished results), EHV-I (Allen & Coogle, 1988) and VZV (Davison & Scott, 1986) also contain an unfavourable nucleotide at position +4.

Analysis of the predicted CHV gC amino acid sequence

The deduced amino acid sequence of the CHV gC homologue is presented in Fig. 2. Hydropathicity
Table 2. Homology between the predicted amino acid sequences of nine herpesvirus gC glycoproteins*

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* Values were obtained using the ALIGN Plus program and are expressed as percentage identity. The entire gC amino acid sequence was used. See Table 1 for alignment parameters. References: FHV (J.-C. Audonnet, unpublished results), EHV-1 (Allen & Coogle, 1988), EHV-4 (Nicolson & Onions, 1990), PRV (Robbins et al., 1986), BHV-1 (Fitzpatrick et al., 1989), VZV (Davidson & Scott, 1986), MDV (Ihara et al., 1989) and HSV-1 (McGeoch et al., 1988).

Table 3. Homology between the predicted amino acid sequences of the ORFs located adjacent to the gC gene in eight herpesviruses*

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* Values were obtained using the FASTA and RDF2 programs (Pearson & Lipman, 1988). A k-tup of 1 was used. Values in parentheses represent the number of standard deviations between the FASTA score and the mean of the scores obtained from 100 randomly permuted versions of the potentially related sequence. References: FHV (J.-C. Audonnet, unpublished results), EHV-1 (Telford et al., 1992), EHV-4 (Nicolson & Onions, 1990), MDV (Ihara et al., 1989), HTV (Kato et al., 1989), HSV-1 (McGeoch et al., 1988) and VZV (Davidson & Scott, 1986).

Analysis of the predicted CHV gC amino acid sequence revealed the presence of two prominent hydrophobic peaks (data not shown). The first peak, located at the N terminus, may represent a potential signal sequence. However, analysis with PSIGNAL did not identify the N terminus of this polypeptide as a potential signal sequence. Nevertheless, this region does contain elements of a typical signal sequence, namely a basic N-terminal region, a hydrophobic core (residues 6 to 20) and a relatively polar C-terminal region (Fig. 2). The second hydrophobic peak, located at the C terminus, with a predicted membrane-spanning segment between residues 426 and 451 (using the method of Klein et al., 1985), probably functions as a membrane anchor region.

Alignment of the CHV gC amino acid sequence with homologous sequences from other herpesviruses revealed a moderate level of similarity throughout the entire sequence, with the exception of the N terminus (data not shown). This alignment also revealed that most of the cysteine residues are conserved. For example, CHV gC contains 10 cysteine residues, eight of which are conserved in all alphaherpesviruses. The only cysteine residues in CHV gC that are not conserved are located in the putative transmembrane or intracellular domains. These results suggest that the gC glycoproteins may have similar tertiary structures. Alignment with other gC sequences also revealed the relative conservation of potential N-linked glycosylation sites. The significance of this conservation relative to the structure and function of the gC CHV glycoprotein is not known.

Identification and sequencing of ORF 2

Nucleotide sequence analysis of the region downstream from the CHV gC gene revealed the presence of a second ORF (Fig. 2). This ORF (ORF 2) starts at position 1699 and ends at position 2226. The predicted translation product is 175 amino acids long. Comparison of this amino acid sequence with the SWISS-PROT (Release 23) database revealed homology with ORFs located downstream from other alphaherpesvirus gC genes (Table 3). These homology scores suggest that the ORFs located...
downstream from the gC gene in CHV, FHV, EHV-1, EHV-4, MDV, herpesvirus of turkey (HVT) and possibly HSV-1 are evolutionarily related. Conversely, the ORF (gene 13) located next to the VZV gC gene does not exhibit significant homology with any of the other comparably positioned ORFs. Furthermore, VZV gene 13 is oriented on the genome in the opposite direction relative to all the other ORF 2-like genes (Davison & Scott, 1986). These results are consistent with the proposed functions of the proteins encoded by these two groups of genes; VZV gene 13 encodes a thymidylate synthetase (Davison & Scott, 1986), whereas the HSV-1 ORF 2-like gene (UL45) encodes a putative virion protein (Visalli & Brandt, 1993). Therefore, it appears that the ORF located next to the gC gene in CHV, FHV, EHV-1, EHV-4, MDV, HVT and possibly HSV-1 encodes a protein that is structurally and functionally unrelated to the protein encoded downstream from the VZV gC homologue.

Analysis of the CHV ORF 2 nucleotide sequence

Numerous potential TATA box, CAAT box and polyadenylation signal sequences are found in the 5' and 3' non-coding regions of ORF 2 (Fig. 2). The nucleotide sequence surrounding the ORF 2 initiation codon (AATATGG) is favourable with respect to Kozak’s rules at positions -3 and +4.

Identification and sequencing of the CHV gD gene

Employing the same method used to map the CHV gC homologue, a 7 kb PstI fragment encoding an ORF with homology to herpesvirus gD glycoproteins was identified. The nucleotide sequence of this ORF is presented in Fig. 3. The putative CHV gD gene starts at position 201 and ends at position 1238. The predicted translation product is 345 amino acids long. Comparison of this amino acid sequence with the sequence of other gD glycoproteins revealed extensive homology, indicating that this ORF encodes the CHV gD homologue (Table 4).

Table 4. Homology between the predicted amino acid sequences of six herpesvirus gD glycoproteins*

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* Values were obtained using the ALIGN Plus program and are expressed as percentage identity. The entire gD amino acid sequence was used. See Table 1 for alignment parameters. References: FHV (J.-C. Audonnet, unpublished results), EHV-1 (Flowers et al., 1991), PRV (Petrovskis et al., 1986), BHV-1 (Tikoo et al., 1990) and HSV-1 (Lasky & Dowbenko, 1984).
Analysis of the CHV gD nucleotide sequence

Potential polyadenylation signal sequences are found downstream from the CHV gD gene. However, TATA box and CAAT box sequences are not found upstream from the CHV gD gene, although numerous TATA box-like sequences are found (Fig. 3). The nucleotide sequence surrounding the CHV gD initiation codon (AAAATGA) is favourable with respect to Kozak's rules at position -3, but not at position +4 (Fig. 3). This is not unusual, as the CHV gB and gC genes (see above), as well as gD genes of FHV (J.-C. Audonnet, unpublished results), EHV-1 (Audonnet et al., 1990; Flowers et al., 1991), PRV (Petrovskis et al., 1986) and BHV-1 (Tikoo et al., 1990) also contain an unfavourable nucleotide at position +4.

Analysis of the predicted CHV gD amino acid sequence

The deduced amino acid sequence of the CHV gD homologue is presented in Fig. 3. Hydropathicity analysis of the predicted CHV gD amino acid sequence revealed the presence of two prominent hydrophobic peaks (data not shown). The first peak, located at the N terminus, may represent a potential signal sequence. In fact, PSIGNAL identifies a potential cleavage site between residues 16 and 17. The second hydrophobic peak, located near the C terminus, with a predicted membrane-spanning segment between residues 309 and 328 (using the method of Klein et al., 1985), probably functions as a membrane anchor region.

Alignment of the CHV gD amino acid sequence with homologous sequences from other herpesviruses revealed a moderate level of similarity throughout the entire sequence, with the exception of the N terminus (data not shown). This alignment also revealed that the vast majority of cysteine residues are perfectly conserved. For example, CHV gD contains six cysteine residues, all of which are perfectly conserved in all alphaherpesviruses. These results suggest that the gD glycoproteins may have similar tertiary structures. This alignment also revealed that the potential N-linked glycosylation sites are well conserved. It is not known whether the CHV gD glycosylation sites are utilized. However, it is known that all the potential HSV-1 gD glycosylation sites are used (Sodora et al., 1991).

Genomic organization

The gB, gC and gD genes were not mapped to specific locations on the CHV genome. However, nucleotide sequence analyses of the regions flanking these genes indicate that the genomic organization of CHV is similar to that of other alphaherpesviruses. For example, the ORF located immediately upstream from the CHV gB gene has homology with gene 30 of VZV (Davison & Scott, 1986) and with UL28 of HSV-1 (McGeoch et al., 1988), both of which are located immediately upstream from the gB homologue in those viruses (data not shown). ORF 2, located immediately downstream from the CHV gC gene, has homology with the ORFs located immediately downstream from the gC homologue in FHV (J.-C. Audonnet, unpublished results), EHV-1 (Telford et al., 1992), EHV-4 (Nicolson & Onions, 1990), HVT (Kato et al., 1989) and perhaps HSV-1 (McGeoch et al., 1988) (see above). Additionally, the ORF located immediately downstream from the CHV gD gene has homology with the gI gene of EHV-1 (Audonnet et al., 1990) and the gp63 gene of PRV (Petrovskis et al., 1986), both of which are located immediately downstream from the gD homologue in those viruses (data not shown).

Discussion

In this report we present the nucleotide sequence of the CHV gB, gC and gD genes. These genes are predicted to encode polypeptides of 879, 459 and 345 amino acids, respectively. Comparison of the predicted amino acid sequence of these putative glycoproteins with the gB, gC and gD amino acid sequences of other herpesviruses indicates that CHV is an alphaherpesvirus, a conclusion that is consistent with the previous classification of this virus according to biological properties. This analysis also revealed that the homology among gB homologues is greater than that among gC or gD homologues, suggesting that the structural and functional constraints on gB may be greater than those on gC or gD.

Alignment of homologous gB, gC and gD polypeptides revealed that the vast majority of cysteine residues are perfectly conserved. These results suggest that these cysteine residues, due to their ability to form disulphide bonds, are important in maintaining the structural and functional integrity of the gB, gC and gD glycoproteins. In HSV-1 gD, it has been shown that cysteine 1 forms a disulphide bond with cysteine 5, that cysteine 2 forms a disulphide bond with cysteine 6 and that cysteine 3 forms a disulphide bond with cysteine 4 (Long et al., 1992). Furthermore, it has been shown that a mutation of any of these residues has a profound effect on the conformation, processing and function of the resulting glycoprotein (Wilcox et al., 1988; Long et al., 1990). Therefore, the conservation of cysteine residues in these glycoproteins may have structural significance.

The high degree of homology among the gB, gC and gD homologues also suggests that these glycoproteins have common functions. It has been shown that the
BHV-1 gB homologue can rescue a gB− PRV strain, indicating that these two glycoproteins are functionally equivalent (Kopp & Mettenleiter, 1992).

Alignment of the gB, gC and gD amino acid sequences also revealed that potential N-linked glycosylation sites are somewhat conserved. N-linked glycosylation is thought to play a role in a variety of functions, such as maintenance of protein conformation and protection against proteolytic degradation. The biological significance of N-linked carbohydrates on herpesvirus glycoproteins is not completely understood. For example, tunicamycin treatment of HSV-1-infected cells has been shown to inhibit the production of infectious virions (Pizer et al., 1980). In addition, endoglycosidase treatment of HSV-1 virions has been shown to decrease infectivity (Kuhn et al., 1988). On the other hand, N-linked glycosylation of HSV-1 gD does not appear to be absolutely essential, since mutagenesis of the glycosylation sites on this glycoprotein does not affect infectivity (Sodora et al., 1991). Therefore, although the glycosylation sites on the gB, gC and gD glycoproteins are relatively well conserved, proper glycosylation of each of these polypeptides may not be absolutely essential.

The G + C content of herpesviruses varies from 33 to 75% (Roizman, 1982). It has been suggested that this extensive variability is due to a non-selective mutational bias based on the presence (or absence) of virus-encoded or -induced enzymes involved in nucleotide metabolism (Honess, 1984). For example, VZV and herpesvirus saimiri both have a relatively low G + C content (46%) and both encode an enzyme, thymidylate synthetase, which is involved in TTP synthesis (Davison & Scott, 1986; Honess et al., 1986). HSV-1, HCMV and EBV, on the other hand, have relatively high G + C content (68%, 57% and 60%, respectively) and do not appear to encode a thymidylate synthetase (Honess et al., 1986). CHV has been determined by DNA density analysis to have the lowest G + C content of any herpesvirus, 33% (Plummer et al., 1969; Roizman, 1982), a value which is consistent with the relatively low G + C content of the nucleotide sequence presented in this report (29%). It is not known whether CHV encodes an enzyme involved in nucleotide metabolism. However, it is known that the ORF located immediately downstream from the CHV gC gene is not homologous to VZV thymidylate synthetase. Therefore, if CHV contains a thymidylate synthetase gene, it is not found at the same genomic location as that of VZV.

The parameters of a protective immune response against a CHV infection have not been completely elucidated. It is known that newborn pups exposed to CHV usually die without forming CHV-specific neutralizing antibodies. It is also known that maternal antibodies or treatment with immune serum from seropositive dogs can protect puppies from a fatal CHV infection (Carmichael, 1970). Therefore, it appears that serum neutralizing antibodies can protect puppies against a fatal CHV infection.

Three CHV glycoproteins, gp145/112, gp80 and gp47, are known to elicit CHV neutralizing antibodies (Xuan et al., 1991). The genes encoding these glycoproteins have not been identified. It is possible that these antigens are encoded by the gB, gC and gD genes identified in this paper. Since several reports have indicated that an immune response against gB, gC and/or gD can provide protection of target species animals against a herpesvirus challenge (Babiuk et al., 1987; Nazarian et al., 1992; Riviere et al., 1992; Brockmeier et al., 1993), it is hoped that the isolation of the CHV gB, gC and gD genes will lead to the development of an efficacious CHV vaccine.

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References


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