Nucleotide Sequence and Characterization of the Marek's Disease Virus Homologue of Glycoprotein B of Herpes Simplex Virus

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SUMMARY

The Marek's disease virus (MDV) homologue of the herpes simplex virus (HSV) gene encoding glycoprotein B (gB) has been identified within BamHI fragments I3 and K3 of the 'highly oncogenic' strain RB1B of MDV. The entire nucleotide sequence of the gene has been determined and its predicted amino acid sequence shown to share gross overall structural features with the gB genes of HSV, varicella-zoster virus (VZV) and other mammalian herpesviruses. In particular, all 10 cysteine residues were conserved in MDV gB and there was extensive homology throughout the gene with VZV, HSV and pseudorabies virus except for the N and C termini. The overall percentage amino acid identity between MDV gB and gB of the alphaherpesviruses had a mean of 50% which was almost twice that between cytomegalovirus and Epstein-Barr virus. Northern blot analysis showed that the main RNA transcribed from this gene is approx. 2.7 kb in size. Antibodies raised against synthetic peptides (residues 250 to 271 and 304 to 330) allowed the identification of a family of serologically related glycoproteins of Mr 110K, 64K and 48K in extracts of MDV-infected cells using immunoblots. Furthermore, the antisera were able to differentiate between the antigens of MDV and herpesvirus of turkeys in immunoblots. Immunofluorescence tests indicated that MDV gB is associated with granules in the cytoplasm and is present at the surface of MDV-infected cells.

INTRODUCTION

Marek's disease virus (MDV) is a cell-associated herpesvirus which causes a lymphoproliferative disease in the chicken. The disease is characterized by malignant transformation of T lymphocytes and is notable for being the first example of a naturally occurring lymphomatous condition caused by a virus that can be prevented by vaccination (for review, see Payne, 1985). For more than a decade the disease has been effectively controlled by vaccination with herpesvirus of turkeys (HVT) which is serologically related to MDV. However, in recent years, losses attributed to the emergence of 'very virulent' strains of MDV have occurred in many parts of the world despite vaccination with HVT (Witter et al., 1980; Powell & Lombardini, 1986). In some instances, the efficacy of HVT can be improved by co-immunization with naturally occurring non-pathogenic serotype 2 strains of MDV (Calnek et al., 1983). The mechanism of this 'synergism' is not understood although it is likely that it is attributable at least in part to the presence of relevant epitopes in serotype 2 virus that are lacking in HVT (for review, see Ross & Biggs, 1986).

Knowledge of the structure of antigens that are likely to be important in conferring protective immunity is essential for a rational approach to the construction of improved vaccines. Because of the cell-associated nature of MDV and the poor yields of enveloped infectious particles in cultured cells, it has not been possible to characterize the structural proteins by analysis of
purified virus particles. Consequently, alternative approaches have been used. We have recently identified 35 MDV genes by comparing the predicted amino acid sequence of random fragments of virus DNA to the sequence of known proteins of human herpesviruses (Buckmaster et al., 1988). The relationship between these proteins and the major precipitating antigens A, B and C (Chubb & Churchill, 1968) has not been established except in the case of the A antigen, a secreted glycoprotein which we have shown to be homologous to glycoprotein C (gC) of herpes simplex virus (HSV) (Binns & Ross, 1989). In this paper we report on the structure and entire sequence of the MDV homologue of the HSV gB. We have been guided in our decision to study gB by the fact that it is essential for infectivity (Sarmiento et al., 1979) and is highly conserved among herpesviruses (Snowden et al., 1985; Cranage et al., 1986; Pellett et al., 1985b; Robbins et al., 1987; Keller et al., 1986; Whitbeck et al., 1988). More importantly, gB has been shown to induce both humoral and cell-mediated immune responses and to confer protective immunity to HSV (Cantin et al., 1987) and pseudorabies virus (PRV) (Marchioli et al., 1987). It is likely to be an important immunogen in Marek's disease (MD).

METHODS

Virus strains. The 'highly oncogenic' strain RB1B of MDV (Schat et al., 1982) was obtained from Professor B. Calnek, Cornell University, Ithaca, N.Y., U.S.A. The virus had been plaque-purified in chicken kidney cells (CKC). It was passaged twice in specific pathogen-free Rhode Island Red (HPRS RIR) chickens and four times in chick embryo fibroblasts (CEF). Its highly oncogenic nature was demonstrated by the development of a high incidence of gross tumours when inoculated into genetically resistant N line White Leghorn chickens.

The FC126 strain of HVT (Witter et al., 1970) which had been passaged 14 times in CEF was plaque-purified and grown in CEF. The HPRS-16 strain of MDV (Purchase & Biggs, 1967) was plaque-purified and grown in CEF.

Tissue culture. CEF were grown in roller bottles in 199 medium (Gibco) supplemented with calf serum, penicillin, streptomycin and fungizone as described previously (Ross et al., 1975).

To study the effect of inhibitors of glycosylation and of glycoprotein processing on MDV gB, monolayers of 5 x 10^6 CEF in 5 cm Petri dishes were infected with 1 x 10^6 infectious centres (approximately 5 x 10^6 infected cells). After adsorption for 3 h, the monolayers were washed three times with phosphate-buffered saline (PBS) to remove most of the inoculum. Tunicamycin (2 μg/ml) or monensin (0.5 μM) were added at the times specified in the figure legends and were maintained until cells were harvested, usually 2 to 3 days after infection when c.p.e. was visible.

Isolation and cloning of viral DNA. Viral DNA was isolated from purified particles as described previously (Lee et al., 1980). BamHI digests of MDV (RB1B) DNA were ligated to BamHI-cut, dephosphorylated pUC13 (Pharmacia). Competent Escherichia coli strain TG1 cells were transformed according to standard procedures (Hanahan, 1983) and were grown on L agar in the presence of ampicillin and X-gal. White colonies were picked and tested for the presence of MDV inserts by hybridization to nick-translated MDV DNA (Grunstein & Hogness, 1975). Positive colonies were cultured in small volumes and plasmid DNA was isolated by the procedure of Holmes & Quigley (1981). The size of the inserts ranged from 1 to 18 kbp. Plasmid DNA for cloning was purified by centrifugation to equilibrium in cesium chloride (Clewell & Helinski, 1969).

Random sequencing of viral DNA. Sonicated fragments of genomic DNA or cloned DNA were cloned into Smal-cut, dephosphorylated M13mp10 (Amersham) and the sequence was determined by the dideoxynucleotide method (Sanger et al., 1977) as described in detail by Buckmaster et al. (1988).

Computer analysis of sequence data. Sequences were read using a sonic digitizer (Graf-Bar, Science Accessories Corporation) and the data were analysed on a VAX computer using the ANALYSEQ program of Staden (1982, 1984). Homologies between sequences were determined initially using FASTP (Lipman & Pearson, 1985).

Optimal alignment of amino acid sequences was achieved using the GAP program of the University of Wisconsin Genetics Computer group (Deverreux et al., 1984). To align the amino acid sequence of the gB gene of all six viruses, the following procedure was used. The sequences were first aligned in pairs and the percentage identical matches determined for every possible combination using the following parameter values: pair, 1.5; gap weight, 2.5; gappen weight, 0.5. Each sequence was then aligned with each of the others in turn, starting with those which gave the highest scores in the first round in descending order of magnitude. The process was repeated until padded output files of equal length were obtained. The Wisconsin program PRETTY was then used to align all the sequences and to derive a majority (> 4) consensus sequence using a threshold of 1.5. Upon inspection of the alignments, some manual editing was necessary to minimize padding in regions separating conserved regions.

Southern blot hybridization. Digests of genomic DNA were separated by electrophoresis in 0.7% agarose gels, transferred to nitrocellulose and hybridized with 32P-labelled probes in the presence of 50% formamide as described previously (Ross et al., 1981). Probes used to identify restriction enzyme fragments that contained the gB
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Initially, Southern blot analysis showed that an M13 clone from HVT genomic DNA homologous to HSV gB hybridized to BamHI fragment I, of MDV DNA (Buckmaster et al.,...
Fig. 1. Gene organization in the region of MDV DNA that encodes gB. (a) Conventional arrangement of MDV genome showing unique sequences (UL and Us), flanking inverted repeats (IRL, TRL, IRs and TRs) and partial restriction enzyme map. (b) Expanded map of relevant BamHI fragments. (c) Location of the gB gene and flanking EcoRI and SalI restriction sites. (d) Direction of gB mRNA transcription.

1988). I3 was then sequenced and the data were analysed for open reading frames (ORFs) and for homology to the gB gene sequence of HSV and varicella-zoster virus (VZV) using FASTP. The results showed that the I3 fragment contained an ORF which comprised approximately two-thirds of the MDV homologue of gB including its N terminus. Subsequently, Southern blot hybridization, using an M13 clone from random fragments of MDV genomic DNA homologous to the C terminus of gB of VZV as a probe, showed that the C terminus of the gB gene of MDV was located in the adjacent BamHI fragment K3 (Fig. 1). These results suggested that the gB gene of MDV spanned the junction between I3 and K3 and extended into these fragments.

Sequencing K3 allowed the remaining part of the gene to be identified. The results obtained also indicated that MDV gB was flanked by EcoRI and SalI restriction enzyme sites which were not present internally and suggested that it would be possible to clone the entire gene in a single fragment. This was achieved by cloning EcoRI and SalI double digestion products of MDV DNA into EcoRI-cut and dephosphorylated pUC13 (Pharmacia) which had also been cut with SalI. The MDV insert was characterized by Southern blot analysis of EcoRI and SalI digests of the recombinant DNA and was 3-9 kbp in size (not shown). Sequencing of random fragments of the recombinant plasmid (MS.B27) provided independent confirmation of the sequence derived by sequencing I3 and K3, particularly the region overlapping the BamHI site between the two fragments.

Analysis of the nucleotide sequence of MDV gB

The nucleotide sequence and the predicted amino acid sequence of the entire gB gene of the RB1B strain of MDV obtained by shotgun sequencing of the cloned BamHI fragments I3, K3 and the plasmid (MS.B27) is shown in Fig. 2. The entire gene was sequenced from both strands. Within the sequence shown there is a single ORF capable of encoding a primary translation product of 865 amino acids containing eight potential N-linked glycosylation sites. The C + G composition of the gene is 42%.

Although the ATG triplet at positions 1 to 3 (Fig. 2) is not particularly favourable for initiation of translation (Kozak, 1986), it is the only ATG in all six ORFs that is likely to act as a translation initiation point to produce a protein of the size expected for gB. Moreover it is noteworthy that a pyrimidine (C) following ATG is to be found at the translational start of gB of
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HSV-1 (Pellett et al., 1985a) and also in the case of the A antigen gene of MDV (Coussens & Velicer, 1988; Binns & Ross, 1989). Exceptions to Kozak's consensus are also found in the case of the gB genes of VZV (ATGT; Keller et al., 1986) and PRV (ATGC; Robbins et al., 1987). The ORF is flanked by consensus sequences for promotion and termination of transcription (Fig. 2). These include a potential promoter element, TATAT, located 140 bp 5' upstream of the start codon and potential CAT boxes (CAAT) approximately 40 bp further upstream of the TATA box. The adjacent sequence, ATTG, may also function as a CAT box as has been suggested for HSV gB (see Hammerschmidt et al., 1988). Downstream of the ORF are two sequence elements AATAAA (24 bp and 154 bp at positions 2623 and 2753) followed by GT-rich sequences which are potential sites for mRNA cleavage and polyadenylation. However, after considering the positions of the potential TATA elements and the size of the gB mRNA (2.7 kb; see below), it is probable that mRNA cleavage occurs primarily after the first AATAAA sequence (position 2623) at the first base of the sequence ATGGTTT, 14 bp downstream of the AATAAA sequence (Bernstiel et al., 1985). Assuming that this is correct and allowing 100 bp for polyadenylation, we would expect that the 3' end of the mRNA is located in the region of position 2640 and the 5' end approximately 90 to 110 bp upstream of the ATG codon. Interestingly, this region contains a GGTTGC sequence approximately 20 bp downstream of the proposed TATA box which could be the start site of transcription as is proposed for the PRV gB (Robbins et al., 1987).

Size of the gB RNA transcript

The results of Northern blot analysis (Fig. 3) show that a major RNA transcript 2.7 kb in size is present in RNA preparations from cells infected with two different strains of MDV. The probe used for hybridization was labelled with 32P by primer extension of an M13 clone derived from the strand complementary to that which encodes gB. The M13 clone used mapped within the gene (positions 30 to 409; Fig. 2) and was 370 nucleotides long. Similar results (not shown) were obtained with RNA blotted on Hybond-mAP (Amersham) indicating that the RNA is polyadenylated. Further work involving S1 nuclease mapping is required to determine the 5' end of the gB mRNA precisely.

Analysis of the gB ORF

The translated sequence (Fig. 2) has many of the characteristics of a transmembrane glycoprotein, a role proposed for HSV gB (Pellett et al., 1985a). Hydrophobicity plots (Fig. 4) identified a hydrophobic sequence close to the N terminus corresponding to the signal sequence of HSV and regions of hydrophobic amino acids near the C terminus which may function as a transmembrane anchor sequence. The putative signal sequence of MDV gB (positions 1 to 21) determined using the criteria of Watson (1984) and von Heijne (1986) comprises charged residues HXXRR followed by nine consecutive hydrophobic residues, IFFLIVILY, a helix-breaking residue, G, followed by amino acids with small uncharged side chains. Prediction of the cleavage site using the criteria of von Heijne (1986) suggests that cleavage might occur at the second serine in the sequence NSSP. It is likely therefore that the signal sequence of MDV is 21 amino acids long and that the cleaved primary non-glycosylated sequence is 844 amino acids long. There was little amino acid similarity between MDV and other herpesviruses in this region. The expected Mr of the cleaved primary amino acid sequence is 95.5K. The broad hydrophobic region near the C terminus of MDV gB (positions 683 to 770 in Fig. 4) representing approximately 69 amino acids (excluding gaps) is similar in length to the transmembrane region proposed for HSV (Pellett et al., 1985a). It is not known whether MDV gB traverses the membrane three times as is postulated for HSV (Pellett et al., 1985a; Claesson-Welsh & Spear, 1987). It is noteworthy that hydrophobicity plots showed three distinct peaks in this region of HSV gB and in the corresponding region of MDV gB. The percentage amino acid identity between MDV and HSV gB is 58% in this region and exceeds the percentage identity between HSV and Epstein–Barr virus (EBV) (calculated from the data of Pellett et al., 1985b). These results, and those of the alignments (Fig. 5) suggest that MDV and HSV may have many common features in this region.
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Fig. 2. DNA sequence and deduced amino acid sequence of MDV gB. Nucleotide positions are numbered with reference to the translation initiation codon ATG (underlined). Potential elements for promotion and termination of transcription are underlined and labelled. Other features such as BamHI and EcoRI restriction enzyme sites, hydrophobic regions, residues used for peptide synthesis and the potential site of signal peptide cleavage (*) have been marked and labelled.

However, the diagrammatic plots of secondary structure (Fig. 4b) did not indicate a predominance of α-helix peptides in this region as postulated by Pellett et al. (1985). By analogy with HSV, the extracellular domain of MDV gB is expected to extend from residues 22 to 682 (Fig. 2) or positions 99 to 780 (Fig. 5), and to contain several antigenic epitopes and regions relevant to penetration, rate of entry and neutralization. Analysis of the sequence of this region of MDV gB revealed several potential N-linked glycosylation sites (NXT/S, where X is any amino acid except proline). Eight of these (Fig. 4 and 5) were particularly favourable although one site (position 505; Fig. 4) which is apparently not favourable was conserved and aligned perfectly in several herpesviruses (NPS at position 597; Fig. 5). There was a high degree of identity between MDV, HSV, PRV and VZV throughout the extracellular domain particularly in the region preceding the transmembrane domains. However, there were differences near the middle where MDV contained 23 extra amino acids compared to HSV (residues 495 to 520; Fig. 5). It is of interest that there is much variation among the other herpesviruses in this region and that the differences in sequence could be associated with cleavage of the glycoprotein during processing (see below).

The 113 amino acids that extend from the transmembrane region to the C terminus of MDV gB have many features of the proposed cytoplasmic domain of HSV gB (Pellett et al., 1985a). It is predominantly hydrophilic (Fig. 4) with charged amino acids accounting for nearly 43% of the residues in this region. The degree of identity between MDV and HSV is remarkably high in the region immediately following the transmembrane domain (17/23 identical) and in a region of HSV (around position 941; Fig. 5) where the Syn mutation maps (23/34 identical). In HSV, the mutation R→S at this position has been associated with the formation of syncytia (DeLuca et al., 1982).

Comparison of MDV gB to homologous genes of mammalian herpesviruses

The predicted translated product of MDV gB was compared to the glycoprotein genes of other herpesviruses. Initially, the search using FASTP revealed significant homology particularly with VZV, PRV and HSV. Subsequently the Wisconsin GAP package and some manual editing...
after inspection (as described in Methods) were used to achieve optimal alignment of all the genes. It was also necessary to introduce gaps because of the differences in the length of the primary translated sequence. The MDV gB was similar in length to that of VZV (868 amino acids), but was smaller than those of cytomegalovirus (CMV) (906 amino acids), PRV (913 amino acids) and HSV (904 amino acids).

The overall percentage amino acid identity values between pairs of viruses are summarized in Table 1. The MDV gene was more closely related to those of PRV, VZV and HSV (51.3, 50.8 and 49.2% identity, respectively) than to the CMV (29%) or EBV (28.2%) homologues. The results suggest further that gB of CMV, a betaherpesvirus, and EBV, a gammaherpesvirus, are as dissimilar from each other (30% similarity) as they are from MDV, PRV and HSV.

Analysis of the alignments of the sequences (Fig. 5) showed that all 10 cysteine residues and several motifs were conserved and were perfectly aligned in gB of the six viruses. Conservation of amino acid sequence was particularly extensive between MDV and the alphaherpesviruses PRV, HSV and VZV. Thus, 410 residues were perfectly matched between MDV and HSV. These were distributed throughout the gene except for the signal peptide, the middle of the gene (positions 490 to 550) and residues near the C terminus. In contrast, only 223 residues were matched between HSV and EBV. Similarly, the number of aligned identical residues between EBV and MDV was only 231. Most of the motifs identified were also found in biologically unrelated proteins when the Leeds database (compiled by the Departments of Biochemistry, Genetics and the Astbury Department of Biophysics, University of Leeds, U.K.) which comprises two-million residues was searched. However, the sequence CYSRP was unique to herpesvirus gB. Its function, if any, is not known at present. It is of interest that one of the regions of greatest diversity is located near the middle of the genes (Fig. 5; positions 490 to 550) where post-translational cleavage during processing is reported to occur in VZV (Keller et al., 1986) and postulated for PRV (Robbins et al., 1987), bovine herpesvirus (Whitbeck et al., 1988) and MDV (see below). Cleavage is thought to occur after two consecutive arginine residues.
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Fig. 4. Comparison of the deduced amino acid sequences of MDV gB and HSV gB. (a) Hydropathic plots determined using the algorithm of Kyte & Doolittle (1982) and the HYDROPUB program of M. E. G. Boursnell (unpublished). Values above the line represent hydrophobic regions. (b) Diagrammatic plots of Chou & Fasman's (1978) predictions of β-sheets (iii), α-helices (ii) and turns (i) using the Wisconsin program. (c) Potential sites of N-linked glycosylation. Height of vertical bars indicates probability of glycosylation.

Table 1. Percentage amino acid identity between the gB of herpesviruses*

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<th>MDV</th>
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* The gB sequences were aligned in pairs using the Wisconsin package as described in Methods.
Sequence and characterization of MDV gB

(Keller et al., 1986). It may be significant that two arginine residues (Fig. 5, positions 522 and 523) are conserved in all the gB's except that of HSV which is thought not to be cleaved.

A search for potential N-linked glycosylation sites (AXT/S, where X is not proline) revealed that some sites which were aligned in PRV, VZV, HSV and EBV (e.g. position 151) were not perfectly matched in MDV or CMV, while others were aligned only in PRV, VZV and MDV (e.g. position 265). Surprisingly, the only site that was conserved and aligned in all cases (position 597) was not a favourable motif for glycosylation since it contained a proline residue. It is the least glycosylated molecule with seven sites.

Fig. 5. Alignment of the gB polypeptides of PRV, VZV, HSV, MDV, EBV and CMV. Dashes have been introduced to generate maximum alignment of identical amino acids as described in Methods. The consensus sequence (Con) indicates residues that are identical in at least four of the six viruses. Identical residues are indicated in capital letters. Potential N-linked glycosylation sites are underlined. Spans 1, 2 and 3 refer to the transmembrane regions of HSV gB. The asterisks (*) indicate the cleavage site of VZV gB during processing of the glycoprotein.

Characterization of the MDV gB

To investigate the nature of the MDV homologue of gB, antisera raised against synthetic peptides were used to characterize its size by immunoblotting and for determining its location in infected cells by immunofluorescence. The sequences selected for synthesis contained a high proportion of hydrophilic amino acids and were notable for their n-helical contents. The best peptides were used to characterize its size by immunoblotting and for determining its location in infected cells by immunofluorescence. The sequences selected for synthesis contained a high proportion of hydrophilic amino acids and were notable for their n-helical contents. The best peptides were used to characterize its size by immunoblotting and for determining its location in infected cells by immunofluorescence. The sequences selected for synthesis contained a high proportion of hydrophilic amino acids and were notable for their n-helical contents.
derived from four independent experiments were 110K (range 108K to 112K), 99K (range 95K to 105K), 64K (range 62K to 66K) and 48K (range 47K to 50K). The specificity of the reaction was demonstrated by the failure of the absorbed sera to react with polypeptides of similar size in uninfected cells (lane 1) or with preimmune serum (not shown). Anti-peptide serum that had been absorbed with HVT-infected cells reacted only with MDV-infected cells, indicating that these polypeptides carry MDV-specific epitopes that are lacking in HVT (Fig. 6b). Polypeptides of approximate Mr 35K present in all lanes were considered to be artefacts caused by non-specific reactions and were disregarded.

An attempt was made to study the relationship between the polypeptides using tunicamycin, an inhibitor of N-linked glycosylation, and monensin, an inhibitor of O-linked glycosylation and glycoprotein processing (Johnson & Spear, 1983). In the presence of monensin, the 110K polypeptide was enhanced and the 64K species was absent (Fig. 6a, lanes 3 and 4). The presence of multiple bands in the Mr range 50K to 60K in monensin-treated preparations suggests that some processing or degradation might have occurred during growth in the presence of the drug for 2 to 3 days. In tunicamycin-treated preparations (lanes 5 and 6, Fig. 6a) the 110K and 64K polypeptides were not present. Instead, smaller polypeptides (94K, 90K and 84K) reacted with anti-peptide sera. The size of the 94K polypeptide is consistent with the calculated size of the unglycosylated primary translated product (95.5K excluding signal peptide). The smaller species could be truncated molecules due to premature termination of translation or to degradation of the unglycosylated primary product (Schwarz et al., 1976). The 48K polypeptide is usually the most abundant species in MDV-infected CEF (lanes 2, Fig. 6b). Its presence in drug-treated
preparations, particularly in tunicamycin experiments, is unexpected. Further work is required to establish its origin. Because of the cell-associated nature of MDV infectivity which precludes synchronous infection, the conclusions that can be drawn are limited. Nonetheless, the results suggest that the 110K polypeptide is probably the fully glycosylated gB product and that the 64K species could result from hydrolysis at the putative peptidase cleavage site (position 522; Fig. 5). Assuming that MDV gB contains eight glycosylation sites and that each glycan has an approximate Mr of 2.5K (Klenk & Rott, 1980), it is possible to calculate that the expected Mr of the glycosylated primary transcript and of the cleaved product is 115K and 64.9K, respectively. These values are in excellent agreement with the observed Mr values.

**Location of gB in MDV-infected cells**

Immunofluorescence tests using the anti-peptide serum revealed virus-specific antigens particularly in cytoplasmic granules probably associated with Golgi bodies in the perinuclear region of infected CEF. The specificity of the reaction was confirmed by the failure of the anti-peptide serum to react with uninfected cells and by the failure of preimmune serum to react with infected cells (not shown). Antigen could be demonstrated at the cell surface in both acetone-fixed or paraformaldehyde-fixed preparations. When antigens were denatured by boiling the coverslip cultures, specific reaction with the anti-peptide serum still occurred as expected since the antiserum reacts with denatured proteins.

**Neutralization tests**

Anti-peptide sera did not neutralize MDV infectivity in the dilution range 1/20 to 1/100, either in the presence or absence of complement. In contrast, virus incubated with convalescent MD antiserum (1/100), neutralized infectivity by 90% (data not shown).

**DISCUSSION**

The MDV homologue of the gB gene of HSV has been identified and its sequence determined. The predicted amino acid sequence of the MD gene has characteristics of the gB genes reported for mammalian herpesviruses and is more closely related to those of the alphaherpesviruses HSV (Pellett et al., 1985a), VZV (Davison et al., 1986) and PRV (Robbins et al., 1987) than to those of the betaherpesvirus CMV (Cranage et al., 1986) or the gammaherpesvirus EBV (Pellett et al., 1985b) at the amino acid sequence level. The existence of common antigenic epitopes in the gB gene products of mammalian herpesviruses has been demonstrated by several groups using both immunoblots and virus neutralization tests (Snowden et al., 1985; Balachandran et al., 1987). Although our results do not prove that avian and mammalian herpesviruses share common antigenic epitopes in gB, it would be surprising if they did not since our sequence analysis did not show that the mammalian herpesviruses were more closely related to each other than to MDV. On the contrary MDV gB was significantly more related to PRV, HSV and VZV gBs than CMV or EBV gBs were to each other and to those of the other mammalian viruses. We emphasize that the relationship was evident only in the predicted amino acid sequence. Homologies between MDV and the mammalian viruses were too low at the nucleotide level to allow useful comparisons to be made.

We were able to find in the predicted amino acid sequence of the MDV gB many features of the primary transcript of HSV gB. These included a potential signal peptide at the N terminus, an external domain extending to a hydrophobic anchor sequence postulated to cross the membrane of infected cells or viral envelope three times (Pellett et al., 1985a; Claesson-Welsh & Spear, 1987), and a domain at the C terminus which is thought to be cytoplasmic or in contact with the interior of the mature virus particle. There were also shared features in the external domain particularly among the alphaherpesviruses which suggested common secondary structure such as the conservation and perfect alignment of all 10 cysteine residues, the presence of several motifs and potential glycosylation sites which were also aligned.

The use of anti-peptide sera has enabled us to identify MDV epitopes in the gB molecule that are not conserved in HVT. Sequencing of the corresponding gene from HVT (unpublished data) supports this conclusion. The fact that the MDV-specific antigens are associated with
membranes in the cytoplasm and at the cell surface suggests that they could be important in conferring immunity to MDV.

While this work was being prepared for publication, Sithole et al. (1988) reported the synthesis and processing of the B antigen of MDV. That report described an antigen identified originally in immunodiffusion tests (Velicer et al., 1978) and which is not necessarily the homologue of the HSV gB. Using in vitro translation and monoclonal antibodies for immunoprecipitation of metabolically and in vitro labelled antigens, they report that the B antigen (MDHV-B in their study) is encoded by a 1.8 kb mRNA mapping within the BamHI H region of MDV. The initial gene product is reported to be unglycosylated pr44 which dimerizes to form pr88 (resistant to boiling in SDS and mercaptoethanol) which is then glycosylated to gp100, a glycosylated intermediate, which is finally processed to gp60 and gp49.

Although the genes reported in our present work and in their study originate from different parts of the genome and encode different sized RNAs, there is a remarkable similarity in the size of the products detected in the two studies. The monoclonal antibody used in their study (IAN86.17) has been reported to neutralize infectivity and to recognize epitopes at the cell surface. In contrast, the anti-peptide sera used in our study did not neutralize infectivity. They may recognize predominantly the primary structure of the antigen and be unable to interact with epitopes in intact virus particles. However, the anti-peptide sera reacted with antigens located in granules in the cytoplasm and at the cell surface. Further work is required to determine whether MDHV-B antigen is in fact the MDV homologue of HSV gB and to establish the relationship between the family of polypeptides noted in our study.

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