Identification of the Gene Encoding Marek's Disease Herpesvirus A Antigen†

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The gene encoding the glycoprotein Marek's disease herpesvirus A antigen (MDHV-A) precursor polypeptide pr47 was delineated by using Northern blot (RNA blot) analysis and hybrid selection of its mRNA with cloned MDHV DNA, cell-free translation of the mRNA, and immunoprecipitation of the polypeptide. The resulting piece of DNA with strongly positive hybrid selection results was a 2.2-kilobase-pair (kbp) *PvuII-EcoRI* restriction fragment localized to the center of the 18.3-kbp MDHV *BamHI* B fragment of the total virus genome. The localization was specific since no other small restriction subfragment of the larger *BamHI* B fragment was able to hybrid select significant MDHV-A mRNA and the gene mapped only in the *BamHI* B fragment of the total virus genome. Northern blot analysis confirmed the localization of the MDHV-A gene on the 2.2-kbp fragment and detected its mRNA as a 1.8-kilobase species, a size consistent with encoding a 47-kilodalton polypeptide. This is the first report of an MDHV gene being mapped to the MDHV viral genome. This opens the way for the use of recombinant DNA technology to study the nature of the gene encoding a secreted virus-specific glycoprotein that could possibly be involved in immunoprevention, immunosuppression, or immunoevasion, immune phenomena known or speculated to be involved in this oncogenic herpesvirus system.

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by Marek's disease herpesvirus (MDHV), which results in T-cell lymphomas and peripheral nerve demyelination (18, 21, 22). The disease was a major cause of economic loss (\$200 million/year) to the poultry industry until the early 1970s (26), when a live vaccine was developed from the antigenically related apathogenic herpesvirus of turkeys (HVT) (20).

The ability of HVT to protect chickens against infection by MDHV may be owing to an antigenic relationship between HVT and MDHV. At least six antigenically active viral proteins are common between MDHV and HVT (7, 28). One of these antigenic proteins, the prominent MDHV A antigen (MDHV-A), was the first MDHV and HVT common antigen to be characterized on a physical, chemical, and molecular basis (5, 15, 16). MDHV-A is a glycoprotein with an apparent molecular weight of 57,000 to 65,000 in its fully glycosylated form (5, 10) and therefore is also designated gp57-65 (10). A combination of cell-free translation, pulse-chase, and tunicamycin inhibitor studies have shown that MDHV-A is synthesized as a 47-kilodalton (kDa) precursor polypeptide that apparently undergoes cleavage to remove a small signal peptide (10). The resulting 44-kDa polypeptide undergoes glycosylation and secretion from the cell in a precisely programmed manner (10). While MDHV-A is primarily secreted from infected cells (10), there is mounting evidence that a small amount is also associated with the plasma membrane in a specific manner (8, 19; R. J. Isfort, unpublished data). Not only is its role in the immunoprevention by HVT still unclear, it could have a role in the pathogenesis of MD, since it has been recently postulated to play an immunoevasive role in protecting virus-producing

cells of the feather follicle epithelium (10). Furthermore, any virus-encoded glycoprotein should be a candidate for causing the early-stage immunosuppression that is reported to occur after MDHV infection and that may be one of the key events that lead to neoplasia (25), especially in view of the observation that various virus particles, either infectious or noninfectious, inhibit mitogenic responses (31).

This study was undertaken to identify the gene that encodes this unusual viral glycoprotein antigen. This was done with the dual purpose of facilitating further molecular characterization of the protein and of allowing expression of the MDHV-A gene in order to better assess its role(s) in the immunosuppression, immunoevasion, or immunoprotection processes.

MATERIALS AND METHODS

Cells and viruses. The preparation, propagation, and infection of small-scale duck embryo fibroblast (DEF) cell cultures with MDHV was generally performed as first reported by Glaubiger et al. (5) and more recently by Isfort et al. (10). One exception was that 0.2% calf serum was present in the maintenance medium, as previously reported for the first stage of this study (11). MDHV strain GA (15) was used at passage level 26.

DNA preparation, gel electrophoresis, and Southern blotting. MDHV DNA in the form of 14 pBR328 and 14 lambda clones was a gift from C. Gibbs (4), currently at Rockefeller University. The MDHV *BamHI* library was a gift from M. Nonoyama, Showa University Research Institute (3). The MDHV DNA in each clone was propagated in bacteria and then extracted by standard methods (17). The DNA (1 µg from each clone) was bound to nitrocellulose by the method of Parnes et al. (23) for subsequent hybrid selection.

For further analysis, the entire MDHV genome, clone

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pBR328-15, and the MDHV *Bam*HI clone B were digested with the appropriate restriction enzymes (Bethesda Research Laboratories) by using the conditions recommended by the manufacturer. The digests were then analyzed by gel electrophoresis at 4 V/cm until the leading dye front was three-fourths of the way down the gel. The gel was then stained in ethidium bromide solution (0.5 µg/ml) for 1 h and photographed under UV light. Southern transfer of the electrophoresed DNA was performed by the methods of Southern (30). Nick translations of DNA fragments to be used as probes were performed by the methods of Rigby et al. (27). Hybridization of the ³²P-labeled probes to the nitrocellulose-bound restriction fragments resulting from Southern blotting was performed by the method of Maniatis et al. (17).

Isolation of cellular RNA. The method of RNA isolation was that of Favaloro et al. (2). This method was modified so that the isolated RNA was not treated with DNase prior to the final ethanol precipitation step and the RNA was extracted five times with phenol-chloroform to remove all traces of vanadyl ribonucleotides (New England Nuclear Corp.).

Hybrid selection of MDHV-specific mRNA. The method for hybrid selection was that of Paterson et al. (24), with the time of hybridization and the concentration of RNA modified to achieve maximal selection. Briefly, hybridization was carried out for 3 h by using 50 µg of total cellular RNA per filter in a 100-µl volume.

Cell-free translation. Cell-free translation in rabbit reticulocyte lysates was performed by using the methods of Jackson and Hunt (12), as already reported in preliminary studies of the MDHV system (10, 11). In the present study, translation after hybrid selection was performed by using the selected RNA with 10 µg of calf liver tRNA added as a carrier.

RNA gel electrophoresis and Northern blotting. Electrophoresis of RNA was performed by the methods of Lehrach et al. (14), as outlined by Maniatis et al. (17). Northern blotting (RNA blotting) after electrophoresis was performed by the transfer of formaldehyde-denatured RNA to nitrocellulose according to the method outlined by Maniatis et al. (17). Nick translation and hybridization with ³²P-labeled probe was as described above for Southern blot analysis.

Immunoprecipitation with RαA. Immunoprecipitations were performed by the methods of Witte and Wirth (33), as adapted to the MDHV system by Glaubiger et al. (5) and used by Isfort et al. (10). The rabbit antiserum against MDHV-A (RαA) used to precipitate MDHV-A was prepared as previously described (15) and has been used extensively for that purpose before (5, 10).

SDS-PAGE. Discontinuous stack sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (13) as adapted to the MDHV system by Glaubiger et al. (5), with the two previously reported modifications (10). (i) All samples were boiled in sample buffer containing 2% SDS and 5% 2-mercaptoethanol prior to gel loading. (ii) Electrophoresis was carried out at a constant current of 30 mA until the marker dye ran off the gel. Standard molecular size markers included ¹⁴C-labeled phosphorylase *b* (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), and carbonic anhydrase (30 kDa) (New England Nuclear). Molecular sizes were calculated by interpolation between standard proteins by the method of Weber and Osborn (32). Fluorography was performed by the methods of Bonner and Laskey (1). Autoradiography of all samples was carried out at -70°C.

RESULTS

In previous cell-free translation studies, it was established that MDHV-A is synthesized as a 47-kDa precursor (pr47) immunoprecipitable with RαA (10). Therefore, it was possible to combine hybrid selection with these methods to identify the gene encoding MDHV-A from a partial genomic library of MDHV DNA (4).

Hybrid selection was performed by using RNA preparations isolated at the time of maximal A antigen synthesis (10). For the initial hybrid selection studies (11), both the plasmid pBR328 and the lambda MDHV libraries containing, respectively, approximately 30 and 50% of the MDHV genome (4; C. Gibbs, unpublished data) were used. By using DNA pools of either 14 MDHV pBR328 clones or 14 lambda MDHV clones, hybrid selection was performed. As indicated in the preliminary report (11), after direct SDS-PAGE analysis of the cell-free translation reaction mixtures, approximately 18 to 20 proteins and 10 to 12 proteins, respectively, were produced by translation of mRNA selected by both the lambda- and pBR328-cloned MDHV DNA pools. For the pBR328 clone pool, no background proteins were made from uninfected DEF mRNA nonspecifically binding to MDHV DNA, whereas the lambda-cloned DNA pool apparently selected mRNA for three DEF proteins (R. J. Isfort, unpublished data). This ability of the lambda clone pools to select DEF messages may be due to the cloning of MDHV DNA-cellular DNA junction sequences from the MSB-1 cell line (6) used as the source of MDHV DNA (4).

When these same cell-free translation reaction mixtures were analyzed by immunoprecipitation, MDHV-A pr47 (10) was immunoprecipitated with the RαA serum from the two mixtures receiving infected-cell RNA (11), indicating that the pBR328- and lambda-cloned MDHV DNA pools both selected a message encoding the MDHV-A polypeptide. The inability to visualize pr47 directly without immunoprecipitation analysis of the cell-free translation products is due to pr47 comigrating with and being obscured by a reticulocyte lysate background protein which migrated at 40 to 50 kDa.

The above preliminary results indicated that the pBR328 clone pool contained DNA sequences encoding for MDHV-A pr47. This, and the inability of the pool to nonspecifically bind uninfected DEF mRNA, made it the library of choice for future work. For further gene identification, the 14 readily available MDHV pBR328 clones of a total of 25 that exist (4) were used individually to hybrid select the MDHV-A mRNA. The direct SDS-PAGE analysis of the cell-free translated proteins whose mRNAs were hybrid selected by DNA sequences contained in each clone is shown in Fig. 1A. At least 10 to 12 different proteins resulted from translation of mRNA selected by the 14 clones, which represent less than 20% of the MDHV genome. pBR328 clone 11 (pBR328-11), apparently selected mRNA encoding for at least nine different proteins. The clone 11 data (lane 11) are from the same gel, but a shorter exposure time was used to improve visualization of the multiple bands. It should be noted that many of the mRNAs selected by pBR328-11 appear to be selected by other pBR328 MDHV clones (Fig. 1A), since similar proteins are seen in other lanes as well. The nature and meaning of this observation are unknown and await further study.

Immunoprecipitation analysis of these cell-free translation products with RαA showed that MDHV pBR328 clone 15 (pBR328-15) contained a DNA sequence which selected for the mRNA of MDHV-A pr47 (Fig. 1B). Another polypeptide specifically immunoprecipitable with RαA and migrating at

FIG. 1. Cell-free translation products of mRNA hybrid selected by using 14 individual pBR328 MDHV DNA clones immobilized on nitrocellulose. (A) Direct analysis of the products of MDHV-specific mRNAs that were hybrid selected and cell-free translated. Lanes: 1 to 15, translation of cytoplasmic RNA isolated 72 h postinfection from MDHV-infected cells and selected by using pBR328 clones 1 to 15; 16, translation of unselected cytoplasmic RNA isolated 72 h postinfection from MDHV-infected DEF; 17, translation of unselected cytoplasmic RNA isolated from uninfected DEF; 18, translation with no added RNA. The DNA of clone 13 (numbers were used for orderly lane identification in this study) was lost, explaining the gap in numbering. A 10- μ l sample of each translation mixture was analyzed per lane to display all the polypeptides produced. Samples were analyzed by SDS-PAGE on 10% polyacrylamide gels, and the autoradiogram of the dried gel was prepared by fluorographic exposure for 2 days. Molecular sizes are shown in kilodaltons. (B) Immunoprecipitation and SDS-PAGE analysis of the products of hybrid-selected and cell-free-translated MDHV-specific mRNAs. Lanes 1 to 17 are the same as in panel A, but the translation products were immunoprecipitated with R α A serum. A 40- μ l sample of each translation reaction mixture was subjected to immunoprecipitation with 2 μ l of R α A. Samples were analyzed by SDS-PAGE on 10% polyacrylamide gels, and the autoradiogram of the dried gel was prepared by fluorographic exposure for 5 days. (C) The clone numbers used to identify the lanes in panels A and B are presented in conjunction with a published partial *Eco*RI map based on identification using letters (29). The asterisk highlights clone 15, which selected mRNA for MDHV-A. Also included for orientation purposes, just as presented by the same authors in relation to their *Eco*RI map (29), are the location of the *Bam*HI-B portion of the *Bam*HI map and the locations of the long and short terminal and internal repeats (TRL, IRL, IRS, and TRS), as detailed by Fukuchi et al. (3).

about 30 to 36 kDa in the same lane on the gel (Fig. 1B) is most likely a product of premature translation termination. As already reported (11), and as will be seen below, this molecule is also made every time pr47 is synthesized by cell-free translation of MDHV-specific mRNA but only when pr47 is made. This relationship is a particularly valuable aid to identification in the rare instance when pr47 is difficult to discern owing to a ubiquitous background band, as is discussed below.

Since many of the *Eco*RI plasmid clones have been partially mapped (29) in relation to a previously reported *Bam*HI map (31), it is possible to present a map providing orientation of the clones (Fig. 1C). The DNA clone 15 (this study), which hybrid selected mRNA for MDHV-A (Fig.

1B), is *Eco*RI fragment D (29). Again, by using the map data of others (3, 29), it is possible to predict that *Eco*RI fragment D is within *Bam*HI fragment B, a correlation shown experimentally below.

Clone pBR328-15 contained two MDHV *Eco*RI fragments, an approximately 6.8-kilobase-pair (kbp) fragment and a 1.5-kbp fragment (Fig. 2A). When each of the two cloned MDHV DNA inserts and the 4.9-kbp vector DNA were tested for their ability to select MDHV-A mRNA, it was discovered that only the 6.8-kbp MDHV *Eco*RI fragment contained the genomic coding sequence for MDHV-A (Fig. 2B, left side). This conclusion is based on the presence of the dark band of pr47 just under the more gray background smear occurring in that region of these gels (also in Fig. 1B).

FIG. 2. Further analysis of clone pBR328-15 that selected MDHV-A mRNA. (A) Restriction map of pBR328-15. (B) Cell-free translation by using subfragments of pBR328-15 followed by immunoprecipitation with R α A sera. Hybrid selections, cell-free translation, immunoprecipitation, SDS-PAGE, and fluorography were performed as described in the legend to Fig. 1, with fluorographic exposures for 7 days.

Once again, the 30- to 36-kDa molecule immunoprecipitable with R α A is detected in the same lane but not in other lanes, confirming that the one DNA fragment but not the other two is selecting MDHV-A-specific mRNA. While the pr47 band is not as apparent in this lane on the left side of Fig. 2B as it is in previous and subsequent experiments, apparently owing to less than optimal efficiency of selection or translation or both this one time, the correctness of these data is confirmed by their reproducibility and extension in the next and subsequent experiments.

Upon further digestion with the restriction enzyme *Pvu*II, the approximately 6.8-kbp fragment could be divided into three subfragments of 0.2, 2.2, and 4.4 kbp as mapped and illustrated in Fig. 2A. When these three fragments were isolated and used for hybrid selection, the results presented

in Fig. 2B (right side) were obtained. It is clear that the fragment which contains the coding region for MDHV-A is the terminal *Eco*RI-*Pvu*II 2.2-kbp fragment (Fig. 2B). Northern blot analysis with the same three subfragments confirmed this localization of the MDHV-A gene; only the 2.2-kbp subfragment detected the MDHV-A mRNA, which will be described below (R. J. Isfort, unpublished data). Note that the 30- to 36-kDa protein immunoprecipitable with R α A was again present.

Since the 2.2-kbp subfragment that selected for the A antigen mRNA lay at the end of the pBR328 *Eco*RI fragment of approximately 6.8 kbp, it is possible that the entire gene for MDHV-A is not encoded on this fragment. Since no other pBR328 clone was able to select message for the A antigen (Fig. 1B), it was necessary to obtain a more complete

FIG. 3. Southern blot analysis of *Bam*HI-digested MDHV DNA probed with either total MDHV DNA (A) or the 2.2-kbp *Eco*RI-*Pvu*II MDHV-A-coding region (B). DNA isolation, restriction enzyme digestion, blotting, hybridization, and nick translations were performed as described in the text.

library of the MDHV genome for further gene identification studies. Fortunately, during the course of this study a nearly complete *Bam*HI library was created (3), and the DNA fragments were made available to us. To identify the *Bam*HI fragment which corresponds to the MDHV pBR328 clone that we identified as encoding MDHV-A, a *Bam*HI digest of purified MDHV DNA was separated by gel electrophoresis, Southern blotted, and used to hybridize with a probe of the labeled 2.2-kbp *Eco*RI-*Pvu*II subfragment of pBR328-15. Only *Bam*HI fragment B contained the sequence corresponding to the 2.2-kbp *Eco*RI-*Pvu*II subfragment (Fig. 3).

To determine where in the large (18.3-kbp) *Bam*HI B fragment the genetic information of the 2.2-kbp *Eco*RI-*Pvu*II subfragment lies, fragment B was first digested with several restriction endonucleases and physically mapped (Fig. 4). The restriction digests were gel electrophoresed, Southern blotted, and hybridized to a radioactive probe of the *Eco*RI-*Pvu*II 2.2-kbp subfragment of pBR328-15 (Fig. 2A). The blot was then hybridized to a radioactive probe of the complete

FIG. 5. Northern blot analysis of control (CON) and infected (INF) total cellular RNA by using the 2.2-kbp *Eco*RI-*Pvu*II fragment as a nick-translated probe for hybridization. A total of 10 μ g of RNA was loaded per lane and electrophoresed, blotted, and probed as described in the text. Exposure was for 1 h.

6.8-kbp *Eco*RI fragment (R. J. Isfort, unpublished data). The combined information served to determine the orientation and directionality of the 6.8-kbp fragment and its 2.2-kbp subfragment in *Bam*HI-B. As is seen in the resultant map (Fig. 4), the 2.2-kbp *Eco*RI-*Pvu*II subfragment lies in the center of the *Bam*HI B fragment. Thus the adjacent sequences are available to determine if any of the MDHV-A gene lies adjacent to the 2.2-kbp *Eco*RI-*Pvu*II subfragment known to contain at least part of the gene.

To delineate the extent of the A antigen gene in the *Bam*HI B fragment, the other restriction fragments of *Bam*HI-B shown in Fig. 4 were isolated and used to hybrid select the MDHV-A mRNA. From these studies, it appears that a 2.4-kbp *Eco*RI-*Sma*I subfragment adjacent to the 2.2-kbp *Eco*RI-*Pvu*II subfragment was also able to hybrid select the MDHV-A mRNA (Fig. 4), although this selection was very weak when compared with that of the 2.2-kbp *Pvu*II-*Eco*RI subfragment. No other DNA sequences in the entire *Bam*HI B fragment were able to hybrid select MDHV-A mRNA, thereby delineating the approximate limits of the A antigen-coding region.

FIG. 4. Map of the *Bam*HI B fragment, showing orientation and position of the MDHV-A-coding region as determined by both hybrid selection and Northern blot analysis. Both hybrid selection and Northern blot analysis were performed as described in the text.

To complete the initial analyses of the MDHV-A gene, Northern blot analysis was performed by using the same RNA preparations used above for hybrid selection. In this case, the 2.2-kbp *PvuII-EcoRI* fragment, the one most strongly positive in hybrid selection, was nick translated and used as a probe. It detected an mRNA band migrating just below the position of 18S RNA marker (Fig. 5), a size of approximately 1.8 kb. When all the other subfragments of the *BamHI* B fragment were used as probes for Northern blot analysis, none of them detected enough of the same 1.8-kb mRNA to be seen on the same short exposure of the gel to X-ray film. The 2.4-kbp *EcoRI-SmaI* fragment was very weakly positive in detecting the same mRNA, a result that could only be seen upon long exposure and is considered borderline (Fig. 4).

DISCUSSION

This study was performed to identify the location of the coding region for MDHV-A within the MDHV genome. Initially, the MDHV-A gene was identified within the library of pBR328 clones (Fig. 1) previously developed and studied in these laboratories (4). The gene was first localized on a 2.2-kbp subfragment of an approximately 6.8-kbp clone designated pBR328-15 (Fig. 2). However, this nomenclature is used for numbering purposes only within this study. The DNA fragment carrying the MDHV-A gene is actually the one designated M (4) or D (29) in other published work in which these clones are used. On the basis of the partial *EcoRI* mapping and other studies of Silva and Witter (29), it appears that the clone D is larger than that reported here and is within the genome region spanned by the *BamHI* B fragment (Fig. 1C).

Further work with the MDHV-A gene in this clone seemed inadvisable for two reasons. (i) The location of the 2.2-kbp subfragment near one end of the larger fragment (Fig. 2) means it was possible that the entire gene was not present on the fragment; and (ii) the pBR328 library contains only 30% of the MDHV genome. Thus, since none of the other pBR328 clones hybrid selected MDHV-A, the real possibility existed that not all of its gene was available. Fortunately, at this time Fukuchi et al. (3) investigated the structure of the MDHV genome by using the GA strain, the same one used in our original cloning studies (4). Fukuchi et al. (3) presented *BamHI*, *BglI*, and *SmaI* restriction endonuclease maps of MDHV DNA. They had successfully cloned 27 of the 29 *BamHI* fragments, and these were then made available for study by others. Since their *BamHI* library constituted nearly 100% of the MDHV genome and was well mapped, it seemed wisest to use it as a potential source of the entire MDHV-A gene for all future studies.

Initial localization of the MDHV-A gene in the *BamHI* library was by Southern blot analysis with the 2.2-kbp *EcoRI-PvuII* subfragment of the pBR328 clone (Fig. 3) used as a probe of the entire set of *BamHI* fragments. Clearly, the MDHV-A genetic material in the 2.2-kbp subfragment hybridized only with the 18.3-kbp *BamHI* B fragment (Fig. 3). Upon further restriction mapping and hybrid selection analysis, it was found that the MDHV-A-coding region lay on a 4.6-kbp *PvuII-SmaI* fragment found within the larger *BamHI* B fragment (Fig. 4). Because early mapping studies revealed an *EcoRI* site, this fragment can be subdivided into a 2.2-kbp *EcoRI-PvuII* fragment and a 2.4-kbp *EcoRI-SmaI* fragment. Although both could hybrid select MDHV-A mRNA, in this study, the extent of selection was much greater with the former than with the latter fragment (Fig. 4). The weak

hybrid selection with the 2.4-kbp *EcoRI-SmaI* fragment was consistent with the very weak positive results obtained with the same fragment by Northern blot analysis with long exposure of the X-ray film (Fig. 4). Recent detailed gene analysis, however, shows that the entire MDHV-A gene is actually confined to the 2.2-kbp *EcoRI-PvuII* fragment (P. M. Coussens and L. F. Velicer, unpublished data). The weak hybridization results with the other fragment might be explained by occasional readthrough transcription, among other possibilities. Because of the inability of the other surrounding subfragments to hybrid select MDHV-A mRNA or to detect the mRNA by Northern blot analysis, the complete A antigen-coding region has been identified within the center of the *BamHI* B fragment. This fragment is in the right half of the unique long region of the virus genome, based on the map of Fukuchi et al. (3).

Based on the size of MDHV-A unglycosylated precursor polypeptide pr47 (10, 11; Fig. 1 and 2), it is possible to predict that its mRNA should be approximately 1.6 kb. Detection of MDHV-A mRNA of 1.8 kb is consistent with this prediction, and the small size difference suggests that splicing is minimal if it occurs at all. The small size difference could reflect the effect of the amino acid composition of the protein or the poly(A) tail of the mRNA or both. Now that the gene has been identified and located, work is in progress that will provide further insight as to the full nature and content of the 1.8-kb mRNA of MDHV-A.

So far, a function for the A antigen has not been found. Its possible role(s) in immunoprevention or immunosuppression or both was mentioned above. Possibly it is not needed for virus growth, since A antigen-negative strains are known and are able to propagate well in tissue culture. However, more rigorous proof that these strains are truly negative may be called for. Recently, the A antigen has been implicated as having a role in host immunoevasion, possibly as a function of its extensive secretion (10).

These studies on identification of the MDHV-A gene are extremely important to the MDHV system in three ways. (i) This is the first gene identified and cloned in this system. (ii) The means now exist to use modern approaches of recombinant DNA technology to study this gene and its glycoprotein product. (iii) Continued progress with the MDHV-A gene should provide methods and strategies for the identification and study of other MDHV genes, such as another glycoprotein antigen called B antigen. The MDHV-B glycoprotein complex and its putative precursor polypeptides have been recently characterized (9), and efforts are currently under way to identify the gene for this antigen by using the methods that have worked for MDHV-A. At the same time, the 2.2-kbp *PvuII-EcoRI* fragment is being subjected to further detailed analysis in order to facilitate its expression via available expression vector systems. On the basis of phosphonacetic acid studies, we now know MDHV-A is a late protein (L. F. Velicer, unpublished data). Thus, current studies should shed much light on the nature of a gene for a unique late glycoprotein antigen that is rapidly secreted from DEF cells but may also have a membrane component (10). In the process, it is expected that more will be learned about the nature of an oncogenic herpesvirus-encoded antigen that may be involved in immunoprevention, immunosuppression, or possible immunoevasion.

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