JOURNAL OF VIROLOGY, Apr. 1988, p. 1416–1422
0022-538X/88/041416-07$02.00/0

Vol. 62, No. 4

Identification and Expression of a Human Cytomegalovirus Glycoprotein with Homology to the Epstein-Barr Virus BXLF2 Product, Varicella-Zoster Virus gpIII, and Herpes Simplex Virus Type 1 Glycoprotein H

MARTIN P. CRANAGE,[1]* GEOFFREY L. SMITH,[1] SUSANNE E. BELL,[1] HELENA HART,[2] CAROL BROWN,[3] ALAN T. BANKIER,[3] PETER TOMLINSON,[3] BART G. BARRELL,[3] AND TONY C. MINSON[1]

Division of Virology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, England[1]; Bioscot Ltd., Edinburgh 9, Scotland[2]; and Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England[3]

Received 14 September 1987/Accepted 21 December 1987

An open reading frame with the characteristics of a glycoprotein-coding sequence was identified by nucleotide sequencing of human cytomegalovirus (HCMV) genomic DNA. The predicted amino acid sequence was homologous with glycoprotein H of herpes simplex virus type 1 and the homologous protein of Epstein-Barr virus (BXLF2 gene product) and varicella-zoster virus (gpIII). Recombinant vaccinia viruses that expressed this gene were constructed. A glycoprotein of approximately 86 kilodaltons was immunoprecipitated from cells infected with the recombinant viruses and from HCMV-infected cells with a monoclonal antibody that efficiently neutralized HCMV infectivity. In HCMV-infected MRC5 cells, this glycoprotein was present on nuclear and cytoplasmic membranes, but in recombinant vaccinia virus-infected cells it accumulated predominantly on the nuclear membrane.

Infection with human cytomegalovirus (HCMV), a betaherpesvirus, is a relatively common occurrence, as shown by seropositivity rates in adult populations (43), but illness is frequently mild or subclinical. However, in those individuals with immature immune systems, such as the fetus and neonate, or in immunosuppressed individuals, such as those undergoing organ grafting or patients with the acquired immune deficiency syndrome, infection with HCMV can cause significant morbidity and mortality (11, 25, 40).

The development of rational strategies of vaccination and immunotherapy to prevent HCMV infection or to limit illness in those at high risk depends upon both a better understanding of the biology of the virus and detailed analysis of virion components that are able to elicit immune responses. It is expected that the target antigens for antibodies that are able to neutralize virus infectivity will be the virus-encoded glycoproteins that are inserted into the envelope of the mature virus particles. Analysis of these proteins has, however, been complicated by several factors. The virus grows slowly and in a limited number of cell types and fails to shut off host cell synthesis; infected cells display an avid immunoglobulin F_c binding activity (13); and the virion glycoproteins have complex product precursor relationships (30) and form multimeric complexes (4, 12, 18, 20).

In several reports monoclonal antibodies have been described that are reactive with HCMV glycoproteins that are able to neutralize virus infectivity in vitro (5, 8, 18, 29, 32, 33). Individually purified or expressed HCMV glycoproteins have also been shown to elicit neutralizing responses in experimental animals (8, 16, 31).

To identify HCMV glycoprotein genes and the encoded proteins, we are screening the HCMV nucleotide sequence for open reading frames with glycoprotein characteristics and then expressing these in vaccinia virus. By using this approach, a HCMV glycoprotein sharing homology with glycoprotein B (gB) of other human herpesviruses was identified (8). Here we describe the identification and expression of another HCMV glycoprotein that is conserved within human herpesviruses and show that, like gB, it also is a target for antibody-mediated virus neutralization in vitro. Interestingly, the glycoprotein has a different cellular localization in HCMV-infected cells compared with that in cells infected with the vaccinia virus recombinant. The implications of this are discussed.

MATERIALS AND METHODS

Cells and viruses. The AD169 strain of HCMV was grown and titrated in MRC5 cells as previously described (8). The vaccinia virus WR strain and TK^- recombinant viruses were grown in BHK cells, and infectivity was determined by plaque assay on CV1 cell monolayers (23).

Construction of recombinant vaccinia viruses expressing the HCMV glycoprotein. A potential HCMV glycoprotein-coding region was isolated from plasmid pAT153 containing the 11.4-kilobase *Hin*dIII L fragment of the HCMV genome (26). The *Hin*dIII L fragment was excised with *Hin*dIII, treated with the *Escherichia coli* DNA polymerase Klenow fragment to create blunt ends, and then digested with *Sma*I, which cuts 96 nucleotides upstream of the translational initiation codon of the glycoprotein gene. A 2.5-kilobase fragment containing the glycoprotein gene was ligated into plasmids pGS62 (35) and pSC11 (7) at the unique *Sma*I site to create plasmids pSB3 and pSB4, respectively. These plasmids each contain the HCMV open reading frame correctly positioned downstream of a constitutively expressed vaccinia virus promoter derived from a gene encoding a protein of 7.5 kilodaltons (kDa) and were used to generate TK^- recombinant vaccinia viruses by previously established methods (7, 22). Analyses of DNA from recombinant viruses by restriction endonuclease digestion, agarose gel electropho-

* Corresponding author.

resis, and Southern blotting demonstrated that the HCMV gene was inserted into the vaccinia virus TK gene and that no other gross genomic alterations had occurred.

Immunoprecipitation. Preparation of [^{35}S]methionine-labeled infected cell lysates and immunoprecipitation were performed as previously described (8), except that, when monoclonal antibodies were used, protein A-Sepharose was reacted with rabbit anti-mouse immunoglobulin (Dako) before it was added to the immunoprecipitation reaction. Samples were electrophoresed through linear 10% or 5 to 12% gradient polyacrylamide gels, and after fixation gels were treated with fluorographic enhancer (Amplify; Amersham International plc) and autoradiographs were prepared.

Immunofluorescence. CV1 and MRC5 cells were grown on glass cover slips and infected with either vaccinia virus or HCMV at multiplicities of 30 and 10, respectively. For surface membrane immunofluorescence, cells were washed in phosphate-buffered saline (PBS) and then fixed in an isotonic solution of 2% paraformaldehyde for 30 min at room temperature. Cells were blocked with PBS containing 1% bovine serum albumin and 5% nonimmune rabbit serum (blocking buffer) at room temperature for 30 min. Antibody was diluted in blocking buffer and left on the cover slips for 60 min at room temperature. Following extensive washing, bound antibody was detected with fluorescein-conjugated rabbit anti-mouse immunoglobulin (Dako) diluted 1/20 in blocking buffer.

For internal staining, monolayers were fixed for 10 min at room temperature with 5% formaldehyde–2% sucrose in PBS. Following three washes with PBS, cells were permeabilized by incubation for 5 min at room temperature in PBS containing 1% Triton X-100, 10% sucrose, and 1% calf serum. Cell monolayers were then incubated in blocking buffer and stained as described above for nonpermeabilized cells.

HCMV neutralization assays. Monoclonal antibody (ascites fluid) was heated at 56°C for 30 min to inactivate endogenous complement. Dilutions of antibody were then incubated with equal volumes of HCMV (750 PFU) for 30 min at 37°C, at which time fresh nonimmune rabbit serum was added to some tubes to a final concentration of 5% as a source of complement. After a further 30 min of incubation, residual virus was assayed by plaque formation on MRC5 cell monolayers.

RESULTS

Identification and comparative analyses of an HCMV glycoprotein-encoding sequence. Putative glycoprotein-encoding genes were sought within the HCMV genome by sequencing of cloned restriction fragments (26) by using the M13 and dideoxy nucleotide chain termination method (34) (strategy and methods have been described by Bankier and Barrell [3]). Open reading frames associated with potential transcriptional regulatory signals were analyzed further for membrane glycoprotein characteristics, including hydrophobic amino-terminal signal sequences, potential N-linked glycosylation sites, and a hydrophobic membrane anchor region near the carboxy terminus of the deduced amino acid sequence.

These criteria were used to identify a putative glycoprotein gene within the 11.4-kilobase *Hin*dIII L fragment of HCMV. This open reading frame has a primary translation product of 743 amino acids containing six potential N-linked glycosylation sites. The predicted polypeptide sequence was analyzed for its hydrophobicity profile by using the ANALYSEP computer program (39). A stretch of N-terminal hydrophobic amino acids consistent with signal peptide and a C-terminal-associated stretch of hydrophobic residues consistent with a transmembrane region were identified.

The predicted translation product of the open reading frame was compared with glycoprotein genes of other herpesviruses by using the FAST P computer program (21), and the matches were analyzed with the DIAGON computer program (38). This revealed homology with glycoprotein H (gH) of herpes simplex virus (HSV) (15, 24), the Epstein-Barr virus (EBV) BXLF2 gene product (1), and varicella-zoster virus (VZV) gpIII (19). In previous analyses (9, 10, 24), it has been established that these three gene products are homologous and that the homology is restricted to the carboxy-terminal region of the molecule. The homology was weak and was confined to the C-terminal half of the reading frame (Fig. 1). The strongest similarity was seen with the EBV homolog. The sequences of the herpesvirus gH proteins are not highly conserved but have the following distinctive features in common: (i) an unusually short carboxy-terminal cytoplasmic region; (ii) a conserved sequence of four amino acids, NGTV (which includes a potential N-linked glycosylation site) approximately 15 amino acids N-terminal to the transmembrane region; and (iii) four cysteine residues whose positions relative to that of the transmembrane region were conserved in all three proteins. These features are all found in the predicted translation product of the HCMV open reading frame shown in Fig. 2. In addition, the view that this open reading frame is a gH homolog is further strengthened by the position of the coding sequence relative to that of the adjacent open reading frames. Thus, in VZV the gene coding for gpIII (the gH homolog) is the open reading frame designated VZV-37 (9) and is preceded by open reading frames 36, 35, and 34. In HCMV the gH reading frame is preceded by homologs of VZV-35 and VZV-34, although there is no homolog to VZV-36, the thymidine kinase gene (B. G. Barrell, unpublished data). Collectively, these data establish that the product of this open reading frame is a gH homolog and is subsequently referred to as HCMV-gH.

Expression of HCMV-gH. The HCMV-gH gene product synthesized in cells infected with the recombinant vaccinia virus that contains the HCMV-gH gene (designated HCMV-gH-VAC) was analyzed by immunoprecipitation of [^{35}S] methionine-labeled infected cell extracts by using a rabbit antiserum sample raised against purified HCMV virions (a gift from Ken Powell). Both recombinant vaccinia viruses (derived from insertion vectors pGS62 or pSC11, which coexpresses β-galactosidase) synthesized a polypeptide of

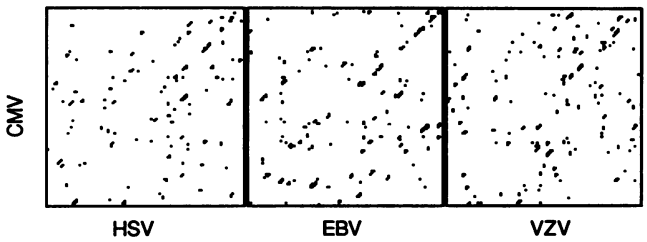

FIG. 1. Comparison of the HCMV-gH protein sequence with those of HSV type 1 gH and the gH homologs of EBV (BXLF2 gene product; gp85) and VZV (VZV-37 gene product; gpIII) by use of the DIAGON program (38). The proportional algorithm was used with a window of 21 amino acids and a score of 235. The N termini of the sequences are in the bottom left-hand corner in each case.

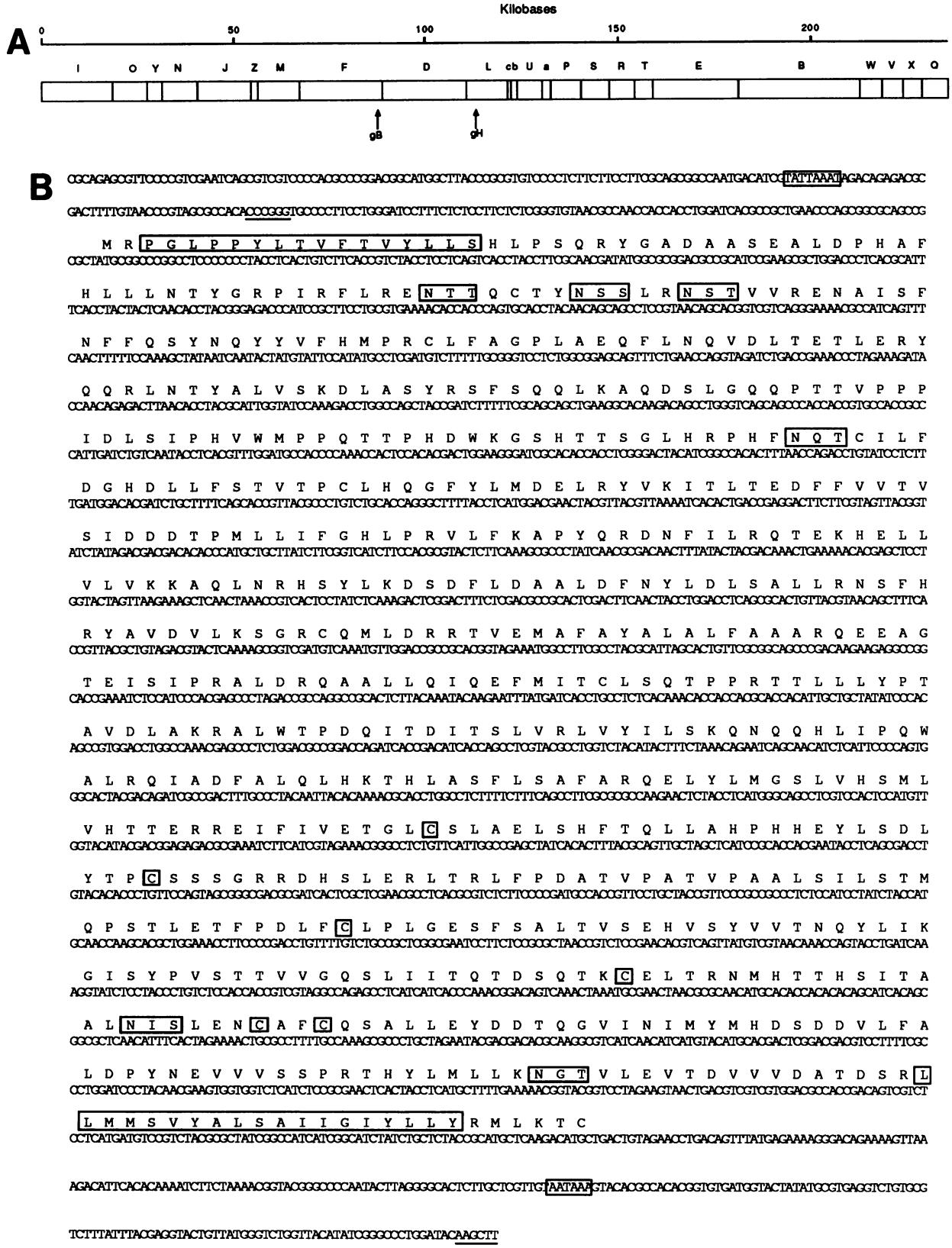

FIG. 2. The position and sequence of the HCMV-gH gene. (A) The *Hin*dIII restriction map of HCMV AD169 is given, and the position of the gH gene is shown. (B) The DNA sequence is of a region of the *Hin*dIII L fragment, including the site at the boundary of the *Hin*dIII L and D fragments. The *Hin*dIII and *Sma*I sites used in the cloning of the HCMV-gH gene are underlined. In the DNA sequence, the putative TATA box and poly(A) addition site for the gene are boxed. In the protein sequence, potential signal and transmembrane sequences are boxed, as are potential glycosylation sites and the six cysteine residues whose positions are conserved. Of the boxed cysteine residues, the most C-terminal pair are conserved in EBV and VZV but not in HSV type 1. The remaining four boxed cysteines are conserved in all four viruses.

86 kDa that was absent in wild-type (WT) vaccinia virus-infected cells (Fig. 3). A control rabbit antiserum sample raised against a HSV peptide did not precipitate this protein, although it did react with the 116-kDa β-galactosidase. A band with a slightly greater electrophoretic mobility than that of HCMV-gH was common to all vaccinia virus-infected cells and probably represents the breakthrough of a vaccinia virus protein. In HCMV-infected MRC5 cell lysates, multiple polypeptides were precipitated with the anti-HCMV antisera, including a prominent 86-kDa band which may represent gH.

Next, a panel of murine monoclonal antibodies raised against HCMV was screened for reactivity to the gH-VAC recombinant viruses. An antibody designated HCMV-16 immunoprecipitated an 86-kDa protein from both HCMV and HCMV-gH-VAC-infected cell lysates (Fig. 4). It was repeatedly noted that the electrophoretic mobility of the protein precipitated from HCMV-infected cell lysates was slightly greater than that seen from HCMV-gH-VAC-infected cell lysates. When cells infected with HCMV or vaccinia virus recombinants were labeled with [^{35}S]methionine in the presence of 10 μg of tunicamycin per ml, the 86-kDa band was no longer detectable by immunoprecipitation with either HCMV-16 monoclonal antibody or rabbit anti-HCMV virion serum (data not shown).

Cellular localization of HCMV-gH. The location of gH in HCMV-gH-VAC-infected cells was analyzed by indirect immunofluorescence by using HCMV-16 ascitic fluid on recombinant and WT vaccinia virus-infected CV1 cells. No expression of the antigen at the cell surface was seen (Fig. 5). However, when cells were permeabilized by treatment with Triton X-100 before antibody was added, diffuse, fibrillar cytoplasmic staining and striking nuclear membrane staining of HCMV-gH-VAC-infected cells were observed.

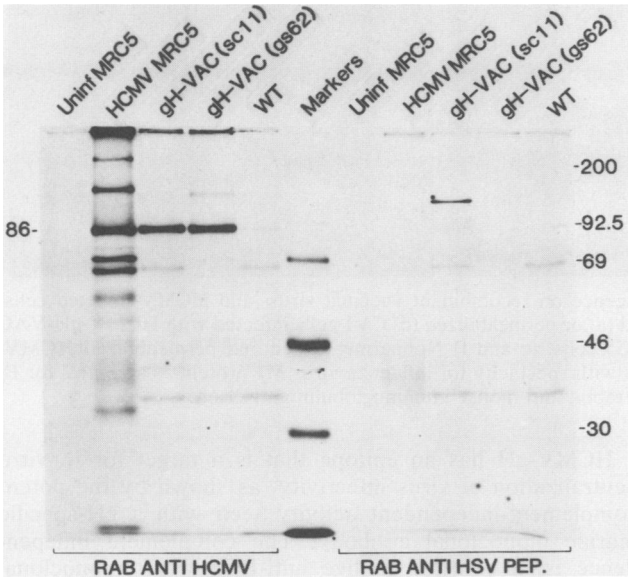

FIG. 3. Detection of HCMV-gH by immunoprecipitation with rabbit anti-HCMV antiserum. Lysates of [^{35}S]methionine-labeled HCMV-infected MRC5 cells, mock-infected (Uninf) MRC5 cells, and HCMV-gH-VAC (produced by using either plasmid pSC11 or plasmid pGS62)-infected or WT vaccinia virus-infected CV1 cells were reacted with either rabbit anti-HCMV serum (RAB ANTI HCMV) or rabbit anti-HSV peptide serum (RAB ANTI HSV PEP.). Proteins were separated by electrophoresis on sodium dodecyl sulfate–10% polyacrylamide gels, and an autoradiograph is shown. Numbers to the right are in kilodaltons.

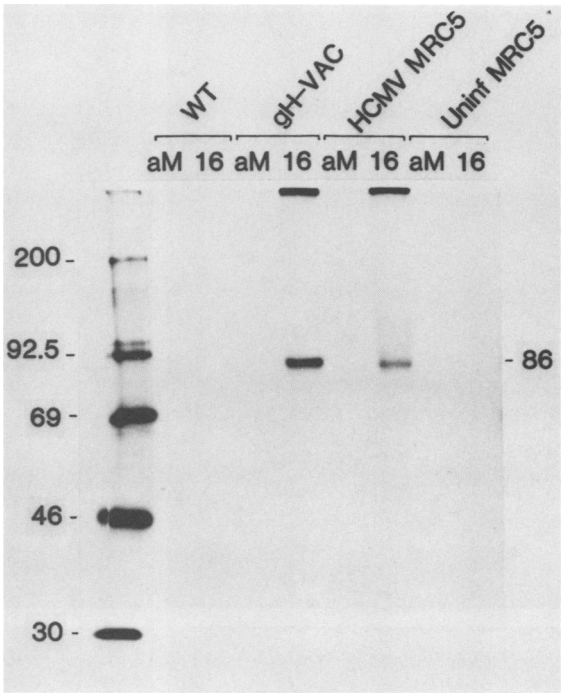

FIG. 4. Immunoprecipitation of HCMV-gH from HCMV-gH-VAC-infected or HCMV-infected cells. [^{35}S]methionine-labeled lysates from HCMV-gH-VAC, WT vaccinia virus, or HCMV-infected or mock-infected (Uninf) MRC5 cells were reacted with monoclonal antibody HCMV-16 or with another γ_1 monoclonal antibody with specificity for the influenza virus M1 protein (aM). Precipitated proteins were separated on a sodium dodecyl sulfate–10% polyacrylamide gel, and an autoradiograph is shown. Numbers on both sides of the gels are in kilodaltons.

In HCMV-infected MRC5 cells, HCMV-16 antibody reacted with nonpermeabilized cells with a distinctive patchy staining pattern. Specific staining was not seen at early times (4 and 24 h postinfection) and was detected on less than 1% of cells at 48 h. By 72 h approximately 80% of cells showed surface staining, and by 96 h this was detected on all the cells. In contrast, a monoclonal antibody (HCMV-37) specific for HCMV gB (8) showed specific surface membrane staining of HCMV-infected cells as early as 24 h after infection. No staining was seen on uninfected cells with either antibody. In permeabilized HCMV-infected cells, HCMV-16 antibody showed strong cytoplasmic staining. Nuclear membrane staining was also present, although it was not as prominent as that in the HCMV-gH-VAC-infected cells. In addition, most cells showed a very prominent cytoplasmic inclusion body that was not seen in the recombinant vaccinia virus-infected cells. Since HCMV-infected cells are known to display avid F_c receptors, another γ_1 monoclonal antibody with specificity for the influenza virus M1 protein (kindly provided by J. Yewdell) was used as a further control. Although this antibody reacted with cytoplasmic vesicle-like bodies, the distribution and intensity of staining was quite different from that with HCMV-16.

HCMV-gH is a target for neutralizing antibody. Monoclonal antibody HCMV-16 (ascites fluid) was shown to neutralize HCMV infectivity strongly in vitro, both in the presence and absence of rabbit complement (Fig. 6). This is in contrast to the complement-dependent neutralization seen with two monoclonal antibodies with specificity for HCMV-gB.

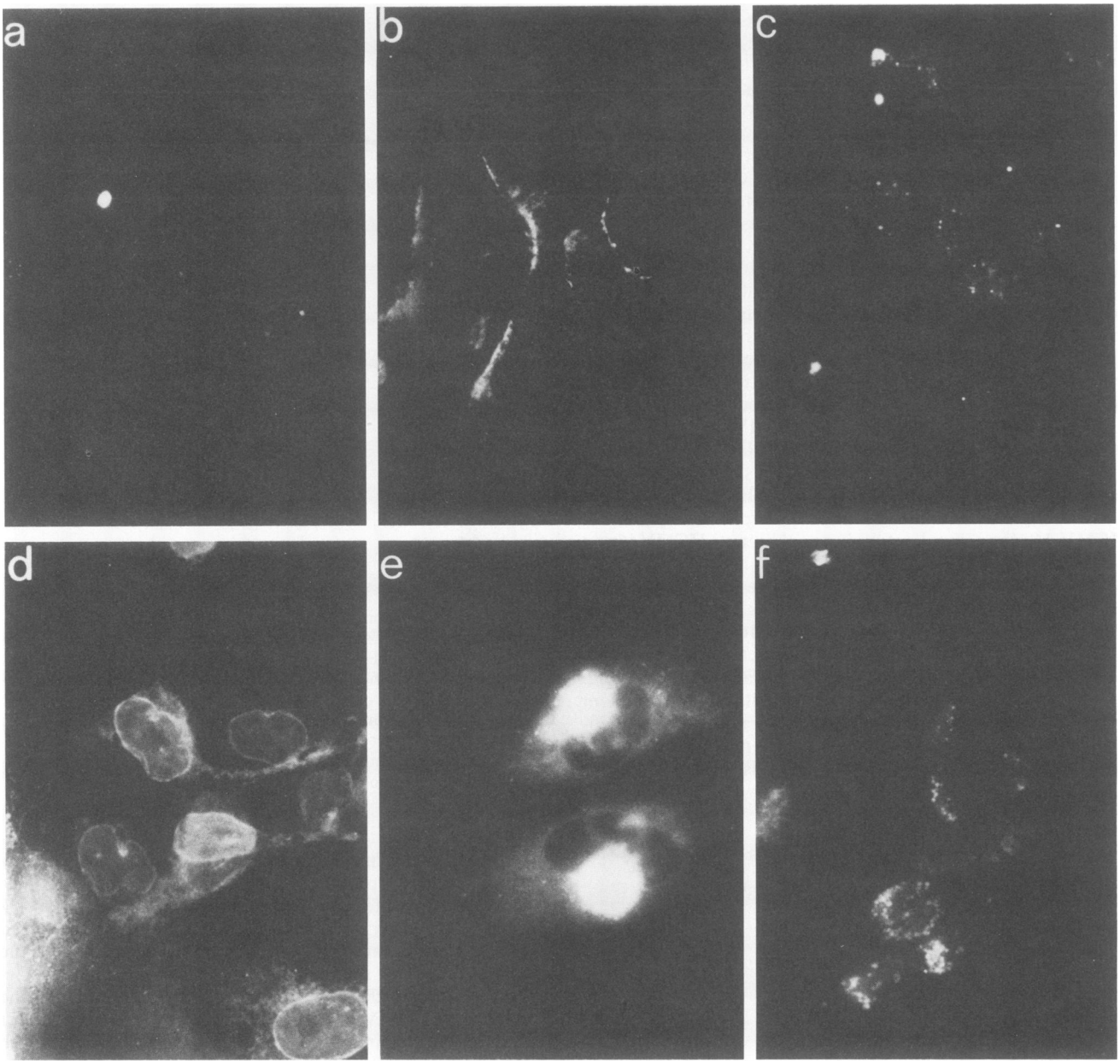

FIG. 5. Cellular localization of HCMV-gH studied by immunofluorescence on recombinant vaccinia virus- and HCMV-infected cells. Monoclonal antibody HCMV-16 was reacted with either nonpermeabilized (a) or permeabilized (d) CV1 cells infected with HCMV-gH-VAC or with nonpermeabilized (b) or permeabilized (e) HCMV-infected MRC5 cells. (c and f) Nonpermeabilized and permeabilized HCMV-infected MRC5 cells, respectively, stained with a γ_1 monoclonal antibody with specificity for influenza virus M1 protein as a control for F_c binding. The antibody reaction was detected with fluorescein-conjugated rabbit anti-mouse immunoglobulin antiserum.

DISCUSSION

An open reading frame within the *Hin*dIII L fragment of the HCMV genome has a predicted primary translation product of 84.4 kDa. This product has the properties of a transmembrane glycoprotein. The gene product was expressed by recombinant vaccinia virus and identified by immunoprecipitation as an 86-kDa protein. The predicted translation product showed weak homology with gH of HSV type 1 and the homologous proteins in EBV and VZV. The view that this gene product is the HCMV-gH homolog is supported by the conserved features of the primary amino acid sequence, the conserved size of the protein, and the position of the gene with respect to adjacent conserved open reading frames.

HCMV-gH has an epitope that is a target for in vitro neutralization of virus infectivity, as shown by the potent complement-independent activity seen with a gH-specific murine monoclonal antibody. The complement independence is in contrast to five anti-HCMV gB monoclonal antibodies studied in this laboratory which neutralize the virus infectivity only in the presence of complement. While these observations are consistent with those of Rasmussen et al. (31–33), who have also described a monoclonal antibody directed against an 86-kDa HCMV glycoprotein displaying complement-independent neutralizing activity, it has yet to be determined whether these proteins are the same.

Both the monoclonal antibody and polyclonal antisera used in this study precipitated polypeptides of approxi-

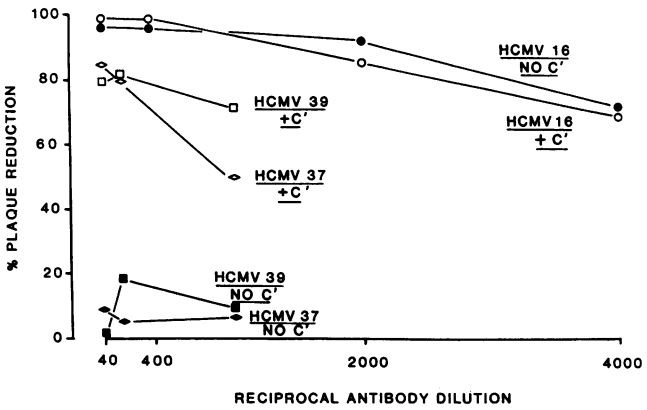

FIG. 6. In vitro complement (C')-independent neutralization of HCMV infectivity with an anti-HCMV gH monoclonal antibody. Virus was incubated with monoclonal antibody HCMV-16, monoclonal antibody HCMV-37, or monoclonal antibody HCMV-39; the last two have specificities for HCMV gB, both in the presence and absence of added rabbit complement. Residual nonneutralized virus was assayed by plaque formation in MRC5 cells, and the percent plaque reduction was calculated.

mately 86 kDa from HCMV and recombinant vaccinia virus-infected cell lysates, although minor variations in mobility were detected. These differences were not due to the host cell, since gH precipitated from recombinant vaccinia virus-infected MRC5 cells was indistinguishable from that from CV1 cells (data not shown). We believe that the 86-kDa species represents a glycosylated form of the gH gene product since in the presence of tunicamycin a protein of this relative molecular mass was no longer seen. Our inability to detect a protein in the presence of tunicamycin suggests that the precursor form of gH may be unstable in the absence of N-linked carbohydrate. Alternatively, the antigenic determinant on gH seen by the monoclonal antibody and polyclonal antiserum may be dependent upon the presence of carbohydrate.

Results of immunofluorescence studies indicated that there are differences in cellular localization. On the basis of the reaction with monoclonal antibody HCMV-16, no evidence of cell surface expression of gH was obtained in the recombinant vaccinia virus-infected cell. This is in marked contrast to results obtained with HCMV-gB (8) and other viral glycoproteins expressed in vaccinia virus, in which the foreign protein is distributed normally in the cells infected with the virus recombinant (2, 35, 36, 41, 42, 44). The failure to obtain surface expression of the HCMV-16 epitope in HCMV-gH-VAC-infected cells, together with the pronounced nuclear membrane staining, suggests that there is a block in the transport of gH to the cell surface. Additionally, the prominent intensely stained cytoplasmic body in HCMV-infected cells was absent in recombinant vaccinia virus-infected cells. The finding of only one body per cell and its positioning adjacent to the nuclear membrane suggests that this may be the Golgi complex. Although the HCMV-infected cell F_c-binding activity seen with rabbit serum is also associated with this cytoplasmic body (unpublished data), an unrelated γ_1 murine monoclonal antibody gave a quantitatively and qualitatively different staining pattern from that seen with a HCMV-gH-specific monoclonal antibody (Fig. 5).

While the translocation of viral glycoproteins to specific cellular compartments is generally determined intrinsically, as suggested by results of experiments in which either heterologous virus vectors or transfection was used to introduce virus genes into cells (for example, see Gething and Sambrook [14] and vaccinia virus recombinants [see above]), it is possible that the cellular distribution of the gene products may also be influenced by other factors. The kinetics of processing of cloned gene products, and hence their transport, may differ from those in infected cells, as has been shown for HSV type 2 gD (17). Johnson and Smiley (17) have also suggested that the distribution of the glycoprotein may be influenced by the presence of other viral gene products. Furthermore, mutants of HSV have been isolated that exhibit greatly reduced cell surface expression of viral glycoproteins, despite normal levels of glycoprotein synthesis (27), and the coding region involved has recently been sequenced and found to be conserved in EBV (28). It is therefore conceivable that the normal transport of HCMV-gH by the Golgi complex to the cell surface requires other HCMV-specific gene products; indeed, HCMV infection is known to modify the ultrastructure of the Golgi complex (37). It is equally possible that the vaccinia virus infection produces an intracellular environment that is detrimental to the proper processing of HCMV-gH, the proper translocation of HCMV-gH, or both.

Homologs of HSV type 1 gH have been found in members of the alpha, beta, and gammaherpesvirus subgroups (15, 24; this report); and it is therefore probable that, together with gB, gH will be found in all herpesviruses. The functions of gH are unknown, but HSV type 1 antibody to gH efficiently neutralizes virus infectivity in the absence of complement and prevents the intercellular spread of infectivity, implying that there is a role for gH in virus entry and exit or in the formation of intercellular junctions (6, 15). Similar observations have been made with antibody against the VZV homolog (19). Efficient neutralization of infectivity by antibody to the HCMV homolog suggests that the gH function is conserved among the herpesvirus subgroups, and it now appears likely that gH represents a neutralizing target in all herpesviruses.

ACKNOWLEDGMENTS

This study was supported in part by funds from Cogent Ltd. and by the Medical Research Council of the United Kingdom.

We are grateful to the generosity of J. D. Oram and P. J. Greenaway for providing clones of HCMV DNA. We thank Joan Williamson and Y. S. Chan for technical assistance.

ADDENDUM

Rassmussen et al. (33) described a monoclonal antibody, 1G6, that precipitated a glycoprotein of HCMV that they referred to as p86. We have used antibody 1G6 (a gift from L. Rassmussen) to immunoprecipitate the HCMV glycoprotein expressed by the recombinant vaccinia virus HCMV-gH-VAC described in this report. The HCMV glycoprotein whose sequence and expression we describe here and which, on the basis of amino acid homology, we have called HCMV-gH, therefore corresponds to the polypeptide species designated p86 by Rassmussen et al. (33).

LITERATURE CITED

1. **Baer, R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, C. Seguin, P. S. Tuffnell, and B. G. Barrell.** 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature (London) **310**:207–211.
2. **Ball, L. A., K. K. Y. Young, K. Anderson, P. L. Collins, and G. W. Wertz.** 1986. Expression of the major glycoprotein G of human respiratory syncytial virus from recombinant virus vectors. Proc. Natl. Acad. Sci. USA **83**:246–250.

3. **Bankier, A. T., and B. G. Barrell.** 1983. Shotgun DNA sequencing, p. 1–34. *In* R. A. Flavell (ed.), Techniques in the life sciences, vol. B508. Elsevier Scientific, Dublin.
4. **Britt, W. J.** 1984. Neutralizing antibodies detect a disulphide-linked glycoprotein complex within the envelope of human cytomegalovirus. Virology **135**:369–378.
5. **Britt, W. J., and D. Auger.** 1985. Identification of a 65,000 dalton virion envelope protein of human cytomegalovirus. Virus Res. **4**:31–36.
6. **Buckmaster, E. A., J. Gompels, and A. Minson.** 1984. Characterisation and physical mapping of an HSV-2 glycoprotein of approximately 115 × 10^3 molecular weight. Virology **139**:408–413.
7. **Chakrabarti, S., K. Brechling, and B. Moss.** 1985. Vaccinia virus expression vector: coexpression of β-galactosidase provides visual screening of recombinant virus plaques. Mol. Cell. Biol. **5**:3403–3409.
8. **Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith.** 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J. **5**:3057–3063.
9. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella zoster virus. J. Gen. Virol. **67**:1759–1816.
10. **Davison, A. J., and P. Taylor.** 1987. Genetic relations between varicella-zoster virus and Epstein-Barr virus. J. Gen. Virol. **68**: 1067–1079.
11. **Durack, D. T.** 1981. Opportunistic infections and Kaposi's sarcoma in homosexual men. N. Engl. J. Med. **305**:1465–1467.
12. **Farrar, G. H., and P. J. Greenaway.** 1986. Characterization of glycoprotein complexes present in human cytomegalovirus envelopes. J. Gen. Virol. **67**:1469–1473.
13. **Furukawa, T., E. Hornberger, S. Sakuma, and S. A. Plotkin.** 1975. Demonstration of immunoglobulin G receptors induced by human cytomegalovirus. J. Clin. Microbiol. **2**:332–336.
14. **Gething, M.-J., and J. Sambrook.** 1981. Cell-surface expression of influenza haemagglutinin from a cloned DNA copy of the RNA gene. Nature (London) **293**:620–625.
15. **Gompels, U., and A. Minson.** 1986. The properties and sequence of glycoprotein H of herpes simplex virus type 1. Virology **153**: 230–247.
16. **Gonczol, E., F. Hudecz, J. Ianacone, B. Dietzschold, S. Starr, and S. A. Plotkin.** 1986. Immune responses to isolated human cytomegalovirus envelope proteins. J. Virol. **58**:661–664.
17. **Johnson, D. C., and J. R. Smiley.** 1985. Intracellular transport of herpes simplex gD occurs more rapidly in uninfected cells than in infected cells. J. Virol. **54**:682–689.
18. **Kari, B., N. Lussenhop, R. Goertz, M. Wabuke-Bunoti, R. Radeke, and R. Gehrz.** 1986. Characterization of monoclonal antibodies reactive to several biochemically distinct human cytomegalovirus glycoprotein complexes. J. Virol. **60**:345–352.
19. **Keller, P. M., A. J. Davison, R. S. Lowe, M. W. Riemon, and R. W. Ellis.** 1987. Identification and sequence of the gene encoding gp111, a major glycoprotein of varicella-zoster virus. Virology **157**:526–533.
20. **Law, K. M., P. Wilton-Smith, and G. H. Farrar.** 1985. A murine monoclonal antibody recognising a single glycoprotein within a human cytomegalovirus virion envelope glycoprotein complex. J. Med. Virol. **17**:255–266.
21. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
22. **Mackett, M., G. L. Smith, and B. Moss.** 1984. General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. J. Virol. **49**:857–864.
23. **Mackett, M., G. L. Smith, and B. Moss.** 1985. The construction and characterisation of vaccinia virus recombinants expressing foreign genes, p. 191–211. *In* D. M. Glover, (ed.), DNA cloning: a practical approach. Methuen Inc., New York.
24. **McGeoch, D. J., and A. J. Davison.** 1986. DNA sequence of the herpes simplex virus type 1 gene encoding glycoprotein gH and identification of homologues din the genomes of varicella-zoster virus and Epstein-Barr virus. Nucleic Acids Res. **14**:4281–4292.
25. **Meyers, J. D.** 1985. Cytomegalovirus infection after organ allografting. Prospects for immunoprophylaxis, p. 201. *In* B. Roizman and C. Lopez (ed.), The herpesviruses. Vol. 4. Plenum Publishing Corp., New York.
26. **Oram, J. D., R. G. Downing, A. Akrigg, C. J. Doggleby, G. W. G. Wilkinson, and P. J. Greenaway.** 1982. Use of recombinant plasmids to investigate the structure of the human cytomegalovirus genome. J. Gen. Virol. **59**:111–129.
27. **Pancake, B. A., D. P. Aschman, and P. A. Schaffer.** 1983. Genetic and phenotypic analysis of herpes simplex type 1 mutants conditionally resistant to immune cytolysis. J. Virol. **47**:568–585.
28. **Pellet, P. E., F. J. Jenkins, M. Ackermann, M. Sarmiento, and B. Roizman.** 1986. Transcription initiation sites and nucleotide sequence of a herpes simplex virus 1 gene conserved in the Epstein-Barr virus genome and reported to affect the transport of viral glycoproteins. J. Virol. **60**:1134–1140.
29. **Pereira, L., M. Hoffman, D. Gallo, and N. Cremer.** 1982. Monoclonal antibodies to human cytomegalovirus: three surface membrane proteins with unique immunological and electrophoretic properties specify cross-reactive determinants. Infect. Immun. **36**:924–932.
30. **Pereira, L., M. Hoffman, M. Tatsuno, and D. Dondero.** 1984. Polymorphism of human cytomegalovirus glycoproteins characterized by monoclonal antibodies. Virology **139**:73–86.
31. **Rasmussen, L., J. Mullenax, M. Nelson, and T. C. Merigan.** 1985. Human cytomegalovirus polypeptides stimulate neutralizing antibody in vivo. Virology **145**:186–190.
32. **Rasmussen, L., J. Mullenax, R. Nelson, and T. C. Merigan.** 1985. Viral polypeptides detected by a complement-dependent neutralizing murine monoclonal antibody to human cytomegalovirus. J. Virol. **55**:274–280.
33. **Rasmussen, L., R. Nelson, D. Kelsall, and T. Merigan.** 1984. Murine monoclonal antibody to a single protein neutralizes the infectivity of human cytomegalovirus. Proc. Natl. Acad. Sci. USA **81**:876–880.
34. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
35. **Smith, G. L., J. Levin, P. Palese, and B. Moss.** 1987. Synthesis and cellular location of the ten influenza polypeptides individually expressed by recombinant vaccinia viruses. Virology **160**: 336–345.
36. **Smith, G. L., M. Mackett, and B. Moss.** 1983. Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. Nature (London) **302**:490–495.
37. **Smith, J. D., and D. DeHarven.** 1973. Herpes simplex virus and human cytomegalovirus replication in WI-38 cells. I. Sequence of viral replication. J. Virol. **12**:919–930.
38. **Staden, R.** 1982. An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. Nucleic Acids Res. **10**:2951–2961.
39. **Staden, R.** 1984. Graphic methods to determine the function of nucleic acid sequences. Nucleic Acids Res. **12**:521–538.
40. **Stagno, S., R. F. Pass, M. E. Dworsky, and C. A. Alford.** 1983. Congenital and perinatal cytomegalovirus infections. Seminars Perinatal. **7**:31–42.
41. **Stephens, E. B., R. W. Compans, P. Earl, and B. Moss.** 1986. Surface expression of viral glycoproteins is polarized in epithelial cells infected with recombinant vaccinia viral vectors. EMBO J. **5**:237–245.
42. **Sullivan, V., and G. L. Smith.** 1987. Expression and characterisation of herpes simplex virus type 1 (HSV1) glycoprotein G by recombinant vaccinia virus: neutralisation of HSV1 infectivity with anti gG antibody. J. Gen. Virol. **68**:2587–2598.
43. **Wentworth, B. B., and E. R. Alexander.** 1971. Seroepidemiology of infections due to members of the herpesvirus group. Am. J. Epidemiol. **94**:496–507.
44. **Wiktor, T. J., R. I. Macfarlan, K. J. Reagan, B. Dietzschold, P. J. Curtis, W. H. Wunner, M. P. Kieny, R. Lathe, J. P. Lecocq, M. Mackett, B. Moss, and H. Koprowski.** 1984. Protection from rabies by a vaccinia virus recombinant containing the rabies virus glycoprotein gene. Proc. Natl. Acad. Sci. USA **81**:7194–7198.