Journal of General Virology (1994), **75**, 183–188. *Printed in Great Britain*

Expression of the murine cytomegalovirus glycoprotein H by recombinant vaccinia virus

Maria Rapp,[1] Pero Lučin,[1] Martin Messerle,[1] Lambert C. Loh[2] and Ulrich H. Koszinowski[3]*

[1] *Department of Virology, Institute for Microbiology, University of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany,* [2] *Department of Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0 and* [3] *Department of Virology, Center for Hygiene, University of Heidelberg, Im Neuenheimer Feld 324, D-69120 Heidelberg, Germany*

The sequence of the gene encoding glycoprotein H (gH) of murine cytomegalovirus (MCMV) strain Smith was determined and compared with the sequence of the gH of MCMV strain K181. Transcriptional analysis showed that gH is encoded by a large mRNA of 5·0 kb, which is synthesized late in infection. A recombinant vaccinia virus expressing the MCMV gH open reading frame was constructed (Vac-gH). Anti-MCMV serum precipitated a protein of 87K from Vac-gH-infected cells. Reactivity with a monoclonal antibody showed the identity of the MCMV gH with a 87K envelope glycoprotein described previously by Loh and Qualtiere. Immunization of mice with the Vac-gH recombinant gave rise to an anti-gH serum, which neutralized MCMV without complement *in vitro*.

Murine cytomegalovirus (MCMV) is a close relative of human cytomegalovirus (HCMV), which is a ubiquitous human pathogen. Most primary infections cause only mild if any symptoms of sickness. However, in an immunologically immature host, such as the fetus, as well as in immunocompromised individuals HCMV is a cause of serious illness and often death (Ho, 1991; Pass, 1991). Because of the species specificity of HCMV, the availability of an appropriate animal model is important. MCMV is widely used as such a model because of the many similarities between human and murine infection with respect to biology and pathogenesis.

To prevent the risks of intra-uterine HCMV infection attenuated virus strains are candidates for vaccines. Treatment of HCMV disease in immunodeficient patients is even more difficult and presently the combined application of drugs and anti-HCMV antibodies is regarded as being most successful. To avoid the risks associated with live vaccines, the application of subunit vaccines based upon the surface glycoproteins of the virus has been proposed. Envelope glycoproteins of herpesviruses are known to be antigens for both cellular and humoral immune response (Spear, 1985). Their property of serving as targets for neutralizing antibodies is of particular interest.

Two glycoproteins are known to be conserved among all herpesviruses, namely the glycoprotein B (gB) and the glycoprotein H (gH). The two glycoproteins are essential and probably play a role in virus entry and cell-to-cell spread. They are conserved in several aspects including genomic location of the genes, amino acid sequence, tertiary structure and probably function. The gB appears to be the major target for the host immune response against HCMV, and a high proportion of the neutralizing antibodies in human antisera are directed against gB (Britt *et al.*, 1990). The recombinant protein induces a neutralizing antibody response (Cranage *et al.*, 1986) and gB is also a target for human cytotoxic T lymphocytes (Borysiewicz *et al.*, 1988).

So far, most investigations concerning glycoproteins have concentrated on gB, whereas much less is known about gH, although it is an essential protein as well and capable of inducing neutralizing antibodies. Recently, the gH gene of the MCMV strain K181 has been identified and sequenced (Xu *et al.*, 1992). After having characterized and expressed the gB gene (Rapp *et al.*, 1992) we examined the sequence and expression of the gH gene of the MCMV strain Smith with the intention of investigating the MCMV glycoproteins further in view of their usefulness as subunit vaccines.

The nucleic acid sequence of the gH gene of the MCMV strain Smith was determined by a modified version of the dideoxynucleotide sequencing method (Tabor & Richardson, 1987). The GCG software

The nucleotide sequence reported in this paper has been submitted to the GenBank database and assigned the accession number L18782.

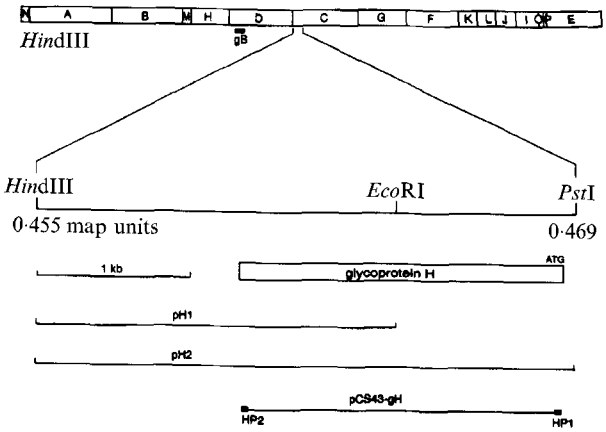

Fig. 1. Location of the gH coding region and plasmids. Top: *Hin*dIII cleavage map of the MCMV (Smith strain) genome. The black bar shows the location of the gB gene. Middle, expanded: restriction enzyme sites used for gH gene characterization and cloning purposes. Below: the gH ORF and subclones of the *Hin*dIII C fragment are indicated. Primers HP1 and HP2 which were used for amplification of the gH ORF from the MCMV *Hin*dIII C fragment are shown as small black bars.

package version 7.0 (Devereux *et al.*, 1984) was used for the analysis of the nucleic acid sequence, for the deduction of the amino acid sequence, and for the comparison with the sequence of strain K181. Assuming no differences between the two MCMV strains regarding the genomic location of the gene, a 2·5 kb *Hin*dIII–*Eco*RI fragment of the *Hin*dIII C fragment of MCMV strain Smith was subcloned (pH1, Fig. 1) and sequenced at the *Eco*RI end. The nucleotide sequence of this region showed 95% identity to the published K181 strain gH sequence. Then, a further subclone was constructed from the *Hin*dIII C fragment (pH2, Fig. 1) which should contain the complete open reading frame (ORF). The DNA sequence was determined on both strands by either using Bal 31-generated subclones of the plasmid pH1 or sequencing of pH1 and pH2 with synthetic oligonucleotide primers. The analysis revealed a single, long ORF of 2175 nucleotides capable of encoding a protein of 725 amino acids. Thus, the gH of MCMV strain Smith is one amino acid longer than that of MCMV strain K181. The identity of the genes is 98% on the nucleotide level and 95% on the amino acid level. The differences are clustered in three short regions (Fig. 2). The amino acid exchanges in region 1 result from several differences in the nucleotide sequences. The differences occurring in regions 2 and 3 are due to short frameshifts. Besides these regions there are only two other amino acid differences. One occurs at position 322, the other at position 664.

Genes of herpesviruses often show rather complicated transcriptional patterns as exemplified by the mRNAs of the HCMV and MCMV polymerase and gB genes

(*a*)

```
REGION 1:
1256  GTTCCAGACGAGGATGCAGCTCACCGAAAAACACAAACCCGCACATTCCAGCAACGCGAT
      ||| |||||||||||||| || ||| || |||||||||| || |  | ||||||| ||||
1415  GTTTCAGACGAGGATGCAAATCGCCGCTAATCACAAACCCACAAAGCCAAGCAACTCGAT

REGION 2:
1721  TCA.GGGCCCTGATGGAGTCGGCGCACCGTCCGGAGAGACGGGCCGAACA
      ||| ||||||||||||||||||||||||||||||||||||| |||||||||
1880  TCAGGGGCCCTGATGGAGTCGGCGCACCGTCCGGAGAGAC.GGCCGAACA

REGION 3:
1970  CTGGACCGCAGGAGTCCCTGTGTCCTGTCGAGGAGCTACCGCGAACGCGGA
      |||||||||| ||| ||||| |||||||||||||||||||||||| ||||
2129  CTGGACCGCA.GAGCCCCTGCGTCCTGTCGAGGAGCTACCGCGAA..CGGA
```

(*b*)

```
  1  MKLSLILSIALCSTRVVYAAGAEAPRISRNTVKLHSYNESRVCRHDESSNQTVSHAAMFT
  1

 61  FNFQDGDGYRVYQVPRCLFNTHAAREVLSSVDMTETLESYRKRFRVYFVVPIYGAYRLVA
 61

121  RSPTAKYPGGVLNPPPASSVTMQDLIVDATNIHTVVPDKLCVITEHPVIFSMKVPCSHQV
121

181  ITWTGYTVTVSLAQKFFVLTIKPTRDHTSENTLAMFFGDVREVDLKAPYTVGAFLLRQTP
181

241  DHDLLVVVKQTAFIQRYMFLTDVVFLQRTLSADYADTSVCLRVLSVLASVVARGKQCGLI
241

301  TRDTVEFFFTYSLCQLMANGTRYQSTAPVSTALWRQSELELFGEFIRHCFKTTTPNPTPA
301                    S
             Region 1
361  FQTRMQLTEKHKPAHSSNAIDVRVLAATYSSGMHAASMADLAFLLRSTRIPPNVNTDALL
361       IAAN   TKP  S

421  QKLLFTTDAYYRMSLKIPLSGSMRRILIRVDLTVRTQLNESSVARRHFVLLTSMCSPREQ
421
                                        Region 2
481  ISWGELLMNPQRGAPSEIYSPCVSGGRRDYTGPSVRALMESAHRPERRAEQVMSVTEALR
481                                    GPDGVGAPSGET

541  PKRSQMSDEANCVPDSTQGAVITANEKTYLISSDFIVKGLAIPVSNTVVDRNLMITVLDR
541
       Region 3
601  RSPCVLSRSYRERGSVIVMNNITFTERCEFCASTLVEYDEVDGLTSIMHIPSIEVLKYLT
601   A AS.C GATAN

661  DPENDILVATPRVHYLLLTANGTVFEVTDILVNVRPSMPYSVVVALVIIAILMALGLYRL
660    D

721  CRQKR  725
720        724
```

Fig. 2. (*a*) Comparison of parts of the nucleotide sequence of the MCMV strain Smith (above) and K181 (below) gH ORFs. The three diverging regions are indicated. (*b*) Comparison of the deduced amino acid sequences of the MCMV strains Smith (above) and K181 (below) gH homologues. The sequences are displayed in the one-letter amino acid code. Only amino acid differences are shown in the K181 strain sequence.

(Kouzarides *et al.*, 1987; Elliott *et al.*, 1991; Rapp *et al.*, 1992). To elucidate the situation for the gH gene, whole-cell RNA was prepared from MCMV-infected cells at different times after infection following published procedures (Chirgwin *et al.*, 1979), and Northern blot hybridization was performed. A double-stranded probe specific for the gH region revealed two fragments of 5·0 kb and 5·8 kb (data not shown). To discriminate between the gH encoding transcript and other transcripts of that region, strand-specific probes were prepared from a 1·1 kb fragment from the 3′-terminal half of the gH

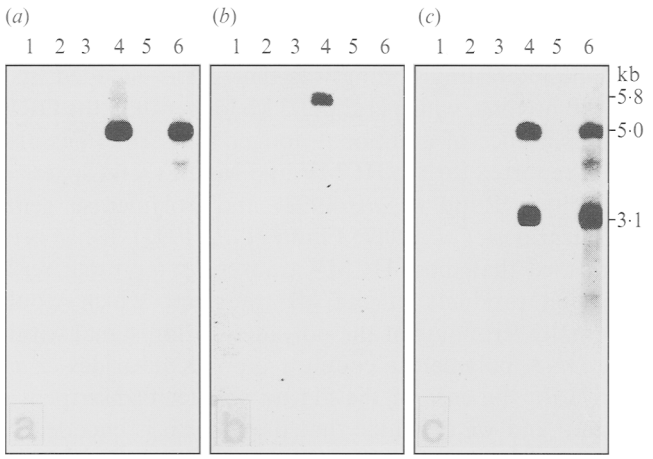

Fig. 3. Transcriptional analysis of gH gene expression kinetics. RNA was prepared at different times post-infection and analysed by Northern blot hybridization with different strand-specific probes: (*a*) probe a, (*b*) probe b and (*c*) probe c. Lanes 1, mock-infected; lanes 2, 4 h p.i.; lanes 3, 8 h p.i.; lanes 4, 16 h p.i.; lanes 5, 24 h p.i. in the presence of phosphonoacetic acid; lanes 6, 24 h p.i. Primers used for the generation of strand-specific probes are shown as small black bars. The arrows indicate the direction of the primer extension.

ORF (Fig. 3, probes a and b) and 'run-off' synthesis was carried out (Stürzl & Roth, 1990). Probe a, which is specific for the coding strand, reacted with the 5·0 kb band. The probe specific for the non-coding strand (b) hybridized with a transcript of 5·8 kb. Probe c, which is located in the *Hin*dIII D fragment about 2·0 kb downstream of the gH ORF (Fig. 3*c*) also reacted with the 5·0 kb RNA plus an additional RNA of 3·1 kb. All RNAs are expressed late in infection, i.e. after 16 h post-infection (p.i.) (Keil *et al.*, 1984). Since they were not synthesized after the DNA polymerase inhibitor phosphonoacetic acid had been added to infected cells we conclude that the transcription of the gH gene is restricted to the late phase of infection.

A vaccinia virus recombinant (Vac-gH) expressing the complete gH ORF was generated as follows. For the construction of the recombinant plasmid pCS43-gH a 2·1 kb fragment was amplified by PCR from the *Hin*dIII C fragment of MCMV (Ebeling *et al.*, 1983) using the primers HP1 and HP2 which included a *Bam*HI restriction site for cloning purposes (Fig. 1). The generation of a fragment of the correct size was confirmed by gel electrophoresis. The fragment was isolated, cut with *Bam*HI and inserted into the vaccinia

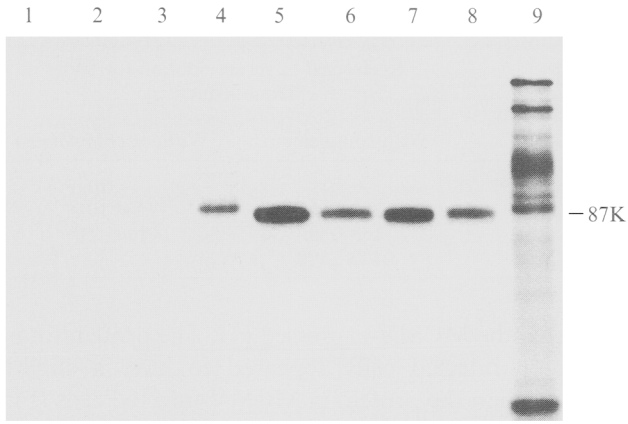

Fig. 4. Detection of gH in MCMV- and Vac-gH-infected cells. Lanes 1, 4 and 9, MCMV-infected cells; lane 2, mock-infected; lane 3, Vac-infected; lanes 5 and 7, VacgH1-infected; lanes 6 and 8, VacgH2-infected cells. Proteins were precipitated by a pre-immune serum (lane 1), an anti-MCMV serum (lanes 7 to 9) and the monoclonal antibody 8D1.22A (lanes 2 to 6). The position corresponding to an M_r of 87K is indicated on the right.

virus recombination vector pCS43 (Arnold *et al.*, 1990) downstream of the vaccinia virus early-late promoter p7.5. The correct position of the start codon was confirmed by sequencing of the resulting plasmid pCS43-gH. The vaccinia virus recombinants were produced following established procedures (Mackett *et al.*, 1984) using the DNA of vaccinia virus strain Copenhagen and the temperature-sensitive mutant ts7 (Drillien & Spehner, 1983).

To detect gH expression in Vac-gH-infected cells two peptides representing amino acids 476 to 495 (PepgHI) and 504 to 523 (PepgHII) were selected from the deduced amino acid sequence of the ORF and anti-peptide sera were generated in rabbits. Western blotting with these sera confirmed the expression of the gH in Vac-gH-infected cells (data not shown). To characterize the gH gene products further, immunoprecipitations were carried out as described previously (Keil *et al.*, 1985). Proteins of MCMV-infected cells were labelled with [^{35}S]methionine from 20 to 28 h p.i. Similarly, proteins of Vac-gH-infected cells were labelled from 4 to 8 h p.i. Samples of cell lysates were incubated with anti-MCMV serum or ascitic fluid of the monoclonal antibody 8D1.22A (Loh & Qualtiere, 1988). The precipitated proteins were separated on 7·5% SDS–polyacrylamide gels and visualized by fluorography. In MCMV- and Vac-gH-infected cells, a protein with the M_r of 87K was recognized by the monoclonal antibody 8D1.22A (Fig. 4). Anti-MCMV serum also precipitated a 87K protein from Vac-gH-infected cells. In MCMV-infected cells a number of proteins are recognized by this serum, among them a protein with an M_r similar to the 87K protein precipitated from Vac-gH-infected cells, which probably

Table 1. *Neutralization of MCMV by different antisera*

Antiserum	Reciprocal of serum dilution for 50% plaque reduction	
	With complement	Without complement
Anti-Vac-pp89	< 10	< 10
Anti-Vac-gB	200–700	< 10
Anti-Vac-gH	150–280	100–250
Anti-MCMV	2400–2800	40

represents the MCMV-expressed gH. The protein found in Vac-gH-infected cells appeared to have a slightly lower M_r than the protein in MCMV-infected cells (Fig. 4). The positive reaction with the neutralizing monoclonal antibody 8D1.22A showed that the 87K envelope glycoprotein described previously (Loh & Qualtiere, 1988) is indeed the MCMV gH.

To determine the neutralizing capacity of an anti-gH serum raised in mice by infection with the Vac-gH recombinant a plaque reduction assay was performed as described previously (Rapp *et al.*, 1992). An anti-Vac-gB serum and an anti-MCMV serum raised in mice by infection with MCMV were included in the plaque reduction assay for comparison. As a negative control a recombinant vaccinia virus expressing the immediate early protein pp89 was used, which does not induce a neutralizing antibody response (Jonjić *et al.*, 1988). Sera against the vaccinia virus recombinants were generated by immunizing the mice intravenously with two doses of 1×10^8 p.f.u. MCMV given 3 weeks apart. Animals were bled 3 weeks after the boost. Whereas the anti-Vac-pp89 serum was not able to neutralize the virus, the anti-gH, anti-gB and anti-MCMV antisera showed considerable neutralizing activity (Table 1). The anti-gH serum was capable of neutralizing MCMV in the presence or absence of complement. The complement-independent neutralizing activity of the anti-gH serum was about fivefold higher than that of the anti-MCMV serum.

Our data show that the sequences of the gH of the MCMV strains Smith and K181 are almost identical. The differences are clustered in three regions. The lower percentage of identity at the amino acid level (95%) compared to the nucleotide level (98%) is caused by two small frameshifts that result in completely different amino sequences (Fig. 2*a* and *b*, regions 2 and 3). However, we cannot rule out the possibility that these differences are due to sequencing mistakes, since they are located in GC-rich regions with palindromic structures. We believe that our sequence is correct since it was obtained by sequencing both strands of this region several times and using deaza-dGTP to avoid compressions. The identity found between the gH of different MCMV strains is comparable to that found with the HCMV gH (Pachl *et al.*, 1989). In the case of the MCMV gB the comparison of the nucleotide sequence of the Smith strain gB to the 3′-terminal part of the K181 strain gB sequence also showed a high level of identity (97%) (Rapp *et al.*, 1992; Elliott *et al.*, 1991). However, as only part of the K181 strain gB sequence has been published, it is difficult to assess the full extent of the differences. For HCMV the gH sequence is more conserved between different strains than is the gB sequence (Chou, 1992), which is apparently the case for MCMV also.

One interesting finding was that gH is encoded by a 5·0 kb mRNA, which is about 3 kb longer than the ORF. The existence of additional, longer transcripts has also been reported for the HCMV and MCMV gB (Spaete *et al.*, 1988; Rapp *et al.*, 1992) and polymerase genes (Kouzarides *et al.*, 1987; Elliott *et al.*, 1991). It has been proposed that these HCMV transcripts represent read-through products of the gB transcript which would normally terminate at the polyadenylation signal within the DNA polymerase coding region (Kouzarides *et al.*, 1987). In the case of the gH no smaller transcript was found, and we conclude that the protein is encoded by the 5·0 kb transcript. That means that the gH transcript does not terminate at the potential polyadenylation signal sequence immediately downstream of the ORF as proposed by Xu *et al.* (1992) but continues through to the next gene and probably stops after the dUTPase gene.

To conduct *in vivo* protection experiments the gH ORF was inserted into the vaccinia virus genome. The protein expressed by the recombinant vaccinia virus Vac-gH had approximately the same size (87K) as the gH protein in MCMV-infected cells (Fig. 4). The reaction with the monoclonal antibody 8D1.22A revealed the identity of the gH with the 87K protein described by Loh and Qualtiere. They have shown that this 87K protein is a glycoprotein and is present on the surface of the virion as determined by surface iodination reaction (Loh & Qualtiere, 1988). The small difference in size between the vaccinia virus-expressed gH and the authentic protein in MCMV-infected cells may be due to different glycosylation of the protein in Vac-gH-infected CV-1 cells in contrast to MCMV-infected mouse embryo fibroblasts. Alternatively, the difference may reflect improper processing of gH in Vac-gH-infected cells. Several authors have found that the gH homologues of other herpesviruses need an accessory small protein for proper folding, subsequent processing and transport to the cell surface (Hutchinson *et al.*, 1992; Spaete *et al.*, 1993). This small glycoprotein was named gL in herpes simplex virus (HSV) (Hutchinson *et al.*, 1992). The vaccinia virus-expressed HCMV gH for example also shows a slightly reduced electrophoretic mobility compared to the authentic protein and is not transported to the cell surface (Cranage *et al.*, 1988). In immunoprecipitation

experiments with iodinated surface proteins we also found no evidence for the surface expression of the MCMV gH in Vac-gH-infected cells (data not shown). In cells that co-express gH and gL, transportation of the HCMV gH to the cell surface can be observed (Spaete *et al.*, 1993). To what extent the improper processing of the gH has an effect on its antigenic properties remains to be established.

Typically for herpesviral gH homologues the MCMV gH is recognized by a monoclonal antibody (8D1.22A) that shows complement-independent neutralization (Loh & Qualtiere, 1988). In addition, immunization with the Vac-gH recombinant induced considerable complement-independent neutralization activity in mice. In fact, the neutralizing capacity of the Vac-gH-induced antiserum was about fivefold higher than that of the anti-MCMV serum without complement. These results suggest that there are fewer anti-gH antibodies in the anti-MCMV serum than in the monospecific anti-gH serum. This is in accordance with the fact that gH is apparently less abundant in MCMV-infected cells than in Vac-gH-infected cells (data not shown). Furthermore, these data show that, even in the absence of the MCMV gL, the Vac-gH recombinant expresses the protein in a form that is strongly immunogenic and antigenic. This is apparently not the case for the HSV gH, because no or only a little neutralization activity was induced by the HSV gH expressed by vaccinia virus or baculovirus (Blacklaws *et al.*, 1990; Forrester *et al.*, 1991; Ghiasi *et al.*, 1992). Thus, the gL is not a stringent prerequisite for the formation of immunogenic domains of gH giving rise to neutralizing antibodies.

Generation of the vaccinia virus recombinants Vac-gB and Vac-gH provides a tool for studying the immune response against individual glycoproteins. Since the experimental vaccine induces a stronger protein-specific *in vitro* neutralization than the authentic MCMV-expressed protein, *in vivo* protection experiments will determine the active and passive protection potential of the two proteins. As outlined above, the two glycoproteins, especially gB, are extremely conserved in several aspects. Thus, the results of these studies will also be of general interest in view of the evaluation of the two glycoproteins as candidates for subunit vaccines.

We thank A. Lüske for the production of vaccinia virus recombinants and antisera. This work was supported by EC grant BIOT-0191-C and the Deutsche Forschungsgemeinschaft, through SFB 322-C6, and grant Ko 571/11, and grant MT-8196 from the MRC of Canada to L. Loh.

References

ARNOLD, B., MESSERLE, M., JATSCH, L., KÜHLBECK, G. & KOSZINOWSKI, U. H. (1990). Transgenic mice expressing a soluble foreign H-2 class I antigen are tolerant to allogeneic fragments presented by self class I but not the whole membrane-bound alloantigen. *Proceedings of the National Academy of Sciences, U.S.A.* **87**, 1762–1766.

BLACKLAWS, B. A., KRISHNA, S., MINSON, A. C. & NASH, A. A. (1990). Immunogenicity of herpes simplex virus type 1 glycoproteins expressd in vaccinia virus recombinants. *Virology* **177**, 727–736.

BORYSIEWICZ, L. K., HICKLING, J. K., GRAHAM, S., SINCLAIR, J., CRANAGE, M. P., SMITH, G. L. & SISSONS, J. G. (1988). Human cytomegalovirus-specific cytotoxic T cells. *Journal of Experimental Medicine* **168**, 919–931.

BRITT, W. J., VUGLER, L., BUTFILOSKI, E. J. & STEPHANS, E. B. (1990). Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant vaccinia virus-infected cells in analysis of the human neutralizing antibody response. *Journal of Virology* **64**, 1079–1085.

CHIRGWIN, J. M., PRZYBALA, A. E., MACDONALD, R. J. & RUTTER, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294–5299.

CHOU, S. (1992). Molecular epidemiology of envelope glycoprotein H of human cytomegalovirus. *Journal of Infectious Diseases* **166**, 604–607.

CRANAGE, M. P., KOUZARIDES, T., BANKIER, A. T., SATCHWELL, S., WESTON, K., TOMLINSON, P., BARRELL, B., HART, H., BELL, S. E., MINSON, A. C. & SMITH, G. L. (1986). Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. *EMBO Journal* **5**, 3057–3063.

CRANAGE, M. P., SMITH, G. L., BELL, S. E., HART, H., BROWN, C., BANKIER, A. T., TOMLINSON, P., BARRELL, B. G. & MINSON, T. C. (1988). 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. *Journal of Virology* **62**, 1416–1422.

DEVEREUX, J., HABERLI, P. & SMITHIES, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Research* **12**, 387–395.

DRILLIEN, R. & SPEHNER, D. (1983). Physical mapping of vaccinia virus temperature-sensitive mutations. *Virology* **131**, 385–393.

EBELING, A., KEIL, G. M., KNUST, E. & KOSZINOWSKI, U. H. (1983). Molecular cloning and physical mapping of murine cytomegalovirus DNA. *Journal of Virology* **47**, 421–433.

ELLIOTT, R., CLARK, C., JAQUISH, D. & SPECTOR, D. (1991). Transcription analysis and sequence of the putative murine cytomegalovirus DNA polymerase gene. *Virology* **185**, 169–186.

FORRESTER, A. J., SULLIVAN, V., SIMMONS, A., BLACKLAWS, B. A., SMITH, G. L., NASH, A. A. & MINSON, A. C. (1991). Induction of protective immunity with antibody to herpes simplex virus type 1 glycoprotein H (gH) and analysis of the immune response to gH expressed in recombinant vaccinia virus. *Journal of General Virology* **72**, 369–375.

GHIASI, H., KAIWAR, R., NESBURN, A. B. & WECHSLER, S. L. (1992). Baculovirus-expressed glycoprotein H of herpes simplex virus type 1 (HSV-1) induces neutralizing antibody and delayed type hypersensitivity responses, but does not protect immunized mice against lethal HSV-1 challenge. *Journal of General Virology* **73**, 719–722.

HO, M. (1991). *Cytomegalovirus: Biology and Infection*. New York: Plenum Press.

HUTCHINSON, L., BROWNE, H., WARGENT, V., DAVIS-POYNTER, N., PRIMORAC, S., GOLDSMITH, K., MINSON, A. C. & JOHNSON, D. C. (1992). A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. *Journal of Virology* **66**, 2240–2250.

JONJIĆ, S., DEL VAL, M., KEIL, G. M., REDDEHASE, M. J. & KOSZINOWSKI, U. H. (1988). A nonstructural viral protein expressed by a recombinant vaccinia virus protects against lethal cytomegalovirus infection. *Journal of Virology* **62**, 1653–1658.

KEIL, G. M., EBELING-KEIL, A. & KOSZINOWSKI, U. H. (1984). Temporal regulation of murine cytomegalovirus transcription and mapping of viral RNA synthesized at immediately early times after infection. *Journal of Virology* **50**, 784–795.

KEIL, G. M., FIBI, M. R. & KOSZINOWSKI, U. H. (1985). Characterization of the major immediate-early polypeptides encoded by murine cytomegalovirus. *Journal of Virology* **54**, 422–428.

KOUZARIDES, T., BANKIER, A. T., SATCHWELL, S. C., WESTON, K., TOMLINSON, P. & BARRELL, B. G. (1987). Sequence and transcription analysis of the cytomegalovirus DNA polymerase gene. *Journal of Virology* **61**, 125–133.

LOH, L. C. & QUALTIERE, L. F. (1988). A neutralizing monoclonal antibody recognizes an 87K envelope glycoprotein on the murine cytomegalovirus virion. *Virology* **162**, 498–502.

MACKETT, M., SMITH, G. L. & MOSS, B. (1984). General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. *Journal of Virology* **49**, 857–864.

PACHL, C., PROBERT, W. S., HERMSEN, K. M., MASIARZ, F. R., RASMUSSEN, L., MERIGAN, T. C. & SPAETE, R. R. (1989). The human cytomegalovirus strain Towne glycoprotein H gene encodes glycoprotein p86. *Virology* **169**, 418–426.

PASS, R. F. (1991). Clinical importance of CMV. In *Progress in Cytomegalovirus Research*, pp. 3–15. Edited by M. P. Landini. Amsterdam: Elsevier.

RAPP, M., MESSERLE, M., BÜHLER, B., TANNHEIMER, M., KEIL, G. M. & KOSZINOWSKI, U. H. (1992). Identification of the murine cytomegalovirus glycoprotein B gene and its expression by recombinant vaccinia virus. *Journal of Virology* **66**, 4399–4406.

SPAETE, R. R., THAYER, R. M., PROBERT, W. S., MASIARZ, F. R., CHAMBERLAIN, S. H., RASMUSSEN, L., MERIGAN, T. C. & PACHL, C. (1988). Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. *Virology* **167**, 207–225.

SPAETE, R. R., PEROT, K., SCOTT, P. I., NELSON, J. A., STINSKI, M. F. & PACHL, C. (1993). Co-expression of truncated human cytomegalovirus gH with the UL 115 gene product or the truncated human fibroblast growth receptor results in transport of gH to the cell surface. *Virology* **193**, 853–861.

SPEAR, P. G. (1985). Glycoproteins specified by herpes simplex viruses. In *The Herpesviruses*, pp. 315–356. Edited by B. Roizman. New York: Plenum Press.

STÜRZL, M. & ROTH, W. K. (1990). 'Run-off' synthesis and application of defined single-stranded DNA hybridization probes. *Analytical Biochemistry* **185**, 164–169.

TABOR, S. & RICHARDSON, C. C. (1987). DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proceedings of the National Academy of Sciences, U.S.A.* **84**, 4767–4771.

XU, J., DALLAS, P. B., LYONS, P. A., SHELLAM, G. R. & SCALZO, A. A. (1992). Identification of the glycoprotein H gene of murine cytomegalovirus. *Journal of General Virology* **73**, 1849–1854.

(*Received 30 June 1993; Accepted 6 September 1993*)