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Multiple Independent Loci within the Human Cytomegalovirus Unique Short Region Down-Regulate Expression of Major Histocompatibility Complex Class I Heavy Chains

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Reduction of major histocompatibility complex class I cell surface expression occurs in adenovirus-, herpes simplex virus-, human cytomegalovirus (HCMV)-, and murine cytomegalovirus-infected cell systems. Recently, it was demonstrated that the down-regulation mediated by HCMV infection is posttranslational, as a result of increased turnover of class I heavy chains in the endoplasmic reticulum (M. F. C. Beersma, M. J. E. Bijlmakers, and H. L. Ploegh, J. Immunol. 151:4455–4464, 1993; Y. Yamashita, K. Shimokata, S. Saga, S. Mizuno, T. Tsurumi, and Y. Nishiyama, J. Virol. 68:7933–7943, 1994. To identify HCMV genes involved in class I regulation, we screened our bank of HCMV deletion mutants for this phenotype. A mutant with a 9-kb deletion in the S component of the HCMV genome (including open reading frames IRS1 to US9 and US11) failed to down-regulate class I heavy chains. By examining the effects of smaller deletions within this portion of the HCMV genome, a 7-kb region containing at least nine open reading frames was shown to contain the genes required for reduction in heavy-chain expression. Furthermore, it was determined that at least two independent loci within the 7-kb region were able to cause class I heavy-chain down-regulation. One of these, US11, encodes a 32-kDa glycoprotein which causes down-regulation of class I heavy chains in the absence of other viral gene products. Hence, a specific function associated with a phenotype of the HCMV replicative cycle has been mapped to a dispensable gene region. These loci may be important for evasion of the host's immune response and viral persistence.

Human cytomegalovirus (HCMV) is a betaherpesvirus which causes clinically serious disease in immunocompromised and immunosuppressed adults, as well as in some infants infected in utero or perinatally (1). The 230-kb double-stranded DNA genome of HCMV (strain AD169) was entirely sequenced (15) and has at least 200 open reading frames (ORFs). The functions of some HCMV proteins are either known or predicted on the basis of their homology with other virus (especially herpes simplex virus [HSV]) and cellular proteins. However, for the majority of the HCMV ORFs, the functions of the proteins they encode are unknown.

To study HCMV gene function, our strategy has been to construct HCMV deletion mutants and assess their in vitro growth properties (28, 29). This strategy involves site-directed replacement mutagenesis of the selected HCMV gene(s) by a prokaryotic reporter gene, usually the β-glucuronidase gene. In this fashion, the recombinant virus can be isolated only if the replaced viral gene(s) is nonessential. Using this strategy, we have identified 18 HCMV ORFs which are dispensable for viral growth in tissue cultured human fibroblasts (26, 28, 29). Several additional nonessential HCMV genes have been determined by analogous methods in other laboratories (8, 33, 50). Nonessential genes may be required for viral growth in other cell types or for efficient replication or persistence in the infected host.

Many viruses have evolved mechanisms which may modulate or counteract the effect of the immune system of their hosts (20). Although the cellular targets of the viral genes identified so far vary, some target the major histocompatibility complex (MHC) class I heavy-chain proteins (38, 39). Within the past few years, reports from several laboratories have shown that infection by HCMV results in the down-regulation of cellular MHC class I heavy chains (3, 6, 9, 54, 56, 57). This phenomenon has been reported for other DNA viruses, including adenovirus, murine cytomegalovirus (MCMV), and HSV (2, 10, 12, 13, 17, 25, 58). In the adenovirus type 2 and HSV type 1 (HSV-1) systems, the product of a viral gene dispensable for replication in vitro is sufficient to cause the posttranslational down-regulation of MHC class I heavy chains (2, 10, 58). The HCMV UL18 ORF encodes a protein which resembles the heavy chain of MHC class I (5). It was hypothesized that this protein may be involved in HCMV-induced cellular heavy chain down-regulation by competing for association with β-2-microglobulin (9). However, studies with UL18 deletion mutants have dispelled this hypothesis (8). The gene(s) involved in class I heavy-chain down-regulation by MCMV has not yet been identified.

In this study, we begin to address the genetic basis for the down-regulation of MHC class I heavy chains in HCMV-infected cells. Our results demonstrate that (i) down-regulation of MHC class I was observed throughout the infectious cycle, beginning at very early times postinfection (3 h); (ii) deletion of 7 kb within the unique short (U_S) region of the HCMV genome, containing at least nine ORFs (US2 to US11), results in the loss of the wild-type down-regulation phenotype; (iii) there are two independent loci within the 7-kb region containing genes which function independently of the other to cause

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the phenotype; and (iv) the US11 gene product, from one locus, is sufficient for class I heavy-chain down-regulation. Identification of the HCMV gene products which are involved in MHC class I down-regulation is pivotal in determining the mechanism of this important immune evasion strategy. The potential importance of this discovery is threefold: (i) elimination of this region from HCMV may allow for a better immune response to a live virus vaccine; (ii) genes sufficient for class I down-regulation may be of use in gene therapy to minimize the host's immune response (e.g., gene therapy strategies using recombinant adenoviruses); and (iii) knowledge of how the HCMV genes regulate class I may help elucidate early steps in antigen presentation and requirements for stable MHC class I expression.

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MATERIALS AND METHODS

Virus and cells. HCMV strain AD169 was obtained from the American Type Culture Collection and propagated according to standard protocols. Human foreskin fibroblast (HFF) cells were isolated in this laboratory and used below passage 20 (27). U373-MG astrocytoma cells were obtained from the American Type Culture Collection. Both cell types were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES).

DNA sequence. The numbering system of Chee et al. (15) for the HCMV strain AD169 DNA sequence (GenBank accession number X17403) was used in this report.

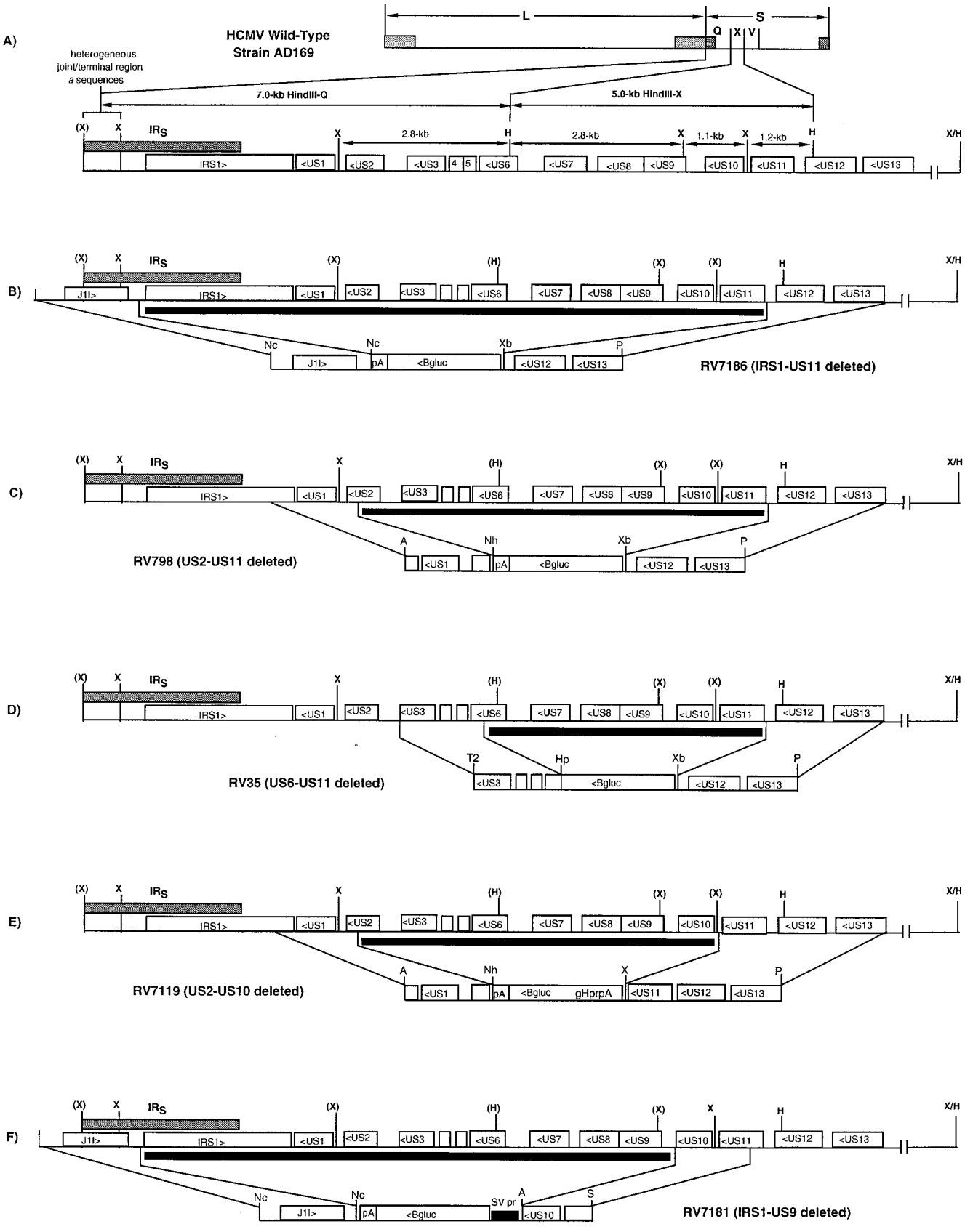
Plasmids. Plasmids used for creation of HCMV mutants were constructed by using the strategies described previously (28, 29). Generally, the β-glucuronidase reporter gene was surrounded on each side by 1.5 kb of HCMV sequences which flank the gene(s) to be deleted from the virus. In each case, the plasmid DNA was linearized with a restriction enzyme which cuts within the prokaryotic backbone prior to transfection. The HCMV strain AD169 genomic DNA fragments were derived from pHind-G, pHind-X, and pXba-P, which contain the *Hin*dIII G (bases 176844 to 195837), *Hin*dIII X (bases 195837 to 200856), and *Xba*I P (bases 200391 to 206314) DNA fragments, respectively (29, 41). pUS7/US3 contains the 1.7-kb *Pst*I-*Pst*I HCMV fragment (bases 196447 to 194741 in pIBI30 vector [International Biotechnologies, Inc.]) derived from pHind-G and pHind-X. To replace HCMV ORFs US11 through IRS1 by β-glucuronidase (i.e., RV7186; Fig. 1B), pBgdUS11/IRS1 was constructed. Sequentially, this plasmid contains the 1.8-kb *Pst*I-*Xba*I fragment (bases 202207 to 200391; from pXba-P), β-glucuronidase, a 288-base simian virus (SV40) fragment containing the early and late polyadenylation signals (from pRcCMV [Invitrogen]), and the 1.7-kb *Nco*I-*Nco*I fragment (bases 189763 to 188062; from pHind-G). To replace HCMV ORFs US11 through US2 by β-glucuronidase (i.e., RV798; Fig. 1C), pBgdUS11/US2 was constructed. Sequentially, this plasmid contains the 1.8-kb *Pst*I-*Xba*I fragment (bases 202207 to 200391), β-glucuronidase, the 255-base fragment containing the US10 polyadenylation signal (bases 199276 to 199021; from pHind-X), and the 1.3-kb *Nhe*I-*Apa*I fragment (bases 193360 to 192033; from pHind-G). To replace HCMV ORFs US11 through US6 by β-glucuronidase (i.e., RV35; Fig. 1D), pBgdUS11/US6 was constructed. Sequentially, this plasmid contains the 1.8-kb *Pst*I-*Xba*I fragment (bases 202207 to 200391), β-glucuronidase, and the 1.5-kb *Hpa*I-*Sst*II fragment (bases 195589 to 194062; from pHind-G). Replacement of HCMV ORFs US11 and US10 or ORF US11 (singly) by β-glucuronisdase (i.e., RV67 and RV699, respectively) was described previously (29). To replace ORFs US10 through US2 by β-glucuronidase (i.e., RV7119; Fig. 1E), pBgdUS10/US2 was constructed. Sequentially, this plasmid contains the 2.52-kb *Pst*I-*Xho*I fragment (bases 202207 to 199683; from pHind-X and pXba-P), a 180-base *Sma*I-*Hae*III fragment containing the HSV-1 gH promoter (40), β-glucuronidase, the 255-base US10 polyadenylation signal fragment, and the 1.3-kb *Nhe*I-*Apa*I fragment (bases 193360 to 192033). To replace HCMV ORFs US9 through IRS1 by β-glucuronidase (i.e., RV7181; Fig. 1F), pBgdUS9/IRS1 was constructed. Sequentially, this plasmid contains the 1.1-kb *Sal*I-*Apa*I fragment (bases 200171 to 199021; from pHind-X), the 351-base SV40 early promoter (from pRcCMV), β-glucuronidase, the 288-base SV40 polyadenylation signal fragment, and the 1.7-kb *Nco*I-*Nco*I fragment (bases 189763 to 188062). To replace HCMV ORFs US6 through IRS1 by β-glucuronidase (i.e., RV7177; Fig. 1G), pBgdUS6/IRS1 was constructed. Sequentially, this plasmid contains the 1.7-kb *Nco*I-*Nco*I fragment (bases 188062 to 189763), β-glucuronidase, the 255-base US10 polyadenylation signal fragment, and the 1.8-kb *Bsm*I-*Sau*I fragment (bases 196222 to 198030; from pHind-X). To replace HCMV ORFs US3 and US2 by β-glucuronidase (i.e., RV47; Fig. 1H), pBgdUS3/US2 was constructed. Sequentially, this plasmid contains the 1.7-kb *Pst*I-*Pst*I fragment (bases 196447 to 194741; from pUS7/US3), the 180-base *Sma*I-*Hae*III fragment containing the HSV-1 gH promoter, β-glucuronidase, the 255-base US10 polyadenylation signal fragment, and the 1.3-kb *Nhe*I-*Apa*I fragment (bases 193360 to 192033). To replace HCMV ORF US1 by β-glucuronidase (i.e., RV5122; Fig. 1I), pBgdUS1 was constructed. Sequentially, this plasmid contains the 1.8-kb *Aat*II-*Sst*I fragment (bases 190884 to 192648; from pHind-G), the 180-base *Sma*I-*Hae*III fragment containing the HSV-1 gH promoter, β-glucuronidase, the 255-base US10 polyadenylation signal fragment, and the 1.6-kb *Sph*I-*Sph*I fragment (bases 192934 to 194544; from pHind-G). To replace HCMV ORF IRS1 by β-glucuronidase (i.e., RV46; Fig. 1J), pBgdIRS1 was constructed. Sequentially, this plasmid contains the 1.7-kb *Nco*I-*Nco*I fragment (bases 188062 to 189763), β-glucuronidase, the 255-base US10 polyadenylation signal fragment, and the 1.2-kb *Nar*I-*Xho*I fragment (bases 191830 to 193003; from pHind-G). To delete HCMV ORFs US11 through US2 without insertion of a reporter gene (i.e., RV799; Fig. 1K), pdUS11/US2 was constructed. Sequentially, this plasmid contains the 1.8-kb fragment *Pst*I-*Xba*I fragment (bases 202207 to 200391), β-glucuronidase, the 65-base *Nru*I-*Apa*I fragment containing the US10 polyadenylation signal (bases 199086 to 199021; from pHind-X), and the 1.3-kb *Nhe*I-*Apa*I fragment (bases 193360 to 192033). For stable expression of the HCMV US11 gene, several expression plasmids, each containing the selectable puromycin resistance gene, were used. Parental plasmids pIE and pIEsp were described previously (31), and both contain essentially the HCMV major immediate-early enhancer-promoter, followed by a polylinker and the HSV-1 thymidine kinase gene polyadenylation signal. The difference between these plasmids is that pIEsp also contains the first exon (untranslated) and first intron from the HCMV major immediate-early region, between the enhancer-promoter and the polylinker. In both plasmids, a 1.1-kb puromycin resistance gene cassette was inserted within the prokaryotic plasmid backbone sequences, 964 bases upstream of the promoter-enhancer, to yield pIE-puro and pIEsp-puro, respectively. The puromycin resistance gene cassette used contains sequentially the SV40 early promoter, the puromycin resistance gene (34), and the 65-b *Nru*I-*Apa*I fragment containing the US10 polyadenylation signal. A 708-base *Xba*I-*Xho*I fragment (bases 199683 to 200391; from pHind-X) containing the US11 coding region was modified with *Eco*RI adapters and inserted in the proper orientation into the unique *Eco*RI site of the polylinker of pIE-puro and pIEsp-puro to yield pIE-US11-puro and pIEsp-US11-puro, respectively. p2.7E-US11-puro was derived from pIEsp-puro. Essentially, most of the major immediate-early enhancer-promoter and first exon-intron sequences, as well as some polylinker sequences, were removed and replaced by a 1.48-kb fragment containing the 768-base HCMV 2.7-kb RNA early promoter (27) and the 708-base US11 gene.

Isolation of recombinant mutant HCMV. Recombinant mutant HCMV was created and isolated as described previously (28, 29). Recombinant viruses were plaque purified three times. HCMV mutant RV799 is β-glucuronidase negative and was isolated by using a modification of the above-referenced procedure. In this case, β-glucuronidase-positive HCMV mutant RV134 was the parent virus (29). Thus, RV134 genomic DNA was used instead of wild-type strain AD169 DNA in the transfections. Primary plaques appearing on the primary transfection plates were picked at random and replated on HFF cells. After 10 days, the medium was removed and the infected cells were overlaid with X-glu (5-bromo-4-chloro-3-indolyl-β-D-glucuronide [Biosynth])-containing agarose (29). Viruses which had converted from a blue- to a white-plaque phenotype (β-glucuronidase-negative mutant virus) were picked 4 days later and purified (36). The proper genomic organization of each HCMV mutant was verified by DNA blot hybridization analysis (data not shown) as described previously (29).

Antibodies. Rabbit polyclonal antisera reactive with HCMV US11 proteins and HCMV UL80 proteins were described previously (29, 31). Murine monoclonal antibodies W6/32 (4), specific for a conformation-dependent epitope on the heavy chain of human MHC class I proteins, and Ber-T9, specific for the human transferrin receptor, were purchased from Dako. Murine monoclonal antibody TP25.99 (18), specific for a conformation-independent epitope on human MHC class I heavy chains, was obtained from S. Ferrone (Department of Microbiology, New York Medical College, Valhalla, N.Y.). Murine monoclonal antibody 2H2.4 (46), specific for the HCMV 72-kDa IE1 protein, was purchased from Dupont-NEN.

Radiolabeling and immunoprecipitation of infected cell proteins. Pulse-chase radiolabeling was done according to the standard protocol (45). HCMV-infected HFF cells (multiplicity of infection of 5) were pulse-labeled with 200 μCi of [^{35}S]methionine and [^{35}S]cysteine (NEN-DuPont) per ml in methionine- and cysteine-free DMEM at the indicated time postinfection. The radioactive medium was removed, the cells were washed twice in complete DMEM, and chases were done for the indicated time in complete DMEM. Proteins were extracted by using triple detergent lysis buffer (45). The cleared protein extracts (supernatant after centrifugation for 2 min at 15,000 × *g* and 4°C) were retained for immunoprecipitation according to the standard protocol (45). Proteins binding to antibodies were pelleted by using protein A-Sepharose (Pharmacia). For immunoprecipitations of the human transferrin receptor, rabbit anti-mouse immunoglobulin G (IgG; Pierce) was added prior to protein A-Sepharose. The washed immunoprecipitates were boiled in the presence of 2-mercaptoethanol and electrophoresed in denaturing polyacrylamide gels. The gels were fixed and soaked in 1 M sodium salicylate fluor (45) prior to drying and autoradiography.

Immunofluorescence. Cell surface expression of MHC class I molecules was quantitated by flow cytometry. HFF cells were treated with 50 U of human gamma interferon per ml for 48 h prior to virus infection. Cell monolayers were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RV35 (US6-US11 deleted)
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RV7181 (IRS1-US9 deleted)

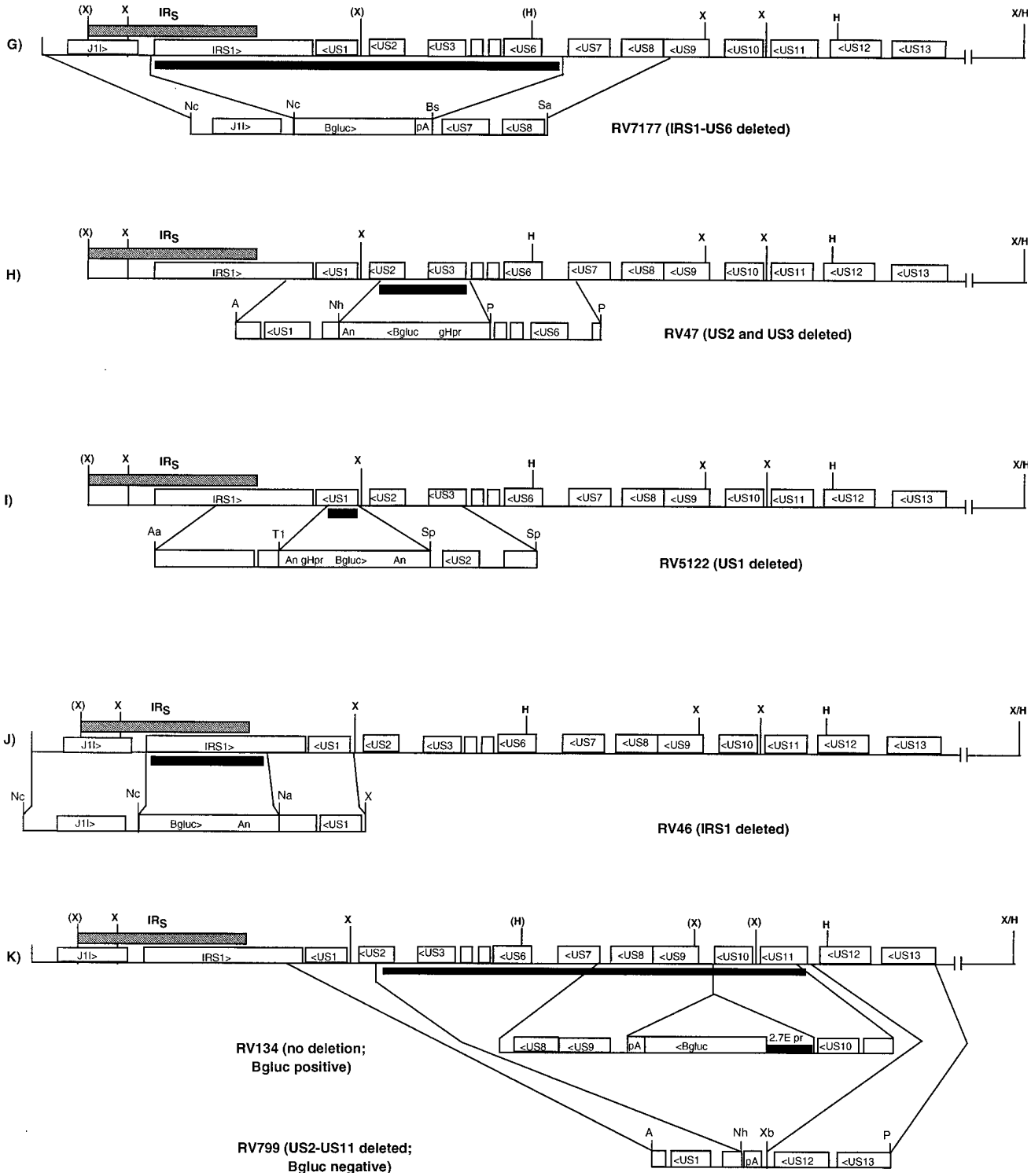

FIG. 1. Organization of recombinant virus genomes. (A) The first line is a schematic of the overall organization of the HCMV wild-type genome. Unique region sequences are shown by a line, while repeated region sequences are indicated by shaded boxes. Relevant *Hin*dIII fragments, within the L and S components, are indicated by letter designation (41). The second line is an expansion of the wild-type *Hin*dIII-Q and -X regions of the S component. The significant ORFs and their orientations are shown as as open boxes (15). The position of each IRS repeated sequence is indicated by a shaded rectangle. (B to J) Genomic organization of the indicated HCMV mutant. In each case, the first line shows the organization of the parental AD169 wild-type genome, and the second line represents the organization of relevant sequences of the linearized plasmid used to make the recombinant virus. The slanted lines indicate the boundaries of the viral flanking sequences which may be involved in homologous recombination to create the desired mutation. The region deleted in each mutant is indicated by a shaded box below the first line. (K) Derivation and organization of RV799, presented as in panels B to J except that a third line represents the organization of the relevant sequences of the linearized plasmid used to make RV799 from the RV134 parent (second line). Restriction sites: *Apa*I (A), *Aat*II (Aa), *Bsm*I (Bs), *Hin*dIII (H), *Hpa*I (Hp), *Nar*I (Na), *Nco*I (Nc), *Nhe*I (Nh), *Pst*I (P), *Sal*I (S), *Sau*I (Sa), *Sph*I (Sp), *Sst*I (T1), (T2), *Xba*I (Xb), and *Xho*I (X). Sites in parentheses no longer exist in the recombinant virus. Bgluc, β-glucuronidase; gHprpA, HSV-1 thymidine kinase polyadenylation signal and glycoprotein H (gH) promoter; SV pr, SV40 promoter; An, polyadenylation signal; gHpr, HSV-1 gH promoter; 2.7E pr, HCMV 2.7-kb RNA early promoter.

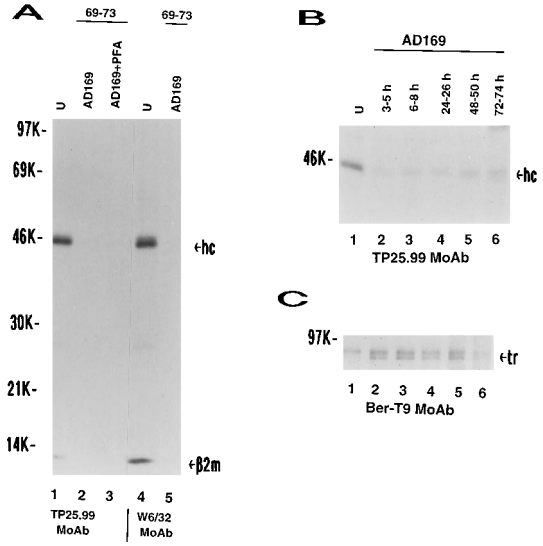

FIG. 2. Expression of MHC class I heavy chains in HCMV wild-type strain AD169-infected cells. HFF cells were uninfected (U) or infected at a multiplicity of infection of 5 PFU per cell in the absence or presence of phosphonoformate (PFA; 200 μg/ml) and radiolabeled for either 4 h at late times postinfection (69 to 73 h) (A) or for 2 h at the indicated time postinfection (B and C). Proteins were harvested immediately after radiolabeling, and class I heavy chains (hc) were immunoprecipitated (A and B) with either the TP25.99 or W6/32 monoclonal antibody (MoAb), as indicated. The extracts used in panel B were also subjected to immunoprecipitation with an anti-human transferrin (tr) receptor monoclonal antibody (Ber-T9) as a control (C). β2m, β-2-microglobulin.

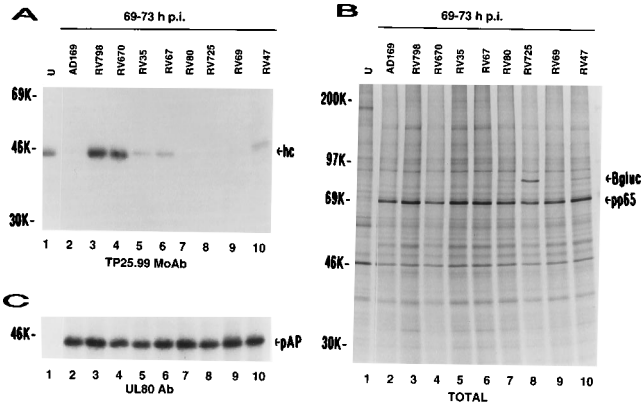

FIG. 3. Analysis of heavy-chain expression in cells infected with HCMV mutants. HFF cells were uninfected (U) or infected with the indicated virus (multiplicity of infection of 5 PFU per cell) and radiolabeled for 4 h at late times postinfection (p.i.) (69 to 73 h). Proteins were harvested immediately after radiolabeling. (A) Class I heavy chains (hc) were immunoprecipitated with murine monoclonal antibody (MoAb) TP25.99. (B) Total radiolabeled proteins to verify approximately equivalent radiolabeling efficiency. (C) To verify equal progression through the viral replicative cycle, UL80 proteins were immunoprecipitated with a rabbit polyclonal antibody (Ab; BX49) which is specific for the assembly protein and protease molecules which contain the assembly protein domain (31). Bgluc, β-glucuronidase; pAP, assembly protein precursor.

washed and infected with HCMV at a multiplicity of 5 PFU/ml. At 72 h postinfection, mock- and HCMV-infected cells were harvested, fixed in 1% paraformaldehyde, and stained with mouse IgG or W6/32 antibody (1 μg/10^6 cells) followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Boehringer Mannheim, Indianapolis, Ind.). Percent positive cells (in a total of 5,000) were calculated on the basis of forward angle light scatter versus log-integrated 90°C light scatter by using the Immuno program Coulter MDADS I (Coulter, Hialeah, Fla.). In other experiments, surface expression of MHC class I molecules was detected by immunofluorescence in situ. This procedure were done in 60-mm-diameter tissue culture plates according to the standard protocol (24). Uninfected or infected HFF cells were fixed with 4% paraformaldehyde. After addition of 3% bovine serum albumin in phosphate-buffered saline, the cells were held overnight at 4°C. The cells were treated sequentially with the following antisera, each for 30 min at room temperature: 10% HCMV-negative human serum (to block any Fc receptors); the indicated primary antibody; and fluorescein isothiocyanate-conjugated anti-mouse or anti-rabbit IgG, as appropriate.

Stable transformation. Either U373-MG cells or low-passage-number HFF cells (passage 5) were transfected (with US11 expression plasmids [see above]) and selected (for resistance to puromycin), and stably transfected cell lines were isolated by using a procedure described previously (34). Prior to transfection, the plasmids were linearized within the prokaryotic plasmid backbone sequences. Cell lines expressing gpUS11 were identified and by Western blot (immunoblot) analysis as described previously (31).

RESULTS

Class I heavy-chain down-regulation in HCMV-infected human fibroblasts. Because of differences in the findings among laboratories (6, 9, 56), we sought to ascertain the timing and nature of MHC class I heavy-chain down-regulation in our HFF cell culture system. Because viral peptides are presented at the cell surface by class I complexes synthesized and assembled after infection (39), we assessed the status of class I proteins synthesized at various times postinfection by immunoprecipitation of metabolically radiolabeled proteins (Fig. 2), using the conformation-independent and conformation-dependent class I heavy-chain monoclonal antibodies TP25.99 and W6/32, respectively. At late times postinfection, reduction in expression of class I heavy chains was detected both in the presence and in the absence of the viral DNA synthesis inhibitor phosphonoformate (Fig. 2A). This result was consistent with previous reports which indicated that viral immediate-early or early gene functions are sufficient for heavy-chain reduction (6, 54, 56). In addition, it was demonstrated that heavy-chain down-regulation was detected at very early times, 3 to 5 h postinfection (Fig. 2B). Since this effect was observed with the conformation-independent antibody, the reduction reflects the fate of newly synthesized heavy chains, not just lack of tripartite complex (i.e., class I heavy chain, β-2-microglobulin, and peptide) formation.

In adenovirus type 2-infected cells, a nonessential viral early gene is required for down-regulation of cell surface expression of MHC class I heavy chains (2, 10). It was reported recently that a dispensable immediate-early gene is sufficient for retention in the endoplasmic reticulum of class I heavy chains in HSV1-infected cells (58). By analogy, we hypothesized that the gene(s) required for reduction of class I heavy-chain expression in HCMV may also be nonessential. Several previously constructed HCMV deletion mutants, representing 18 nonessential ORFs (UL33, IRS1, US1 to US13, US27, US28, and TRS1), were screened for heavy-chain expression by flow cytometry and immunoprecipitation analyses (data not shown). Only RV670 (28), a mutant deleted of a 9-kb region within the S component of the HCMV genome, did not retain the wild-type down-regulation phenotype (Fig. 3A, lane 4). This mutant is deleted of at least 11 ORFs, IRS1 through US11 (except for US10), which includes the US6 family of genes (US6 to US11) which putatively encode glycoproteins (15). To confirm this observation, two additional independently derived mutants which have the same deletion as RV670, as well as a new mutant, RV7186, deleted of the entire IRS1–US11 region (Fig. 1B), were tested. Each was phenotypically identical to RV670 and stably expressed class I heavy chains (data not shown).

Previously, we constructed HCMV mutants deleted of US6 family ORFs, either individually or in groups (28), and similar deletion mutants within the adjacent IRS1–US3 region (Fig. 1H to J). By immunoprecipitation using the conformation-independent antibody, all of these mutants were shown to

retain the ability to down-regulate class I heavy chains (Fig. 3A, lanes 5 to 10, and data not shown) at late times postinfection. Control experiments indicated that radiolabeling was equivalent between the different infected cell cultures (Fig. 3B) and that infection proceeded to late times equally, as judged by pp65 (UL83) (Fig. 3B) and UL80 protein (Fig. 3C) expression. These data, summarized in Fig. 4B, suggested two alternatives: (i) that more than one viral gene may be sufficient for the reduction in class I heavy chains; or (ii) a gene(s) between US3 and US6, deleted in RV670 and RV7186 but not the other mutants, may be required for the phenotype.

Identification of a 7-kb region of the HCMV genome required for MHC class I down-regulation. To further localize the region containing the gene(s) involved in MHC class I heavy chain down-regulation, additional HCMV replacement mutants containing deletions of multiple genes within the IRS1–US11 gene region were created. One of these mutants, RV798 (Fig. 1C), is deleted of genes from US2 to US11. In HFF cells infected by RV798 and analyzed at late times postinfection, MHC class I heavy chains were not down-regulated as they are in wild-type strain AD169-infected cells (Fig. 3); in fact, a slight stimulation was consistently observed. Several independently derived deletion mutants identical to RV798 were examined similarly; all lacked the ability to down-regulate class I heavy chains (data not shown). To further confirm that the 7-kb HCMV US2–US11 region contained the gene(s) required for heavy chain down-regulation, we constructed mutant RV799, which has the same US2–US11 deletion as does RV798 but was created by a different strategy (Fig. 1K). RV798 was derived directly from wild-type strain AD169 by inserting a β-glucuronidase marker gene in the place of ORFs US2 to US11. In contrast, the parent of RV799 is RV134, a mutant which has a β-glucuronidase expression cassette inserted within the US9–US10 intergenic region (29). To create RV799, we designed a plasmid which upon recombination with the RV134 genome would simultaneously delete ORFs US2 to US11 and the β-glucuronidase expression cassette. The proper RV799 HCMV mutant was isolated as a white plaque in the presence of the β-glucuronidase substrate, since it is β-glucuronidase negative. As expected, RV799, but not the RV134 parent, was phenotypically identical to RV798 (Fig. 5). Thus, since RV798 and RV799 were created by different strategies from parents which down-regulate MHC class I heavy chains, this finding confirms that the gene(s) required for the phenotype is located within the 7-kb US2–US11 region (bases 193119 to 200360).

Several types of experiments were done to characterize the class I heavy-chain proteins synthesized in cells infected with HCMV mutants which did not down-regulate these molecules. Proper maturation of class I heavy chains in uninfected cells yields endoglycosidase H-resistant molecules. In contrast, class I heavy chains synthesized in AD169-infected cells are entirely endoglycosidase H sensitive (6, 57). Therefore, we compared the maturation kinetics of MHC class I heavy chains in RV798-infected cells with that in uninfected cells. Class I heavy chains synthesized in RV798-infected cells at either early or late times postinfection were converted to the mature endoglycosidase H-resistant form, similar to those synthesized in uninfected cells (Fig. 6A). The stability of class I heavy-chain proteins in RV798-infected cells was analyzed in pulse-chase studies (Fig. 6B). The half-lives of these molecules were determined to be greater than 3 h in both uninfected cells and RV798-infected cells, compared with less than 0.5 h in wild-type HCMV-infected cells (6, 57). To determine whether the proper cell surface expression of class I heavy chains was restored in these mutant-infected cells, immunofluorescence assays were done. Using either the conformation-dependent (W6/32) or conformation-independent (TP25.99) monoclonal antibody surface expression of MHC class I heavy chains was detected in uninfected and RV670-, RV798-, and RV799-infected HFF cells but was greatly reduced in wild-type HCMV-infected HFF cells (Fig. 7 and data not shown). The cumulative data (Fig. 6 and 7) indicate that unlike the case for wild-type HCMV-infected cells, maturation and surface expression of MHC class I heavy-chain proteins are not impaired, and their stability is not reduced, in cells infected with the US2–US11 deletion mutants of HCMV.

Two independent loci within the US2–US11 region contain genes which are involved in class I heavy-chain down-regulation. The regions of the HCMV genome deleted in RV798 and RV35 are US2 to US11 and US6 to US11, respectively (Fig. 1C and D). In RV35-infected HFF cells, MHC class I heavy chains are down-regulated, but in RV798-infected cells, they are not (Fig. 3, lanes 3 and 5). The implication from these data is that one or more genes involved in MHC class I heavy-chain down-regulation map within the approximately 2-kb region containing ORFs US2 to US5 (Fig. 4A; bases 193119 to 195607). To determine if genes within the US2–US5 region are required for MHC class I heavy-chain down-regulation, HCMV replacement mutants RV7177 and RV7181 were constructed (Fig. 1F and G). HCMV ORFs IRS1 to US6 and IRS1 to US9, respectively, are deleted in these mutants; hence, the US2–US5 region is absent from both mutants. Experiments in infected HFF cells at late times postinfection indicated that both mutants retained the ability to efficiently down-regulate class I heavy-chain expression (Fig. 8). Therefore, when present in the HCMV genome, genes within the US2–US5 region are sufficient for reduction of MHC expression (e.g., RV35), although their presence is not required for the phenotype (e.g., RV7177 and RV7181). Furthermore, the cumulative data (summarized in Fig. 4B and C) indicate that there are no HCMV genes within the identified 7-kb US2–US11 region (i.e., the region deleted in RV798) which are absolutely required for MHC class I heavy-chain down-regulation in infected HFF cells. This finding suggests that genes from another portion of the US2–US11 gene region are also sufficient for this phenotype.

HFF cells infected with the IRS1–US9 deletion mutant (RV7181) down-regulate MHC class I heavy-chain expression, in contrast to cells infected with the US2–US11 deletion mutant (RV798) (Fig. 8). As expected, approximately wild-type levels of both US11- and US10-encoded proteins were detected in cells infected with RV7181 by Western blot analysis (data not shown). These data suggested that a second locus, composed of the US10 and US11 genes (Fig. 4A; bases 199083 to 200360), is involved in down-regulation of heavy-chain expression by a mechanism resulting in a short intracellular half-life (~0.5 h) of these proteins (Fig. 6B, lanes 12 to 16). However, the expression of US10 from the context of the HCMV genome is not sufficient for heavy chain down-regulation. HCMV mutant RV670 is deleted of all of the other ORFs in the 7-kb US2–US11 gene region (28) but contains US10. The US10 gene product is expressed at slightly greater steady-state levels in RV670-infected cells than in wild-type HCMV-infected cells (data not shown). MHC class I heavy chains were not down-regulated in RV670-infected HFF cells (Fig. 3A). Thus, the US11 gene product is implicated as being involved in heavy-chain down-regulation.

US11 encodes a 32-kDa endoglycosidase H-sensitive, N-linked glycoprotein (gpUS11) which is expressed throughout infection, beginning at very early times (i.e., 3 h) (29, 30). Two approaches were taken to confirm the data which suggested that gpUS11 plays an important role in MHC class I heavy-

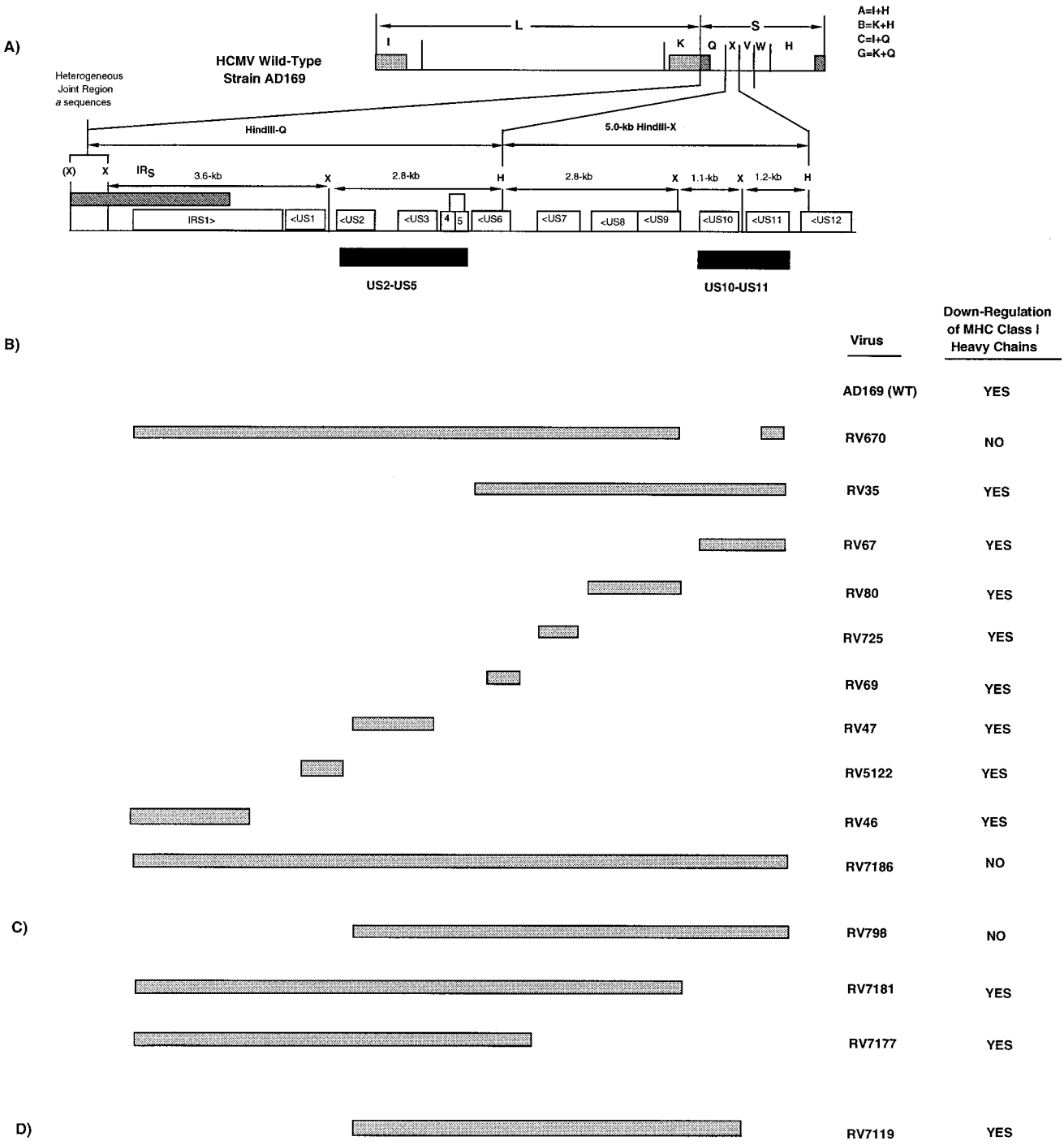

FIG. 4. Summary of MHC class I heavy-chain expression data from HFF cells infected with wild-type and mutant HCMV. (A) The first line shows the overall organization of the HCMV wild-type (WT) genome, and the second line is an expansion of the wild-type *Hin*dIII-Q and -X regions of the S component. The ORFs are indicated by unshaded rectangles; the unlabeled ORF overlapping US4 and US5 is US4.5. The locations of the loci which contain genes which are sufficient for MHC class I heavy-chain down-regulation are shown. One locus contains ORFs US2 to US5 (bases 193119 to 195607), and the other locus contains ORFs US10 and US11 (bases 199083 to 200360). (B to D) The deletions within the various HCMV mutants are indicated by shaded rectangles. (B) RV670 is deleted of IRS1 to US9 and US11, RV35 is deleted of US6 to US11, RV67 is deleted of US10 and US11, RV80 is deleted of US8 and US9, RV725 is deleted of US7, RV69 is deleted of US6, RV47 is deleted of US2 and US3, RV5122 is deleted of US1, RV46 is deleted of IRS1, and RV7186 is deleted of IRS1 to US11. (C) RV798 is deleted of IRS1 to US6. (D) RV7119 is deleted of US2 to US10. MHC class I heavy-chain down-regulation results are from immunoprecipitation experiments (using the heavy-chain conformation-independent monoclonal antibody, TP25.99) in which HCMV-infected HFF cells were radiolabeled at late times postinfection.

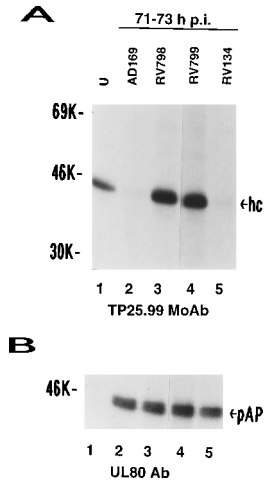

FIG. 5. Immunoprecipitation of class I heavy chains from RV798-, RV799-, RV134-, or wild-type AD169-infected cells. HFF cells were uninfected (U) or infected with the indicated virus (multiplicity of infection of 5 PFU per cell) and radiolabeled for 2 h at late times postinfection (p.i.) (71 to 73 h). Proteins were harvested immediately after radiolabeling. (A) Class I heavy chains (hc) were immunoprecipitated with murine monoclonal antibody (MoAb) TP25.99. Progression through the viral replicative cycle (B) was verified as described for Fig. 3. Ab, antibody; pAP, assembly protein precursor.

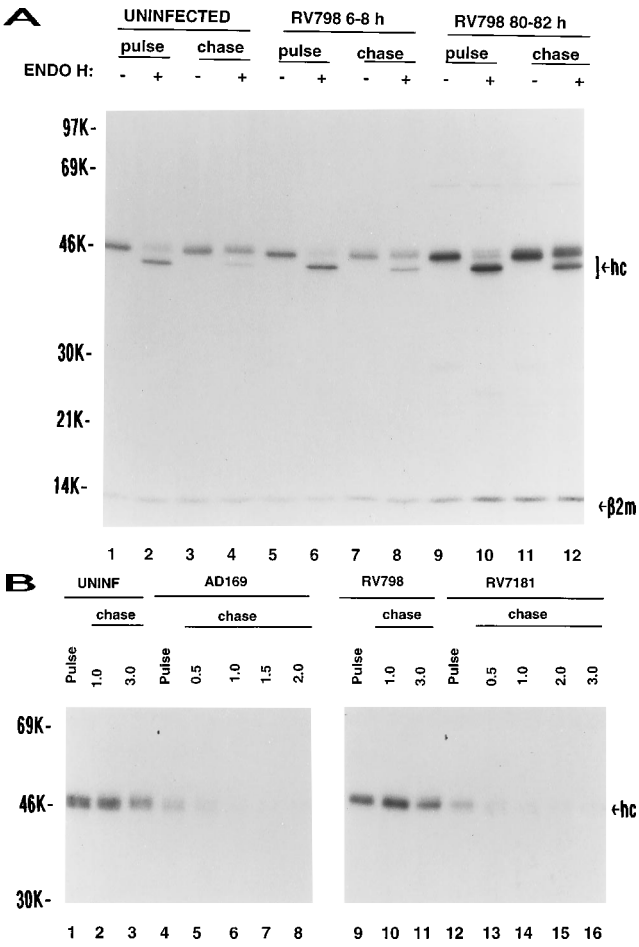

FIG. 6. (A) Endoglycosidase (ENDO) H sensitivity of MHC class I heavy chains. HFF cells were infected with RV798 (multiplicity of infection of 5 PFU per cell) and radiolabeled for 2 h at early times (6 to 8 h) or late times (80 to 82 h) postinfection. For comparison purposes, uninfected cells were radiolabeled for 2 h. Proteins were harvested either immediately after radiolabeling (pulse) or after a 2-h chase (chase) in complete unlabeled medium. Class I heavy chains (hc) were immunoprecipitated with murine monoclonal antibody TP25.99. Immunoprecipitated proteins were incubated for 6 h in either the presence (+) or absence (−) of 1.5 mU of endoglycosidase H prior to SDS-polyacrylamide gel electrophoresis and fluorography. (B) Intracellular stability of MHC class I heavy chains. Uninfected (UNINF) HFF cells or HFF cells infected with either wild-type AD169, RV798, or RV7181 at a multiplicity of 5 PFU per cell were radiolabeled for 0.5 h at late times postinfection (Pulse lanes) and harvested for immunoprecipitation experiments using the TP25.99 monoclonal antibody or chased for the indicated time (in hours) in unlabeled medium prior to harvest and immunoprecipitation.

chain down-regulation. One strategy was to construct recombinant virus RV7119 (Fig. 1E), which was deleted of US2 to US10, leaving US11 intact. In wild-type HCMV, US11 is expressed from a 1.5-kb bicistronic message containing both ORF US11 and ORF US10, although US11 is the 5′-most ORF (22, 27). RV7119 was constructed such that US11 expression is controlled by its natural promoter but is on a monocistronic transcript. This message terminates at the HSV-1 thymidine kinase gene polyadenylation signal which overlaps the HSV-1 gH promoter of the β-glucuronidase expression cassette, inserted downstream of US11. By Western blot analysis, we have determined that the amount of gpUS11 in RV7119-infected cells is similar to the amount in wild-type HCMV-infected cells (data not shown). Radiolabeling and immunoprecipitation experiments indicated that class I heavy-chain proteins were down-regulated in RV7119-infected cells at late times postinfection (Fig. 9A).

The second strategy was to construct cell lines stably expressing gpUS11 and determine the relative level of MHC class I heavy-chain expression. The US11 gene was cloned into two expression vectors such that it was under the control of either the HCMV major immediate-early enhancer-promoter or the HCMV 2.7-kb RNA early promoter (21, 35), yielding pIE-US11-puro and p2.7E-US11-puro, respectively. pIE-US11-puro and pIEsp-US11-puro transfected into U373-MG cells, and were pIE-US11-puro and p2.7E-US11-puro were transfected into low-passage-number HFF cells. HFF-derived cell lines designated 111-7-2, 111-7-3, and 111-7-11 were selected after transfection with p2.7E-US11-puro. These cell lines were analyzed for expression of gpUS11 and MHC class I heavy chains by both radiolabeling-immunoprecipitation studies (data not shown) and Western blot analyses (Fig. 10). The results indicated that class I heavy-chain expression was inversely related to the level of gpUS11. Cell lines 111-7-2 and 111-7-11 expressed relatively low levels of US11 protein when uninfected; however, these levels increased substantially after infection with RV798 (ΔUS2–US11). This increase after infection was expected since the US11 gene is controlled by a strong early promoter in p2.7E-US11-puro (27, 35). MHC class I heavy-chain proteins were not detected in the US11-expressing uninfected 111-7-2 or 111-7-11 cells. Class I heavy-chain proteins were detected in very low amounts in RV798-infected 111-7-2 and 111-7-11 cells. In contrast, uninfected 111-7-3 and parental HFF cells, which do not express US11, expressed high levels of class I heavy chains. In these cells, steady-state class I heavy-chain expression increased after infection with RV798. This is due either to autocrine or paracrine stimulation by interferons (39) or to transcriptional stimulation of the class I promoter by HCMV proteins (11). Control experiments indicated that levels of the cellular transferrin receptor were equal between parental HFF, 111-7-2, 111-7-3, and 111-7-10 cells (data not shown). Results of experiments using HFF-derived cell lines selected after transfection of pIE-US11-puro were

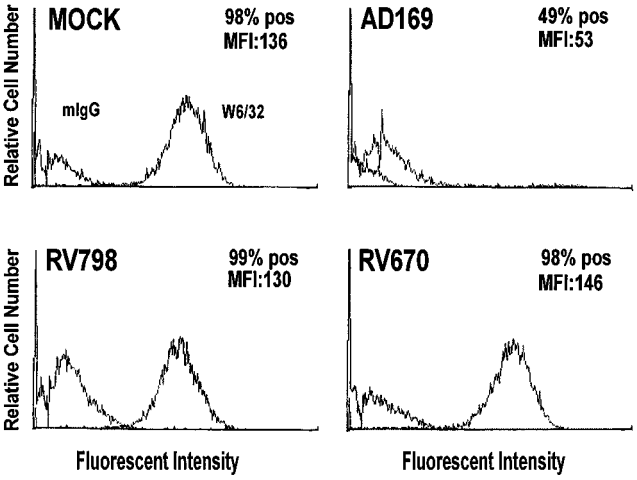

FIG. 7. Detection of cell surface MHC class I in HCMV-infected cells by immunofluorescence-flow cytometry. HFF cells were uninfected (mock) or infected for 72 h with the indicated virus and processed as described in Materials and Methods. The primary antibodies were monoclonal antibody W6/32 (specific for a conformational-dependent epitope of the class I heavy chain and β-2-microglobulin complex) or isotype-matched mouse IgG (negative control). Percent positive (pos) cells (out of a total of 5×10^3) and mean fluorescent intensity (MFI) are shown.

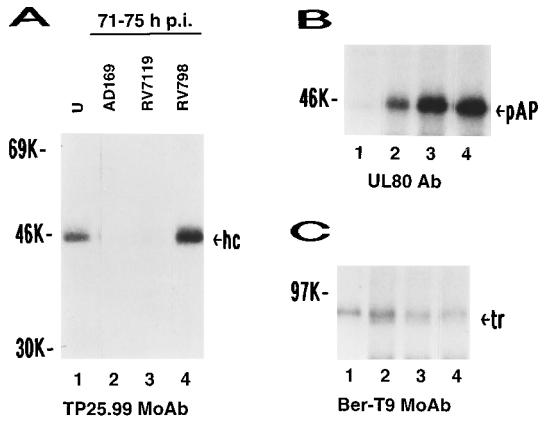

FIG. 9. Immunoprecipitation of class I heavy chains from RV7119-, RV798-, or wild-type AD169-infected cells. HFF cells were uninfected (U) or infected with the indicated virus (multiplicity of infection of 5 PFU per cell) and radiolabeled for 2 h at late times postinfection (p.i.) (71 to 75 h). Proteins were harvested immediately after radiolabeling. (A) Class I heavy chains (hc) were immunoprecipitated with monoclonal antibody (MoAb) TP25.99. Progression through the viral replicative cycle (B) was verified as described for Fig. 3. Ab, antibody; pAP, assembly protein precursor. (C) Immunoprecipitation of the cellular transferrin (tr) receptor glycoprotein.

consistent with the data for the 2.7E-US11-puro stable transfectants presented in Fig. 10 (data not shown). Likewise, results for stably transfected U373-MG cells confirmed that class I heavy-chain expression was inversely related to the level of US11 expression (data not shown). The cumulative data from Fig. 9 and 10 indicate that expression of the HCMV US11 gene is sufficient for MHC class I heavy-chain protein down-regulation.

Down-regulation of MHC class I heavy-chain expression at early times postinfection by HCMV mutants. MHC class I

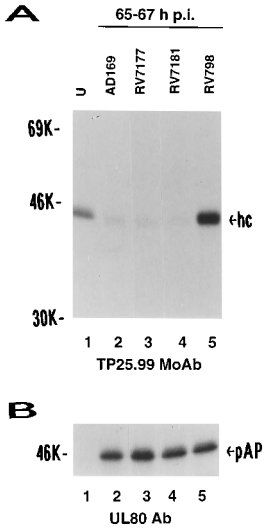

FIG. 8. Immunoprecipitation of class I heavy chains from RV798-, RV7181-, RV7177-, or wild-type AD169-infected cells. HFF cells were uninfected (U) or infected with the indicated virus (multiplicity of infection of 5 PFU per cell) and radiolabeled for 2 h at late times postinfection (p.i.) (65 to 67 h). Proteins were harvested immediately after radiolabeling. (A) Class I heavy chains (hc) were immunoprecipitated using with monoclonal antibody (MoAb) TP25.99. Progression through the viral replicative cycle (B) was verified as described for Fig. 3. Ab, antibody; pAP, assembly protein precursor.

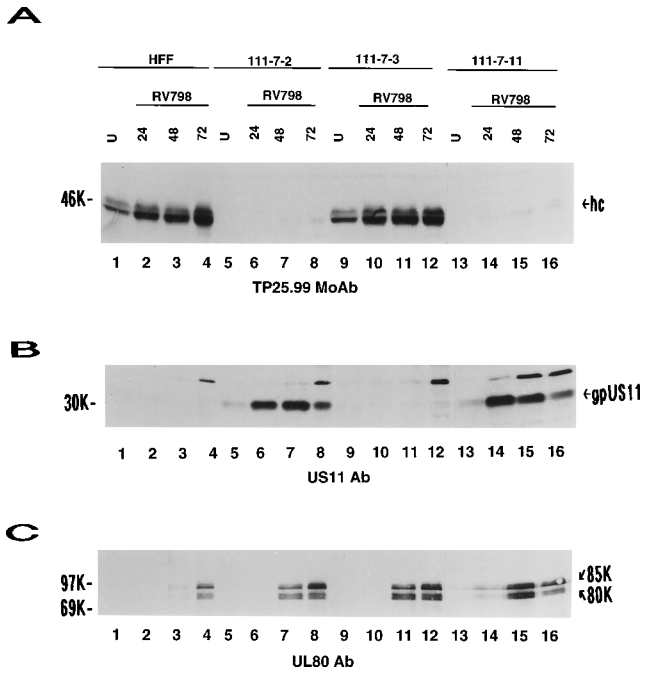

FIG. 10. Western blot analysis of MHC class I heavy-chain (hc) expression and gpUS11 expression in stably transfected HFF cells. Parental HFF cells (lanes 1 to 4) or stably transfected HFF 111-7-2 (lanes 5 to 8), 111-7-3 (lanes 9 to 12), or 111-7-11 (lanes 13 to 16) cells were uninfected (U) or infected with the US2–US11 deletion virus RV798 (multiplicity of infection of 5 PFU per cell). At the indicated times (hours), total cellular proteins were harvested, electrophoresed, and blotted. The relevant regions from the same gel were cut apart and probed with monoclonal antibody (MoAb) TP25.99 (A), US11 antibody (Ab), (B), or UL80 antibody (C) as a control. 85K and 80K, UL80-derived protease molecules described previously (31). Similar to gpUS11-expressing cell lines 111-7-2 and 111-7-10, the cell line designated 111-7-3 was transfected with p2.7E-US11-puro, but it does not express gpUS11. It is included as a control, along with parental HFF cells. The reason that for the lack of gpUS11 expression in 111-7-3 was not investigated but is presumed to be due to the loss or disruption of the US11 transcription unit during integration into the cellular genome.

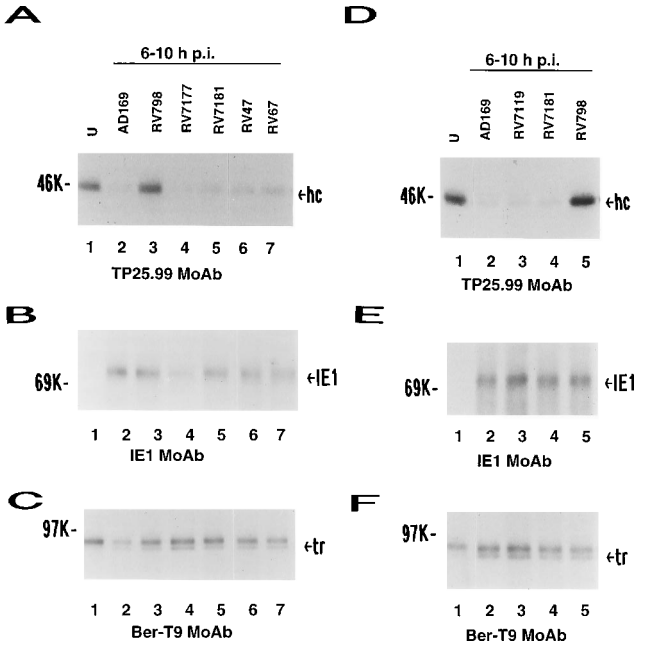

FIG. 11. Analysis of heavy-chain expression in cells infected with HCMV mutants at early times postinfection. HFF cells were uninfected (U) or infected with the indicated virus (multiplicity of infection of 5 PFU per cell) and radiolabeled for 4 h from 6 to 10 h postinfection (p.i.). Proteins were harvested immediately after radiolabeling. (A and D) Class I heavy chains (hc) were immunoprecipitated with monoclonal antibody (MoAb) TP25.99. (B and E) To verify approximately equal infection, the 72-kDa IE1 immediate-early protein was immunoprecipitated with monoclonal antibody 2H2.4. (C and F) Immunoprecipitation of the cellular transferrin (tr) receptor with monoclonal antibody Ber-T9 to verify approximately equal expression of this glycoprotein.

heavy-chain expression in wild-type HCMV-infected cells was shown to begin at very early times postinfection (Fig. 2B). To determine if any of the mutants were deficient for this early down-regulation, immunoprecipitation experiments were performed with extracts from infected HFF cells radiolabeled from 6 to 10 h postinfection. The level of class I heavy chains was reduced during this early period postinfection with each of the mutants, except for RV798 (ΔUS2–US11) (Fig. 11A and D). Control experiments demonstrated that the different mutant-infected cells were equally infected (Fig. 11B and E). Expression of another cellular glycoprotein, the transferrin receptor, was not differentially affected by the various mutants (Fig. 11C and F). Thus, expression of either the US2–US5 region gene(s) or the US11 gene is independently sufficient for down-regulation of MHC class I heavy-chain expression at both early and late times postinfection.

DISCUSSION

Viral genes important in influencing pathogenesis are often nonessential for growth in cell culture (20). Some of these dispensable viral gene products function to reduce cell surface expression of MHC class I heavy chains in cells infected with adenovirus type 2 and HSV-1 (2, 10, 58). Reduction in the expression of MHC class I heavy-chain proteins also occurs during infection by cytomegaloviruses, including MCMV (12, 13, 17) and HCMV (3, 6, 9, 54, 56, 57). In HCMV-infected cells, this reduction is known to be at the posttranslational level in that class I heavy chains have a much higher turnover rate in infected cells than in uninfected cells (Fig. 7B) (6, 54, 57). In this study, we have begun to elucidate the genetic basis for the down-regulation of MHC class I heavy-chain expression observed in HCMV-infected cells. As result of examining our preexisting and newly constructed HCMV deletion mutants (representing 18 ORFs dispensable for viral replication in tissue culture) for the ability to down-regulate MHC class I heavy chains, a 7-kb region of the U_S portion of the HCMV genome containing ORFs US2 to US11 was shown to contain genes which are required for this phenotype (summarized in Fig. 4). Within this region are two loci which contain genes that function independently to cause class I heavy-chain down-regulation. Furthermore, we report that down-regulation occurs throughout the course of infection, beginning at early times (3 h) postinfection (Fig. 2C).

One locus involved in MHC class I heavy-chain down-regulation is composed of the US10 and US11 genes (Fig. 4). The US10 promoter overlaps the 3′ end of the US11 gene (27), and the expression characteristics of, as well as the glycoproteins encoded by, US10 and US11 are similar (30). However, US10 expression is not sufficient for down-regulation of class I heavy-chain expression, as indicated by data for HCMV mutant RV670-infected cells (Fig. 3A), in which slightly greater than wild-type levels of the US10 gene product are expressed (26). Data from two types of experiments indicate that the US11 gene product is the relevant gene in this locus. First, an HCMV deletion mutant (RV7119) which expresses US11 and is deleted of US2 to US10 down-regulates class I heavy-chain proteins (Fig. 9). Also, stably transfected cells which express US11 have very low steady-state levels of class I heavy-chain protein (Fig. 10). Thus, the US11-encoded protein is sufficient to cause MHC class I heavy-chain down-regulation, even in the absence of other viral proteins.

Expression of RNA and protein from US11 begins at early times postinfection and proceeds throughout the course of infection by HCMV (27, 30), kinetics which parallel the decrease in MHC class I expression (Fig. 2C). We have hypothesized that gpUS11, a 32-kDa glycoprotein (with N-linked sugar residues) encoded by US11 (30), is retained in the endoplasmic reticulum or *cis* Golgi (37). Immunofluorescence data were consistent with this hypothesis in that gpUS11 immunoreactivity was not detected on the cell surface but was detected in the cytoplasm of permeabilized HCMV-infected cells (30). Mechanistically, the probable localization of gpUS11 in the endoplasmic reticulum is logical, since this is the site of class I heavy-chain degradation in wild-type HCMV-infected cells (57). Although computer analysis did not detect any significant homology between the two proteins, gpUS11 is similar in some respects to the 25-kDa glycoprotein (E3-19K) encoded from the E3 region of adenovirus type 2. In addition to being nonessential for viral replication, E3-19K has endoglycosidase H-sensitive N-linked sugar residues and is retained in the endoplasmic reticulum. E3-19K has also been shown to directly bind to MHC class I heavy chains, thereby preventing their transport to the cell surface (2, 10, 39). In contrast to E3-19K, a direct association between gpUS11 and class I heavy chains has not been detected by coimmunoprecipitation in our experiments to date (data not shown).

The second locus involved in MHC class I heavy-chain down-regulation is composed of ORFs US2 to US5 (Fig. 4). From the translated DNA sequence, it has been proposed that US2 and US3 encode membrane glycoproteins (15). Differentially spliced mRNAs are transcribed from US3 throughout the viral replicative cycle and encode proteins with transcriptional transactivating function (16, 51, 52, 55). Other small ORFs are also present in this subregion (i.e., between the ORFs US3 and US6), but their expression characteristics or protein function(s) have not been reported. There is a 1.0-kb early mRNA

transcribed from this region of the HCMV genome (22), but the protein-coding potential of this message is currently unknown. Studies to identify which of the US2–US5 genes are involved in heavy-chain down-regulation are in progress.

The mechanism by which gpUS11 or the product of the relevant US2–US5 region gene results in MHC class I heavy-chain down-regulation is under investigation. Mutants reported herein which are deleted of one locus, but not the other, facilitate such studies. The mechanism of posttranslational heavy-chain down-regulation by either HCMV locus may be different than that caused by adenovirus type 2, HSV-1, or MCMV, in which case class I heavy chains are not transported to the cell surface but are stable intracellularly (2, 10, 13, 17, 58). In the case of the HCMV mutant-infected HFF cells, the expression of either locus results in an increased rate of intracellular turnover of class I heavy-chain molecules (Fig. 7B and unpublished data), as reported for HCMV wild-type-infected cells (6, 54, 57).

The existence of at least two independent loci whose expression results in down-regulation of MHC class I expression may emphasize the importance of this phenotype for successful infection or persistence in the human host. The presence of two genes in the same virus strain which can act independently to cause heavy-chain down-regulation has not been reported in other systems. However, there may be a two-gene system in MCMV, since infection of some mouse cell types with this virus results in a profound decrease in both the synthesis and transport of MHC class I heavy chains (12), and these events are independently regulated (47). There are at least three explanations for a dual gene arrangement in HCMV. Each gene product may have different intracellular targets, thereby down-regulating the expression of class I heavy chains by different mechanisms. Because HCMV infection (at low multiplicity of infection) enhances MHC class I expression on neighboring, uninfected cells as a result of the induction and release of cellular beta interferon (3, 23, 49), a second possibility is that cooperation of two genes is necessary in vivo in secondary rounds of infection to overcome the increased MHC class I expression in these newly infected cells. Finally, since HCMV infects a variety of cell types in the human host (1), a third possibility is that there are differences in the efficiency of MHC class I down-regulation by each locus in different cell types. In the case of the HCMV infection of permissive fibroblasts and astrocytoma cells, both mechanisms are functional. When these cells are infected with wild-type or mutant HCMV, down-regulation of MHC class I heavy chains is slightly more efficient when both loci are present than when only one is present (Fig. 3 and unpublished data). With respect to possible cell type specificity, such specificity may be due to differences in relative promoter activity or to qualitative or quantitative differences in cellular proteins which interact with the products of each locus. The latter case may exist with HSV. It was recently reported that the 88-amino-acid US12 gene product (ICP47) of HSV-1 is sufficient for class I heavy-chain sequestering in the endoplasmic reticulum (58). However, expression of heavy chains is not affected in HSV-infected mouse cells, although ICP47 is expressed in those cells and murine heavy chains are down-regulated when expressed in an HSV-infected human fibroblast system (58).

Proper synthesis, folding, intracellular transport, and surface expression of peptide-loaded MHC class I proteins are required for an efficient cell-mediated immune response by cytotoxic T lymphocytes (CTL) (7, 53). Down-regulation of MHC class I molecules by the two HCMV loci identified herein may be responsible, at least in part, for the reduced efficiency of presentation of certain peptides to MHC class I-restricted CTL (19, 54). Virus-specific, $CD8^+$, MHC class I-restricted CTL are protective against cytomegalovirus disease in humans (43, 44) and against lethal infection of mice (32, 42). We have demonstrated that in vivo, MCMV infection interferes with the priming of MHC class I-restricted CTL precursors (14, 48). Although very few class I molecules are necessary for effective antigen presentation, reduced levels within infected cells may set the stage for competition among peptides presented by class I, thereby skewing the repertoire of peptides presented. The ability to limit or regulate viral antigen presentation to CTL may be advantageous for the virus during a primary response or during the initial phases of reactivation from latency. This mode of immune regulation may also contribute to maintenance of viral persistence. HCMV mutants deleted of either locus will allow us to assess functionally the effects of these gene products on antigen presentation to MHC class I-restricted human CTL. An HCMV mutant deleted of the loci involved in MHC class I down-regulation (within the US2–US11 gene region) may be important in the development of a live virus vaccine with an effective anti-HCMV immune response.

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