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Human Cytomegalovirus-Infected Cells Have Unstable Assembly of Major Histocompatibility Complex Class I Complexes and Are Resistant to Lysis by Cytotoxic T Lymphocytes

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Viruses which cause persistence in the naturally infected host are predicted to have evolved immune evasion mechanisms. Human cytomegalovirus (HCMV) causes significant morbidity and mortality in immunocompromised patients yet persists without clinical manifestations in seropositive individuals who have normal immune function. We report that HCMV infection in vitro impairs major histocompatibility complex class I (MHC-I) assembly accompanied by resistance to killing by cytotoxic $CD8^+$ T lymphocytes. Pulse-chase metabolic labelling experiments show that MHC-I complexes continue to be assembled by both uninfected and HCMV-infected cells. However, MHC-I molecules are unstable in HCMV-infected cells and are rapidly broken down. Endoglycosidase H treatment of immunoprecipitates indicates that the breakdown of MHC-I complexes in HCMV-infected cells occurs primarily in a pre-Golgi compartment. Interference with normal MHC-I assembly and expression, if relevant in vivo, may have implications for the restriction of the diversity of the $CD8^+$ cytotoxic T lymphocyte repertoire directed against HCMV antigens and may be an important mechanism of viral persistence.

Human cytomegalovirus (HCMV) is the largest of the human beta-herpesviruses, with a 230-kb genome encoding approximately 200 genes (15). Expression of viral proteins occurs in three phases, with immediate-early (transactivator) proteins expressed at 2 to 6 h postinfection, early (nonstructural) proteins expressed from 6 to 24 h, and finally late (structural) proteins produced after viral DNA replication (15). Primary infection with HCMV is asymptomatic, and 50% of the population in the United Kingdom is seropositive by 20 years of age, although in some ethnic and social groups this rises to 90%. Primary infection is followed by lifelong persistence which is asymptomatic unless the patient is immunosuppressed, in which case HCMV may cause high morbidity and mortality.

Of the different effector arms of the immune response, there is some evidence that the $CD8^+$ cytotoxic T lymphocyte (CTL) compartment may be an important component of the host-virus equilibrium. For instance, development of HCMV-specific CTL in both bone marrow (30, 31) and renal transplant recipients is associated with protection from HCMV disease, and in murine cytomegalovirus (MCMV) infection, $CD8^+$ T lymphocytes protect against lethal challenge with MCMV after vaccination with a recombinant vaccinia virus encoding an MCMV immediate-early protein (26). We and others have been interested in the specificity and precursor frequency of the HCMV-specific $CD8^+$ T-cell response in both normal and immunocompromised subjects, partly in order to determine whether the characteristics of this repertoire yield any clues regarding virus pathogenesis and persistence.

$CD8^+$ T-cell recognition of virus-infected cells occurs through the specific interaction of the T-cell receptor with a virus peptide-loaded major histocompatibility complex class I (MHC-I) molecule. The MHC-I molecule is a heterotrimeric complex consisting of a polymorphic heavy chain bound to both a nonpolymorphic, globular protein β2-microglobulin and to a peptide of 8 to 10 amino acid residues held in the polymorphic peptide binding groove (38). MHC-I assembly occurs in the endoplasmic reticulum (ER), which is the site of synthesis of both MHC heavy chain and β2-microglobulin. Viral peptides incorporated into MHC-I, which both stabilize the tertiary structure and are recognized by CTL, are frequently derived from cytoplasmic or nuclear viral proteins (38). Indirect evidence suggests that such proteins are degraded by the action of cytoplasmic proteinases into peptides, which in turn are transported by MHC-encoded ABC peptide transporters in the ER (33). Mutant cell lines, selected for low expression of MHC-I, contain defective peptide transporter genes. In these cells, although MHC-I is synthesized, it lacks peptide and is unstable at the physiological temperature (28). In addition to association with peptide in the ER, MHC-I molecules form transient complexes with chaperonins (17).

Since $CD8^+$ CTL may control HCMV infection in transplant patients and to explore whether HCMV persists in healthy individuals because of a virus-induced defect in the $CD8^+$ T-cell response, we have studied the effect of HCMV on MHC-I assembly and expression and tested whether this affects sensitivity to CTL lysis. Although there have been previous reports that HCMV decreases expression of MHC-I (3, 4, 11, 42), little is known concerning either the mechanism or the biological consequences. We report that the decreased surface expression of MHC-I in HCMV-infected cells is accompanied by resistance to lysis by CTL. The decreased MHC-I expression may be explained by very rapid turnover of newly synthesized MHC molecules in a pre-Golgi compartment.

MATERIALS AND METHODS

Cell lines and virus. Human fetal lung fibroblasts, MRC5 (ECACC; Porton, Wilts, United Kingdom) and F2002 (Flow Laboratories), were grown in minimal essential medium (GIBCO, Paisley, United Kingdom) supplemented with 10% fetal calf serum and 2 mM glutamine and used between passages 25 and 35. Owens is a primary human dermal

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fibroblast line (9) used between passages 10 and 20. HCMV strain AD169 was propagated and titered in MRC5 fibroblasts as previously described (9).

Influenza virus-specific CTL lines and clones. Peripheral blood mononuclear cells obtained from healthy laboratory volunteers were prepared from fresh, heparinized blood samples by Ficoll-Hypaque (Histopaque; Sigma Chemical Co.) density gradient centrifugation. Peripheral blood mononuclear cells (1.2×10^7) were suspended in 4 ml of serum-free medium (RPMI 1640; GIBCO) supplemented with 2 mM L-glutamine, 1×10^5 IU of penicillin per liter, and 100 mg of streptomycin per liter in 50-cm^3 tissue culture flasks. A total of 10 μl (200 hemagglutination units) of influenza A virus (A/X31; a kind gift from J. J. Skehel and D. B. Thomas, National Institute for Medical Research, Mill Hill, London, United Kingdom) was added to 4 ml of the same medium and incubated together with the peripheral blood mononuclear cells for 1 h at 37°C, after which the virus was inactivated by the addition of 10% heat-inactivated human AB^+ serum. The flasks were incubated in an upright position for 7 days, after which the cells were transferred to 24-well tissue culture plates and restimulated with fresh medium, recombinant human interleukin-2 (10 IU/ml; Boehringer GmbH, Mannheim, Germany), and irradiated (3,000 rads), peptide (M57-68; Neosystem Laboratoire, Strasbourg, France)-pulsed autologous Epstein-Barr virus-transformed B cells. B cells (2×10^5) were added to the T-cell culture which was kept at a cell density of 5×10^5 cells per well. Fresh medium and recombinant human interleukin-2 was added every 3 days, and cells were refed with peptide-pulsed B cells every 7 days.

^{51}Cr-release assays. MRC5 HCMV-infected or mock-infected cells were labelled with 1.8 MBq of ^{51}Cr (Na chromate; Amersham) for 45 min at 37°C in the presence or absence of 20 μg of influenza virus matrix peptide 57-68 per ml. Cells were extensively washed and counted, and aliquots of 5×10^3 cells were dispensed into round-bottom microtiter plates together with various numbers of human leukocyte antigen (HLA) A2-restricted, influenza virus matrix peptide-specific CTL (total volume, 150 μl). Following incubation at 37°C for 5 h, plates were centrifuged and 15-μl supernatants were removed for liquid scintillation counting (Betaplate; Pharmacia). Percent specific lysis was calculated as (release by CTL − spontaneous release in medium alone)/(2.5% Triton X-100 release − spontaneous release in medium alone). Spontaneous release was <20% for all target cells studied.

Immunofluorescence. Cells to be permeabilized were processed essentially as previously described (2). Briefly, following trypsinization, cells were washed and lightly fixed in 0.01% formaldehyde in phosphate-buffered saline (PBS) for 20 min at 4°C. Following four washes with PBS, cells were incubated at 4°C for 5 min in PBS in the presence or absence of digitonin (20 μg/ml). Permeability was checked with trypan blue. Cells were divided into aliquots and incubated in a total volume of 50 μl of PBS–1% bovine serum albumin (BSA) containing monoclonal antibodies W632 (conformationally intact HLA A, B, and C; Seralab, East Grinstead, United Kingdom), BBM1 (HLA-associated and unbound β_2-microglobulin; American Type Culture Collection), HC10 (denatured HLA B [35]), or OKT3 (CD3; American Type Culture Collection) for 30 min at 4°C. Following three washes, cells were resuspended in 50 μl of goat anti-mouse immunoglobulin conjugated to fluorescein isothiocyanate 1/25 dilution; Dako) in PBS-BSA and incubated for a further 30 min at 4°C. Following three washes, cells were fixed in 2% paraformaldehyde and analyzed by flow cytometry with FACScan (Becton Dickinson). In order to compare levels of fluorescence on virus-infected and uninfected cells, mean channel fluorescence was standardized with fluorescein isothiocyanate-conjugated beads (Flow Cytometry Standards Corp., Durham, N.C.).

Analysis of MHC-I molecules by pulse-chase labelling. Pulse-chase labelling experiments were performed by a modification of the method described by Townsend et al. (39). Briefly, subconfluent cultures of MRC5 cells were infected or mock infected with HCMV for 72 h and washed twice in PBS before incubation in methionine-free medium (GIBCO) for 1 h. Monolayers were trypsinized, and 2×10^7 cells per ml were pulse-labelled with [^{35}S]methionine (500 μCi/ml) for 10 min. An aliquot was removed (pulse only), and minimal essential medium supplemented with unlabelled methionine (2 mM) was added. Aliquots were removed at intervals, and washed cell pellets were incubated for 20 min at 4°C in 0.5 ml of lysis buffer (0.5%, Nonidet P-40, 0.5% Mega 9, 150 mM NaCl, 5 mM EDTA, 50 mM Tris [pH 7.5]) containing 1 mM phenylmethylsulfonyl fluoride and 1 mM iodoacetamide. Nuclei and cell debris were removed by centrifugation (11,000 × *g*, 4°C, and 15 min), and the supernatant was precleared overnight at 4°C with 1% fixed *Staphylococcus aureus* organisms (Sigma). The bacteria were removed by centrifugation (11,000 × *g*, 4°C, and 15 min), and antibody W632 (6 μg/ml) was added to the supernatant. Following 90 min of incubation at 4°C, protein A-conjugated Sepharose beads were added and rotated at 4°C for 45 min. The beads were washed four times with wash buffer (0.5% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 50 mM Tris [pH 7.5]). Following the third wash, the bead suspension was equally divided and dry pellets were resuspended in 15 μl of citrate buffer (0.1 M sodium citrate [pH 6.0], 0.02% sodium dodecyl sulfate [SDS] containing 1 mg of aprotinin per ml) in the presence or absence of 1 mU of endoglycosidase H (Boehringer). The suspensions were incubated for 5 h at 33°C. Samples were then boiled in reducing Laemmli sample buffer, and eluted material was separated by SDS–12% polyacrylamide gel electrophoresis (PAGE). Gels were impregnated with Amplify (Amersham), dried, and exposed to X-ray film at −70°C.

Northern (RNA) blot analysis. Total RNA was extracted by the thiocyanate-LiCl method of Cathala et al. (14). Total RNA (15 μg) was denatured and separated on a 1% agarose formaldehyde gel. The RNA was transferred to GeneScreen Plus membranes according to the manufacturer's (DuPont, Wilmington, Del.) instructions. Filters were hybridized with HLA A2 (19), 2.1U (Tap1 [40]), BS2.1 (Tap2 [29]), or γ-actin (25) cDNA (labelled by random priming) and were washed to a final stringency of 0.1× SSC (1× SSC is 150 mM NaCl, 20 mM Na citrate [pH 7])–1% SDS at 60°C. For autoradiographic analysis, Fuji X-ray films were exposed to the filters at −70°C in the presence of intensifying screens.

RESULTS

HCMV infection is associated with down-regulation of MHC-I expression. In contrast to many viruses, following HCMV infection, cellular protein synthesis continues until late in the lytic cycle and in the early phases is increased, possibly reflecting transactivation of cellular genes (6). However, expression of MHC-I on the HCMV-infected cell surface is markedly reduced—fibroblasts infected with HCMV for 72 h have a 10-fold reduction of cell surface MHC-I in comparison with that of uninfected cells (Fig. 1a and b). In order to test whether the decreased surface expression of MHC-I was due to a defect in transport to the cell surface, we compared total levels of MHC-I in infected and uninfected cells by staining permeabilized cells. Figure 1 c and d demonstrate that the

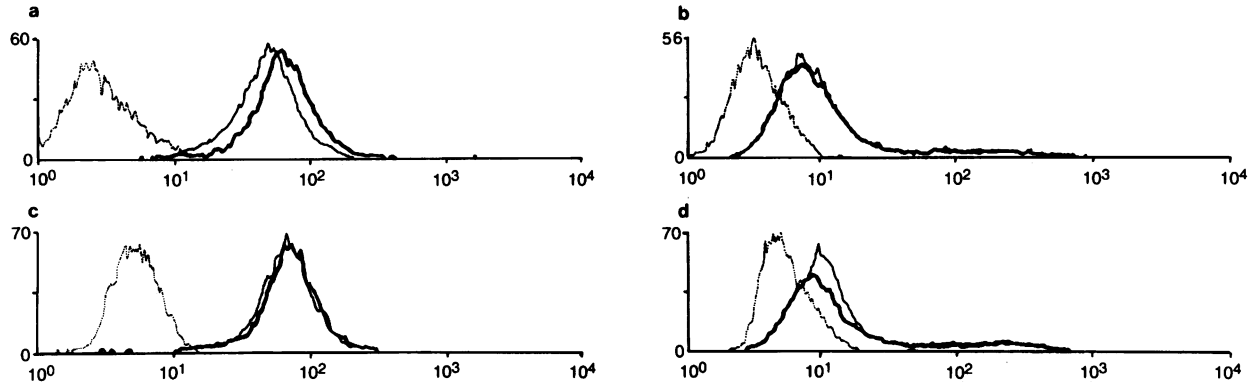

FIG. 1. Flow cytometry analysis of MHC-I expression on cells infected with HCMV. MRC5 cells were infected with HCMV (10 PFU per cell) for 72 h (b and d) or were mock infected (a and c) and were then permeabilized (c and d) or were untreated (a and b) before being stained with W632 (anti-HLA A, B, and C intact heavy-chain–β_2-microglobulin complexes [heavy line]), BBM.1 (anti-β_2-microglobulin, free or heavy chain associated [thin line]), or OKT3 (anti-CD3, irrelevant antibody [dotted line]).

effect of HCMV on expression of MHC-I at the cell surface is also apparent when total cellular levels are compared, indicating that HCMV does not interfere with surface expression by causing intracellular accumulation. The intracellular fraction of MHC-I is very small in both HCMV-infected and uninfected cells (compare Fig. 1a with c and b with d). In order to explore whether the decrease in surface expression is accompanied by a corresponding increase in non-β2-microglobulin-associated, denatured MHC heavy chains, cells were stained with HC10, which recognizes an epitope on denatured HLA B heavy chains. It is clear that the levels of free heavy chain are very low in both HCMV-infected and uninfected cells compared with the levels of heavy chains contained in intact MHC-I complexes (Table 1). Thus, although there is a modest increase in the level of HC10 staining in permeabilized, HCMV-infected cells compared with that in permeabilized, uninfected cells (approximately twofold), this increase is insufficient to explain the decreased surface expression of intact MHC-I.

Down-regulation of surface MHC-I is associated with reduced susceptibility of HCMV-infected cells to CD8$^+$ CTL lysis. In order to determine whether low levels of surface MHC-I in cells infected with HCMV had consequences for the recognition of the infected cell by CD8$^+$ CTL, we examined the ability of HLA A2.1 peptide-specific (influenza virus matrix protein 57-68) CTL to lyse peptide-sensitized, HCMV-infected cells. This indirect approach was used, as no well-defined HCMV peptide-specific CD8$^+$ CTL have been described, thereby making the direct approach less informative in the context of specific peptide antigen recognition. Influenza virus matrix protein peptide (57-68)-specific, HLA A2.1-restricted CD8$^+$ CTL readily lyse peptide-coated HLA A2.1-positive fibroblasts. MRC5 human fibroblasts express HLA A2.1 and as expected, when coated with matrix peptide, are specifically killed by the CTL line (Fig. 2). However, when these targets are infected with HCMV and then peptide coated, they are resistant to lysis, most clearly seen at a high multiplicity of infection (10 to 20 PFU per cell). An active virus infection is required, since no block in killing is found if inactivated virus (UV treated) is used. A similar protection induced by HCMV infection is found when a number of different HLA A2.1-

TABLE 1. Expression of free HLA heavy chains in cells infected with CMV[a]

Treatment	MESF/cell (10^4)					
	Expt 1			Expt 2		
	W632	HC10	OKT3	W632	HC10	OKT3
Uninfected	46.3	1.8	1.4	72.8	1.43	1.18
Uninfected, permeabilized	52.7	5.0	3.1	100.9	8.30	3.06
HCMV treated	5.1	1.9	1.9	8.8	1.31	1.35
HCMV treated, permeabilized	6.1	9.1	3.1	15.0	16.9	3.27

[a] MRC5 cells were uninfected or infected with HCMV (multiplicity of infection, 10) for 72 h. Permeabilized and nonpermeabilized cells were stained with W632 (conformationally intact HLA A, B, and C), HC10 (free, denatured HLA B heavy chains), or OKT3 (irrelevant antibody to CD3) and analyzed by flow cytometry. Mean channel fluorescence was converted to molecules of equivalent soluble fluorochrome (MESF) per cell.

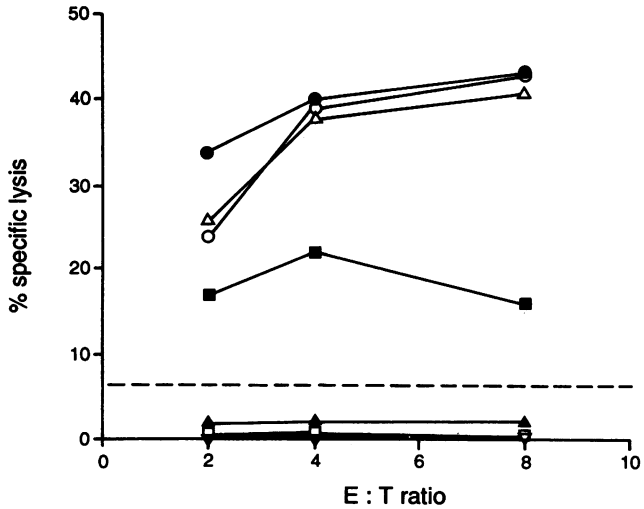

FIG. 2. HCMV infection protects peptide-coated target cells from killing by CD8$^+$ CTL. HLA A2.1-expressing human fibroblasts (MRC5) were infected with HCMV for 48 h at a multiplicity of infection of 20 (□), 10 (▲), 2 (■), or 1 (△) with UV-inactivated virus equivalent to 10 PFU per cell prior to UV inactivation (○) or were mock infected (●). Targets were coated with influenza virus matrix peptide 57-68, ^{51}Cr labelled, and tested as targets (T) in ^{51}Cr-release assay with an HLA A2.1-restricted, matrix peptide-specific CTL line as effectors (E).

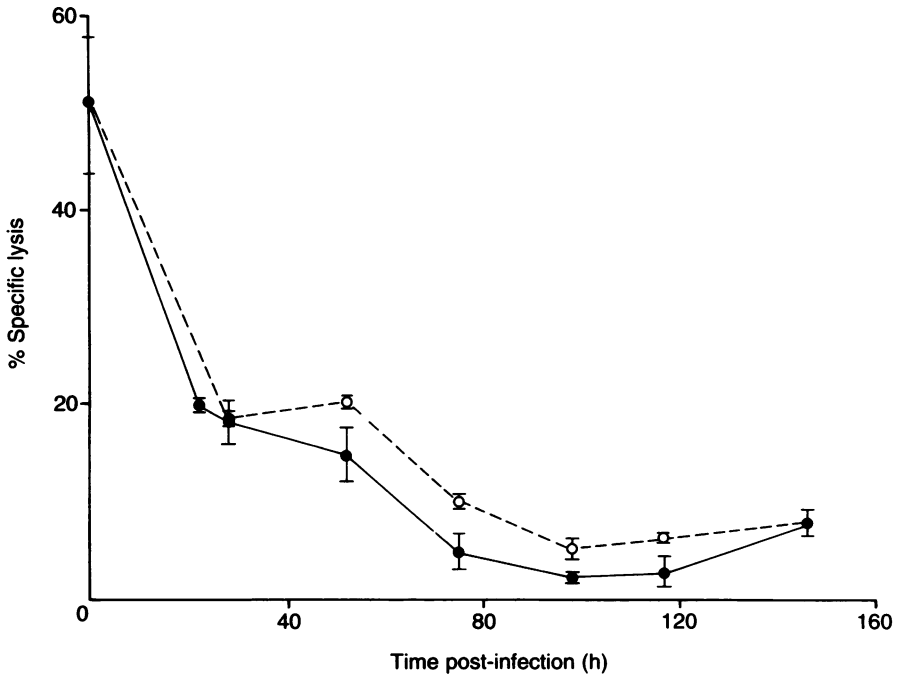

FIG. 3. HCMV infection causes cells to become permanently refractory to CTL-mediated lysis. MRC5 cells were infected with HCMV (10 PFU per cell) in the absence (●) or continuous presence (○) of phosphonoformate (200 μg/ml). At intervals postinfection, cells were trypsinized and used as peptide-coated targets in a ^{51}Cr-release assay.

expressing fibroblasts cell lines are used as targets (data not shown), indicating that HCMV is able to induce protection in these cells irrespective of their origin.

In order to establish whether HCMV-induced protection from lysis is transient, MRC5 cells were infected with HCMV (10 PFU per cell) for various intervals and were then coated with matrix peptide and tested as targets for CTL lysis. A parallel set of cells was infected with HCMV in the continuous presence of phosphonoformate, an inhibitor of viral DNA polymerase, restricting virus protein expression to immediate-early and early proteins (15). Figure 3 shows that the resistance to CTL lysis develops after <24 h postinfection, is maximal by 3 days postinfection, and continues until at least day 5, at which stage HCMV-infected cells are showing severe cytopathic effect. Comparable results were found in the presence or absence of phosphonoformate, indicating that this reduced susceptibility to lysis induced by HCMV is mediated by either immediate-early or early viral products but not late or structural proteins of the virion, since UV-inactivated, noninfectious particles are ineffective (Fig. 2).

MHC-I is assembled normally in HCMV-infected cells but is rapidly turned over. A number of cell lines in which defective surface expression of MHC-I is reduced as a consequence of mutations or deletions in the Tap1 and Tap2 genes located in the MHC-II region have been described. Although MHC-I is assembled in these cells, it is physically unstable and there is reduced surface expression with inefficient viral antigen presentation. In order to determine whether decreased surface expression of MHC-I and reduced susceptibility to lysis caused by HCMV might be due to reduced transcription from genes in the MHC locus, RNA was extracted from HCMV-infected cells and was probed for HLA A2.1, Tap1, and Tap2 transcripts. All HCMV-infected fibroblasts (MRC5, F2002, and a primary human skin fibroblast line, Owens) had elevated levels of Tap1 and Tap2 mRNA compared with those in uninfected cells (Fig. 4) and increased levels of HLA A2 heavy-chain mRNA. Thus, genes in both the MHC-II region and MHC-I region of chromosome 6 appear to be activated in HCMV-infected cells. In contrast, levels of γ-actin mRNA were decreased in HCMV-infected cells relative to those in

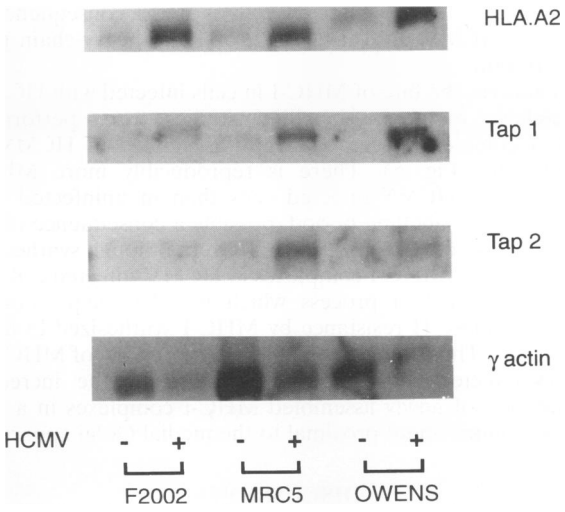

FIG. 4. Northern analysis of MHC gene expression in HCMV-infected cells. Fibroblast lines MRC5, F2002, and Owens were infected with HCMV (10 PFU per cell, 24 h) or were mock infected, and total RNA was extracted. Denatured, separated RNA was transferred to nylon membranes and was probed with ^{32}P-labelled Tap1, Tap2, HLA A2, or γ-actin cDNA as described in Materials and Methods.

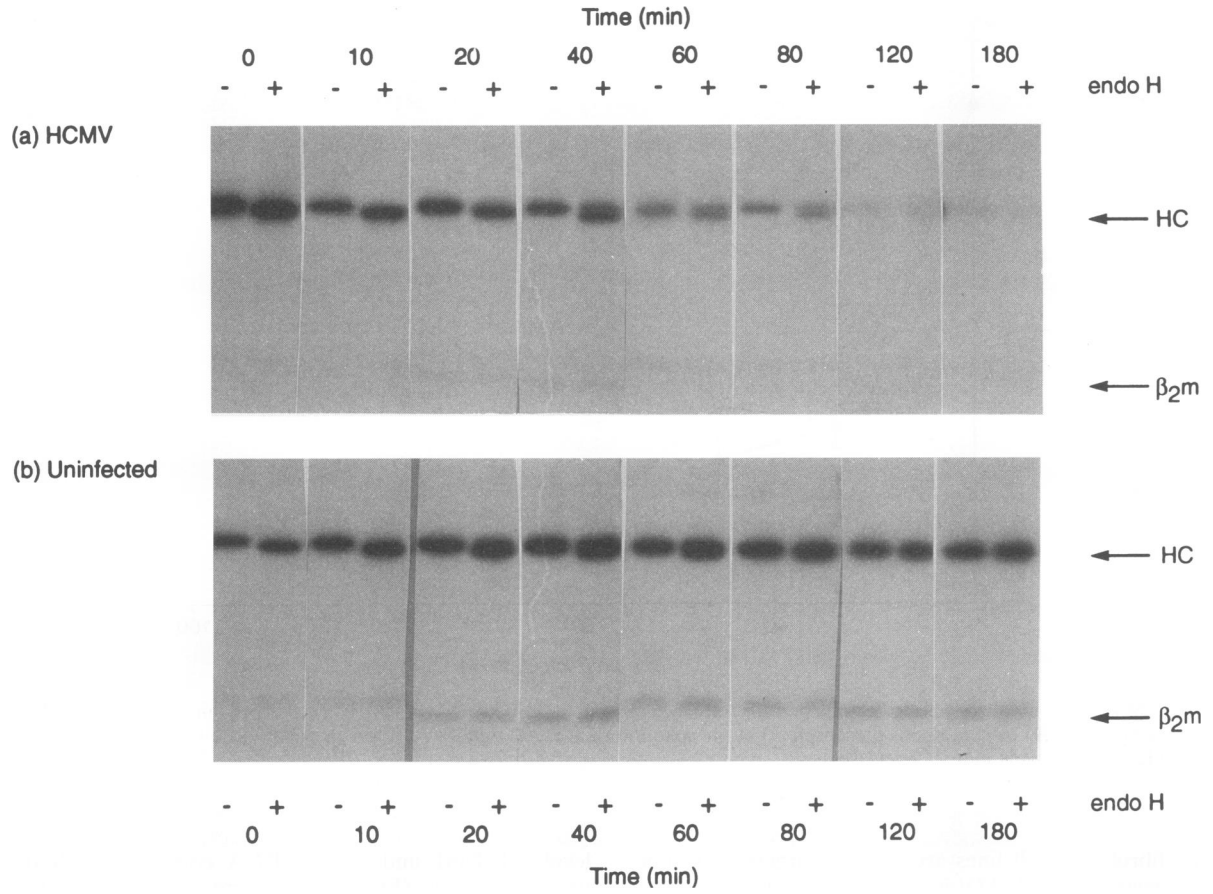

FIG. 5. Pulse-chase metabolic labelling of MHC-I in HCMV-infected and uninfected cells. MRC5 cells infected with HCMV (10 PFU per cell, 72 h) or mock infected were washed and incubated for 1 h in methionine-free medium and then trypsinized. Cells at 2×10^7/ml were pulse-labelled with [^{35}S]methionine for 10 min and then followed by a chase as indicated. W632 immunoprecipitates were divided into two portions and incubated with or without endoglycosidase H (endo H) for 6 h at 33°C before being boiled and separated by SDS–12% PAGE.

uninfected cells. It would seem that the defect in MHC-I expression in HCMV-infected cells is not a consequence of effects of HCMV on Tap1, Tap2, or HLA heavy-chain gene transcription.

To address the fate of MHC-I in cells infected with HCMV, pulse-chase metabolic labelling studies were performed. MHC-I complexes were efficiently assembled in HCMV-infected cells (Fig. 5). There is reproducibly more MHC-I assembled in HCMV-infected cells than in uninfected cells over a 10-min labelling period, possibly a consequence of the large increase in HLA mRNA. However, although synthesized at a faster rate, MHC-I complexes in HCMV-infected cells are rapidly degraded, a process which parallels acquisition of endoglycosidase H resistance by MHC-I synthesized in uninfected cells. Thus, the reduced surface expression of MHC-I in HCMV-infected cells may be explained by the increased degradation of newly assembled MHC-I complexes in a subcellular compartment proximal to the medial Golgi apparatus.

DISCUSSION

HCMV infection disrupts normal expression of the MHC-I molecule in cultured fibroblasts. Although conformationally intact MHC-I molecules are readily assembled, they are rapidly degraded in a subcellular compartment before reaching the Golgi apparatus. This rapid turnover of immature MHC-I glycoprotein probably accounts for the reduction of surface expression detected by surface staining; as expected, the defect in MHC-I expression is detected at earlier times postinfection in metabolic labelling experiments than by antibody staining (data not shown), since any decrease in arrival of newly assembled MHC-I is diluted by the pool of preexisting plasma membrane MHC-I. We have also shown that the failure to export MHC-I to the cell membrane is accompanied by a resistance of HCMV-infected cells to $CD8^+$ CTL lysis, suggesting that the disruption in supply of newly synthesized MHC-I has consequences for T-cell recognition.

A number of laboratories have been interested in the interaction of HCMV with MHC-I. First, it was reported that HCMV bound β_2-microglobulin, and it was proposed that the MHC-I molecule was the receptor for HCMV (24). Subsequent studies suggested that β_2-microglobulin associated with the tegument of ruptured virions (36) and that there was no correlation between MHC-I expression and susceptibility to HCMV infection (5). Second, it was reported that upregulation of MHC-I expression on HCMV-infected fibroblasts occurred (22). However, this increase could be blocked by anti-human beta interferon antibody, suggesting the increase was due to a cytokine effect on the large fraction of uninfected cells in these cultures. More recently, in cultures with a higher number of infected fibroblasts, a decrease in surface MHC-I expression was observed with intracellular accumulation of

MHC-I, by confocal microscopy (3). In contrast, we and others find no evidence for the intracellular accumulation of MHC-I in HCMV-infected cells, either by flow cytometry (Fig. 1) or metabolic labelling (Fig. 5) (4, 11). However, high levels of MHC-I are observed on a small number of cells in HCMV-infected cultures, but these cells are uninfected (data not shown). It has been reported that HCMV infection reduces surface expression of MHC-I even though both polypeptide components of the complex continue to be synthesized (42), in agreement with our findings. Beersma et al. (4) have recently published experiments describing the HCMV-induced instability in MHC-I assembly. Whilst much of their data is in agreement with biochemical data described in this report, Beersma and colleagues additionally demonstrate that the HCMV effect is specific for human MHC-I; thus, in murine L cells transfected with HLA B27, HCMV specifically decreases expression of HLA B27, leaving H-2K^k expression unaltered. This important observation indicates that the HCMV effect is highly specific and distinguishes structural differences between HLA and H-2 heavy chains synthesized within the same cell.

Browne et al. (11) demonstrated that cells infected with HCMV do not synthesize MHC-I, as evidenced by steady-state metabolic labelling experiments. They also showed that the product of the HCMV UL18 gene could bind to β_2-microglobulin when both proteins are coexpressed at high levels in the same cell. This formed the basis for the hypothesis that HCMV-infected cells do not readily assemble and express MHC-I, because the UL18 protein successfully competes with MHC heavy chain for access to β_2-microglobulin. However, it has subsequently been demonstrated that a recombinant HCMV with a defective UL18 gene efficiently down-regulates MHC-I assembly by infected cells (10). Thus, the UL18 gene product is not necessary for HCMV-induced defective MHC-I assembly. At present, the HCMV gene responsible has not been identified and is currently under investigation.

The mechanism underlying the rapid turnover of MHC-I in HCMV-infected cells identified in this study is also unknown. Lack of peptide for MHC-I stabilization is unlikely; first, levels of Tap1 and Tap2 mRNA were increased in HCMV-infected cells, suggesting that production of Tap protein is not inhibited, although we have no direct evidence that functional Tap complexes are present in HCMV-infected cells. Second, addition of the influenza virus matrix peptide to radiolabelled cell lysates or to the medium bathing Tap mutant cells restores stability and surface expression of MHC-I (28), yet this does not rescue MHC-I stability or surface expression in HCMV-infected cells (data not shown). Third, processing mutants with defects in Tap1 or Tap2 are readily lysed by CTL when coated with exogenous synthetic peptide (28), yet cells infected with HCMV are not. Fourth, in the metabolic labelling experiments described here, cell lysates are precleared by overnight incubation at 4°C. Treatment of lysates of RMAS/T2 mutants in this way has been shown to destroy MHC-I heterodimers. In contrast, MHC-I assembled during HCMV infection is not intrinsically unstable but is only unstable in the context of a HCMV-infected cell.

A number of alternative possibilities should be considered. First, the ER-Golgi apparatus vesicle transport pathway may be nonspecifically disrupted by HCMV infection. In this scenario, the flux of viral proteins through the ER may interfere with the regulatory processes involved in assembly, posttranslational modification, and transport of glycoprotein complexes. Alternatively, a HCMV product expressed at immediate-early or early times may block this pathway. The implication of such a defect would be that the maturation and surface expression of a range of cellular glycoprotein complexes would be impaired in cells infected with HCMV. This would seem not to be the case, since we and others have previously shown that surface expression of ICAM 1, LFA 3 (23, 41), and transferrin receptor (8) are increased in HCMV-infected cells and, in the case of the transferrin receptor, are functionally active.

Second, assembly and transport of MHC-I complexes may be specifically affected. The trimolecular association of peptide, β_2-microglobulin, and HLA heavy chain is facilitated by proteins present in the ER. Correct assembly of MHC-I molecules is required for efficient intracellular transport. Williams and Degen (17) have demonstrated that nascent MHC-I molecules associate transiently in the ER with an abundant 88-kDa protein. Dissociation of MHC-I from p88 is rate limiting for transfer to the Golgi apparatus, and efficient transport and p88 dissociation are prevented unless MHC-I is correctly complexed (16). The p88 protein has since been identified as the chaperonin calnexin (20). We do not know whether MHC-I–calnexin association occurs normally in HCMV-infected cells, and this is currently under investigation.

In this study we found a reduced sensitivity of HCMV-infected cells to MHC-I restricted CD8$^+$ CTL lysis. To demonstrate this, we have tested the susceptibility of peptide-coated cells to lysis by influenza virus matrix peptide-specific CTL. That cells infected with HCMV are resistant to lysis is an indication of the severity of the HCMV effect at high multiplicity, since it is known that a few hundred peptide-loaded MHC-I complexes per cell are sufficient to render a target susceptible to T-cell killing. Previously, we and others have shown no intrinsic reduced susceptibility to lysis of HCMV-infected cells by HCMV-specific CTL (8, 9, 31). If the block in antigen presentation caused by HCMV is relevant in vivo, it would be expected that the specificity of the CTL repertoire would be focused on those HCMV antigens capable of being processed and loaded onto MHC-I before the block in stable assembly. This may indeed be the case. First, we have previously reported that a large proportion of CTL precursors recognize peptide fragments derived from the 72-kDa immediate-early protein (1), although Gilbert et al. (21) have suggested that such cells are rare. Furthermore, HCMV-specific CTL also recognize proteins present in the input virion without a requirement for de novo viral protein synthesis, and so it would be predicted that MHC-I complexes could be loaded with fragments of these virion components rapidly following virus entry (32). Conversely, CTL specific for HCMV late proteins are infrequently found in peripheral blood of normal, persistently infected subjects (7). Thus, the temporal generation and MHC-I loading of HCMV peptide fragments may be an important determinant in CTL repertoire diversity.

A number of viruses are able to modify MHC-I expression at both transcriptional (e.g., adenovirus type 12 [34]) and post-transcriptional levels. In the latter instance, adenovirus type 2 encodes an ER resident protein (E3/19k) which binds to and retains nascent MHC-I molecules, preventing surface MHC-I expression in adenovirus type 2-infected cells (12). This has important consequences for CTL recognition of viral antigens synthesized after E3/19k (37). Whether a similar mechanism operates in HCMV-infected cells is not clear, although we find neither intracellular accumulation of MHC-I nor coprecipitation of viral proteins.

Herpes simplex virus type 2 (HSV-2)-infected fibroblasts are relatively poor targets (compared with HSV-2-infected B cells) for both HSV-2-specific CTL and for allospecific killing (27). It has been suggested that these effects may be related to differential effects of HSV-2 on MHC-I expression in these cells.

Cells infected with MCMV are unable to present MHC-I-

bound peptide complexes to T cells during the early phase of MCMV infection. This is associated with a block in maturation of peptide-loaded MHC-I molecules which fail to progress through the medial Golgi apparatus (18), although no alteration in MHC turnover was reported. It has been independently confirmed that MCMV E-gene expression interferes with antigen presentation (13), although in this study the inhibition occurred independently of MHC-I expression and was a selective effect which still enabled MCMV E-derived peptides to be presented. The degree of similarity between strategies for avoidance of recognition by $CD8^+$ lymphocytes adopted by HCMV and MCMV awaits further study.

CMV remains an important cause of morbidity and mortality in the context of immunosuppressed subjects. Evidence suggests that $CD8^+$ CTL mechanisms are important in controlling this infection and maintaining the virus-host equilibrium. However, in order to allow persistent infection to be established and maintained in vivo, evasion of this immune response may be of particular importance. These studies suggest that whilst an effective CTL-mediated response against HCMV may be generated, the changes induced by HCMV in the infected cell may prevent effective clearance of primary infection and thereby allow the establishment and maintenance of the persistent state.

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