Downregulation of MHC class I molecules by human cytomegalovirus-encoded US2 and US11

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CHAPTER 6

Human cytomegalovirus-encoded US2 and US11 target unassembled MHC class I heavy chains for degradation.

Mol. Immunol. : in press (2005)

Human cytomegalovirus-encoded US2 and US11 target unassembled MHC class I heavy chains for degradation.

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Surface MHC class I molecules serve important immune functions as ligands for both T and NK cell receptors for the elimination of infected and malignant cells. In order to reach the cell surface, MHC class I molecules have to fold properly and form trimers consisting of a heavy chain (HC), a β2-microglobulin light chain and an 8 to 10-mer peptide. A panel of ER chaperones facilitates the folding and assembly process. Incorrectly assembled or folded MHC class I HCs are detected by the ER quality control system and transported to the cytosol for degradation by proteasomes. In human cytomegalovirus-infected cells two viral proteins are synthesized, US2 and US11, which target MHC class I HCs for proteasomal degradation. It is unknown at which stage of MHC class I folding and complex formation US2 and US11 come into play. In addition, it is unclear if the disposal takes place via the same pathway through which proteins are removed that fail to pass ER quality control. In this study, we show with a β2m-deficient cell line that US2 and US11 both target unassembled HCs for degradation. This suggests that US2 and US11 both act at an early stage of MHC class I complex formation. In addition, our data indicate that US11-mediated degradation involves mechanisms that are similar to those normally used to remove terminally misfolded HCs.

MHC class I molecules are important reporters for the immune system. They display small fragments of the total cellular protein pool at the cell surface for inspection by cytoxic T cells [1]. In this way they reveal the presence of abnormal proteins expressed by malignant or infected cells. In addition, the absence or presence of MHC class I molecules can be sensed by NK cells and regulate their activation [2].

In order to reach the cell surface, MHC class I molecules have to fold properly and form a trimeric complex that consists of a heavy chain (HC; ~43 kDa), β2-microglobulin (β2m; 12 kDa) and an 8 to 10-mer peptide. The folding and assembly process occurs in an orderly fashion and is facilitated by several ER chaperones.

MHC class I HCs encode a signal peptide, which directs insertion into the ER during translation. Once in the ER, the signal sequence is cleaved off by a signal peptidase. An oligosaccharyl transferase equips the HC with an N-linked oligosaccharide at residue N86. At this stage, free HCs are found in association with the general ER chaperones immunoglobulin binding protein (BiP) [3] and calnexin (CNX), the latter of which is a membrane bound protein with lectin-like activity [4,5]. Bip binds transiently to many newly synthesized proteins and for prolonged times to misfolded proteins or unassembled subunits [6,7]. Binding of CNX is regulated by glucose trimming of nascent N-linked oligosaccharides [8]. CNX generally binds proteins with monoglucosylated (Glc1Man9-7GlnNAc2) oligosaccharides [9]. CNX and BiP predominantly associate with free MHC class I HCs and the assembly with β2m abolishes the interaction of the HC with these chaperones [10-12]. Before binding the light chain, HCs also interact with ERp57, a member of the protein disulfide isomerase (PDI) family, involved in disulfide bond oxidation, reduction and isomerization reactions [13-15]. Mature MHC class I molecules harbor three intra-molecular disulfide bridges, the formation of which is likely to be mainly assisted by ERp57.

After binding β2m, MHC class I molecules are found in association with another, soluble ER chaperone with lectin-like activity, calreticulin (CRT) [16,17]. Like CNX, CRT binds to proteins with Glc1Man9-7GlnNAc2 N-linked oligosaccharides [18,19]. These MHC class I molecules become associated with the peptide loading complex, which besides CRT includes ERp57, tapasin, and the transporter associated with antigen processing (TAP) subunits, TAP1 and TAP2. Tapasin mediates the interaction of HCs with the TAP complex [20-22]. Peptides generated from endogenous proteins by proteasomal degradation are transported from the cytosol into the ER via the TAP complex, where they can be trimmed further by amino-peptidases before

loading onto HC-β2m dimers [23,24]. Trimeric HC-β2m-peptide complexes dissociate from the loading complex and are released into the secretory pathway [25]. In contrast, incompletely assembled MHC class I HCs are recognized by the ER quality control system and are targeted for degradation [26].

During the course of HCMV infection, several viral proteins are synthesized which prevent MHC class I surface expression. These immune evasion proteins can obstruct different steps of the folding and assembly pathway of MHC class I molecules. The unique-short region 3 (US3) gene product retains MHC class I molecules in the ER and specifically affects those types of MHC class I molecules whose surface expression is tapasin-dependent [27]. US6 blocks peptide transport by TAP and thereby prevents the formation of stable trimeric MHC class I complexes [28,29]. Two other HCMV gene products, US2 and US11, both target MHC class I HCs to the cytosol for subsequent proteasomal degradation [30,31].

It is unknown if US2 and US11 make use of the regular ER quality control pathway for disposal of class I molecules. It is also unclear to what extent MHC class I molecules have to be folded and complexed with β2m and/or peptide before US2 and US11 can bind to these proteins. These aspects of US2- and US11-mediated HC degradation are investigated in the present study.

MATERIALS AND METHODS

Cell lines
Wild type FO-1 human melanoma cells [32], which have a defect in β2m gene expression, and FO-1 cells restored for β2m expression [33] were cultured in DMEM (Invitrogen, Breda, The Netherlands), supplemented with 10% FCS (Greiner bv, Alphen aan den Rijn, The Netherlands), 100 U/ml penicillin and 100 g/ml streptomycin (Invitrogen, Breda, The Netherlands). HLA class I molecules expressed by FO-1 cells were genotyped as HLA-A*2501, -B*0801, and -Cw*0701 [34].

Production of retrovirus and transduction
US2 and US11 cDNA fragments, subcloned into the pLZRS-*IRES*-EGFP vector were used for transfection of amphotropic Phoenix packaging cells to produce retrovirus, as described [35-38]. Cells were transduced with retrovirus using retronectin (Takara Shuzo, Otsu, Japan) coated dishes. Transduced cells were sorted for EGFP expression using a FACS Vantage flow cytometer.

Antibodies
The following antisera were used for immuno-precipitations: W6/32 (anti-MHC I complex; [39]), HC10 (anti-MHC I free HC's; [40]), H68.4 (transferrin receptor; Zymed Laboratories, San Francisco, CA), US2(N2) (anti-US2; [41]), and US11(N2) (anti-US11; [42]).

Metabolic labeling, cell lysis, immunoprecipitation and SDS-PAGE
Metabolic labeling, immunoprecipitations and SDS-PAGE were performed as described [43]. Where indicated, media were supplemented with the proteasome inhibitor carboxybenzyl-leucyl-leucyl-leucinal (ZL_3H). For the experiments described in Figure 1, 1mM *N*-ethylmaleimide (NEM; Sigma-Aldrich, Zwijndrecht, the Netherlands) was added to the lysis mix to prevent post-lysis formation of disulfide bonds. Peptide-N-glycosidase F (PNGase F; Roche Diagnostics, Mannheim, Germany) was used according to the manufacturer's protocol. For experiments described in figures 2 and 3, immuno-precipitations were performed on denatured lysates. Cells were lysed in a smaller volume of Nonidet-P40 lysis mix (100 µl /$5x10^6$ cells), and after centrifugation, supernatants were transferred to a new tube with 1/10 volume of 10% SDS and 1/10 volume of 0.1 M DTT. Samples were boiled for 5 min to further denature proteins. Next, the volume was increased 10 times with non-denaturing buffer (1% Triton X-100, 50 mM Tris HCl pH 7.4, 300 mM EDTA, 0.02%NaN_3) supplemented with protease inhibitors and 10 mM iodoacetic acid. Immuno-precipitates were taken up in sample buffer with (Figure 2 and 3) or without β-mercaptoethanol (Figure 1) and boiled for 5 minutes prior to loading onto 12.5 % SDS-PAGE acrylamide gels. Gels were screened with a Bio-Rad Personal Molecular Imager FX and analysed with Quantity One software.

RESULTS

It is unclear at what stage of folding and assembly of newly synthesized MHC class I HCs US2 and US11 come into play to redirect these molecules back to the cytosol for subsequent proteasomal degradation. We evaluated if US2 and US11 can target heavy chains for degradation in an early stage, namely when they are still unassembled. A β2m-negative cell line was used to address this question.

A report by Furman et al. indicated that the redox status influences degradation of class I heavy chains by US2 and US11 [44]. Mature and fully assembled MHC class I complexes contain 3 disulfide bonds: one within the β2m light chain and two within the heavy chain. The disulfide bonds in the heavy chain are located in the membrane-proximal α3 domain and in the α2 domain, the latter of which forms part of the peptide binding groove. Pulse chase experiments with wild type and mutant (C203S+C259S) HLA-A2 revealed that formation of a disulfide bond in the α3 domain of class I was essential for US2-mediated degradation, but not for degradation mediated by US11 [45]. Besides this, several studies indicated that the presence of β2m supports disulfide bond formation in MHC class I HCs [46,47]. In the absence of β2m class I HCs cycle between (fully) oxidized and reduced states [48]. In our current study we make use of the β2m-negative FO-I cell line. Before looking at the effect of US2 and US11 expression on degradation of class I heavy chains, we first investigated the differences in oxidation status of class I HCs in this cell line.

Shortly after synthesis, the majority of free class I HCs is fully oxidised in the absence or presence of β2m

We evaluated the oxidation status of MHC class I heavy chains in β2m-negative (FO-I wild type) and positive (FO-I + β2m) cell lines over time in pulse chase experiments (Figure 1). MHC class I heavy chains were recovered from NP40 lysates (supplemented with the alkylating agent NEM to prevent post lysis formation of disulfide bonds), using either HC10 or W6/32 MoAbs. Samples were separated by SDS-PAGE under non-reducing conditions. Under these circumstances, three distinct bands can be observed of which the intensity and migration patterns differ, with increasing concen-trations of the reducing agent DTT (Figure 1A). The fastest, middle and slowest migrating bands reflect fully oxidized (two disulfide bonds), partially reduced (one disulfide bond) and completely reduced HCs (no disulfide bonds), respectively.

HC10 is specific for free HC's and recognizes all HCs expressed in the β2m negative cells (Figure 1B, lanes 1-4) and only a fraction of the HC pool, likely those still unassembled, in the β2m reconstituted cells (lanes 5-8). W6/32 only recognizes HCs associated with β2m (lanes 13-16) and does not recognize HCs expressed in cells lacking β2m (Figure 1B, upper panel, lanes 9-12). To exclude a contribution of maturation of the N-linked sugar chain on the migration pattern of HC's, part of the samples were treated with PNGase F (Figure 1B, lower panel). In the presence of β2m, all W6/32-reactive material was fully oxidized (lanes 13-16) as well as the majority of

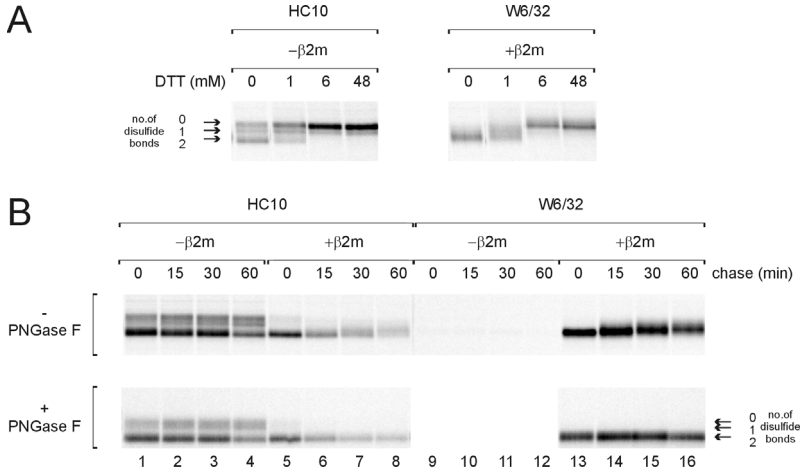

Figure 1. Shortly after synthesis, the majority of free class I HC's is fully oxidised in the presence or absence of β2m. A) FO-I cells, which have a defective β2m gene, and FO-I cells restored for β2m-expression were metabolically labeled with ^{35}S Met/Cys for 60 minutes. Cells were lysed in NP40 lysis mix (supplemented with NEM) and MHC class I HCs were recovered using MoAbs HC10 (anti-free class I HCs) or W6/32 (anti-complexed class I HCs). After immunoprecipitation, samples were split and taken up in sample buffer without or with DTT at the concentrations indicated. Samples were separated by SDS-PAGE (12.5 % gel). **B)** Cells were labeled for 5 minutes and chased for the indicated times. After immunoprecipitation, samples were split and one aliquot was treated with PNGaseF. Samples were taken up in nonreducing sample buffer. Arrows mark the migration pattern of class I HCs with 0, 1, or 2 disulfide bonds.

the HC10-reactive material (lanes 5-8). In contrast, a small amount of fully and partially reduced HC10-reactive HCs were observed in the β2m-negative cells (lanes 1-4). The relative proportion of reduced, partially reduced and oxidized HCs as compared to the total pool varied in the course of the chase in the β2m-negative cells. Right after the pulse and up to 30 minutes later the majority of HCs are fully oxidized (lanes 1-3). After a 60 minutes chase, the total pool of MHC class I is reduced. This is consistent with previous data showing degradation of free HCs in the absence of β2m [49]. At this time point, a decrease is observed in the amount of fully oxidized HCs, and a small increase in the more reduced forms, relative to the total amount of HCs (lane 4). The three distinct conformations are present in more equal amounts after 60 minutes of chase (lane 4).

Since US2 and US11 are known to act within a relatively short time window (minutes after MHC class I synthesis), they are likely to encounter fully oxidized HCs in both β2m-postive and -negative cells.

Unassembled HC's are targeted for degradation by US2 and US11.

Next, we introduced US2 and US11 into the FO-I cell lines to evaluate with pulse chase experiments if these viral proteins can target MHC class I heavy

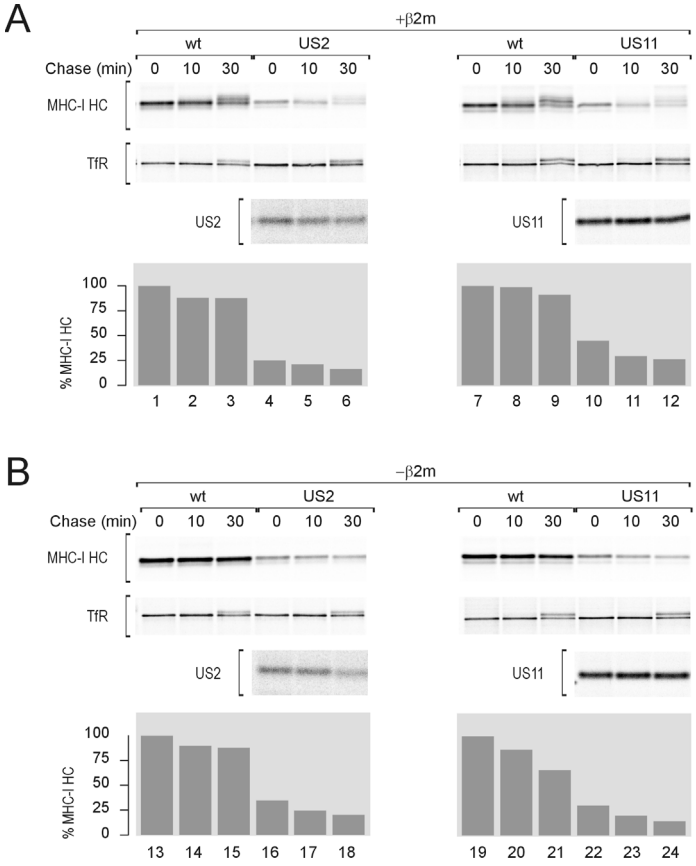

Figure 2. Unassembled HCs are targeted for degradation by US2 and US11. FO-I cells restored for β2m expression (+β2m, panel A) and wild type FO-I cells (-β2m, panel B) were transduced with wt-EGFP, US2-EGFP, or US11-EGFP-encoding retrovirus and sorted for EGFP expression. Cells were metabolically labeled with ^{35}S Met/Cys for 10 minutes and chased for the times indicated. MHC class I HCs, transferrin receptor (TfR), US2 and US11 were recovered from denatured samples, taken up in reducing sample buffer, separated by SDS-PAGE (12.5 % gel) and visualized using a phosphor-imager. The amount of precipitated MHC class I HCs, normalized on the basis of TfR levels, is displayed as a percentage of HC levels found at the onset of chase in wt-EGFP cell lines. Results are based on multiple observations, of which one representative experiment is shown here.

chains for degradation in the absence of β2m (Figure 2). After cell lysis, samples were denatured to ensure that HC10 was able to immunoprecipitate all HCs present in FO-I (+/-β2m) cell samples. Transferrin receptor immunoprecipitates are shown, as an internal control for cell labelling and sample loading. For these experiments, samples were separated by SDS-PAGE under reducing conditions. In FO-I cells reconstituted for β2m expression (Figure 2A), MHC class I heavy chains remained stable over time in the absence of viral proteins (lanes 1-3, 7-9), but are destabilized in the presence of US2 (lanes 4-6) or US11 (lanes 10-12). Note that most of the HCs have already been degraded during the ten minutes pulse, while the transferrin receptor remained stable. Figure 2B shows the effect of US2 and US11 on the stability of HCs in the absence of β2m. In the presence of US2 (lanes 16-18), less HCs could be immunoprecipitated compared to the amount recovered from US2-negative cells, while transferrin receptor levels remained the same in both cell lines (lanes 13-15). The same was observed in US11-expressing FO-I cells (compare lanes 22-24, with 19-21).

Thus, US2 and US11 can target unassembled HCs for degradation, indicating that they can act already at an early stage of MHC class I folding and complex formation.

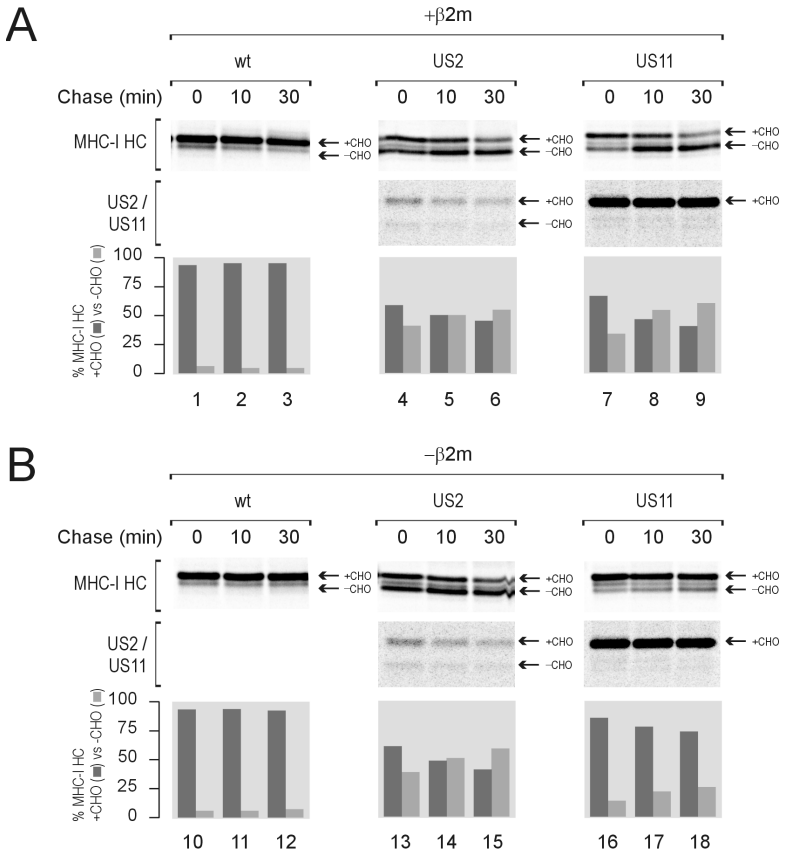

Figure 3. US11 can target HCs to the cytosol in the absence of β2m, but this action is severely compromised when proteasomal activity is blocked. FO-I cells restored for β2m expression (+β2m, panel A) and wild type FO-I cells (-β2m, panel B) were transduced with wt-EGFP, US2-EGFP, or US11-EGFP-encoding retrovirus and sorted for EGFP expression. Cells were metabolically labeled with ^{35}S Met/Cys for 10 minutes and chased for the times indicated, all in the presence of proteasome inhibitor. MHC class I HCs, US2 and US11 were recovered from denatured samples, taken up in reducing sample buffer, separated by SDS-PAGE (12.5 % gel) and visualized using a phosphor-imager. Arrows indicate migration pattern of proteins +/- glycan (CHO). The amount of MHC class I HCs +CHO or –CHO is given as a percentage of the total of MHC class I HCs (+ and – CHO) precipitated from that sample. Results are based on multiple observations, of which one representative experiment is shown here.

US11 can target HCs to the cytosol in the absence of β2m, but this action is severely compromised when proteasomal activity is blocked.

Dislocated MHC class I heavy chains can be visualized using proteasome inhibitors. Visualization is possible due to the fact that the N-linked glycan is removed from retro-translocated HCs by a cytosolic N-glycanase, before HCs are degraded by proteasomes. These breakdown intermediates are characterized by a faster migration pattern in SDS-PAGE [50,51].

To complement the data shown in Figure 2, experiments were performed in the presence of proteasome inhibitor ZL_3H (Figure 3). Figure 3A shows that in β2m expressing cells, HCs remain stable in the absence of viral proteins (lanes 1-3). In both $US2^+$ (lanes 4-6) and $US11^+$ cells (lanes 7-9), a decrease is observed in the amount of glycosylated HCs (HC+CHO) and an increase in the amount of deglycosylated breakdown intermediates (HC-CHO). The results have been quantified and displayed as graphics, with HC+CHO in dark gray and HC-CHO in light gray. Figure 3B shows the results for the β2m-negative cells. A similar conversion from glycosylated HCs to deglycosylated breakdown intermediates could be observed for the $US2^+$ cells (lanes 13-15), compared to the $\beta2m^+$, $US2^+$ cells (lanes 4-6). In contrast, only a minor fraction of HC breakdown intermediates could be observed in the $US11^+$, $\beta2m^-$ cells (lanes 16-18) as compared to the $US11^+$, $\beta2m^+$ cells (lanes 7-9) and the $US2^+$, $\beta2m^-$ cells (lanes 13-15).

These data again show that US2 can target unassembled HCs for degradation and suggest that it can do so equally well in the presence or absence of β2m, with or without proteasome inhibitor. In contrast, proteasome inhibition appears to interfere with the action of US11 in cells lacking β2m.

Inhibition of proteasome activity also delays dislocation of unassembled HCs in β2m negative cells in the absence of viral proteins.

In the absence of β2m, MHC class I HCs become a target for ER quality control mechanisms that ensure disposal of improperly assembled HCs. This has been shown using the β2m-negative Daudi cell line [52]. Pulse chase experiments showed that the dislocation

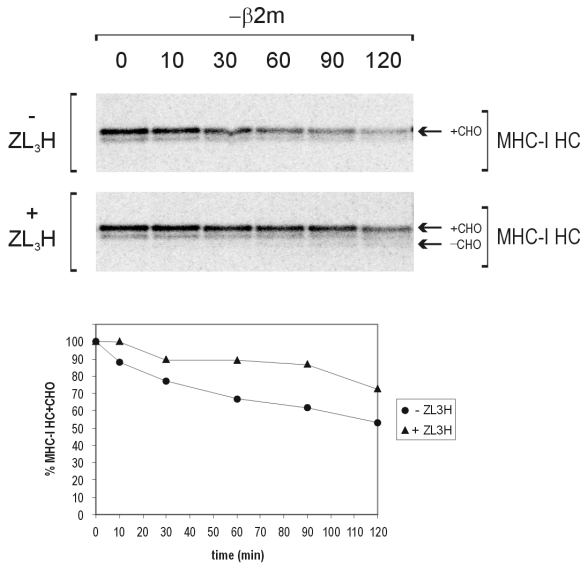

Figure 4. Inhibition of proteasome activity also delays dislocation of unassembled HCs in β2m-negative cells in the absence of viral proteins. Wild type FO-I cells (-β2m) were metabolically labeled with ^{35}S Met/Cys for 10 minutes and chased in the presence or absence of proteasome inhibitor (+/-ZL_3H) for the times indicated. MHC class I HCs were recovered from denatured samples, taken up in reducing sample buffer, separated by SDS-PAGE (12.5 % gel) and visualized using a phosphor-imager. Arrows indicate migration pattern of HCs +/- glycan (CHO). The amount of MHC class I HCs +CHO precipitated at different timepoints (relative to the total amount of HCs at the onset of the chase) is displayed graphically.

and degradation of MHC class I heavy chains takes place at a slower pace, with the first signs of dislocation showing 30 minutes after a 10 minute labelling time. We investigated if the dislocation of unassembled HCs requires proteasomal activity. For this purpose equal amounts of wild type FO-I cells were pulse labelled and chased up to 120 minutes either in the absence or presence of proteasome inhibitor (Figure 4). Equal amounts of glycosylated HCs could be precipitated at the start. Over the course of the chase, some decrease in the amount of glycosylated HCs was observed in cells treated with proteasome inhibitor, accompanied by a slight increase in the amount of deglycosylated HCs. However, this decrease of glycosylated HCs was more pronounced in the absence of proteasome inhibitor.

These results indicate that the quality control-associated dislocation of unassembled HCs is less efficient when proteasomal activity is blocked.

DISCUSSION

HCMV encodes several immune evasion proteins that prevent MHC class I surface expression. These viral gene products can obstruct different steps of the folding and assembly pathway of MHC class I molecules. We investigated at what stage of the assembly process MHC class I HCs are redirected to the cytosol by US2 and US11 for proteasomal degradation.
Previous observations suggest that US2 prefers properly folded and assembled HCs as target; it can be found in association with assembled MHC class I molecules (indicated by its co-precipitation with the conformation-dependent anti-MHC I complex antibody W6/32) [53]. In addition, US2 co-crystallized with class I HC-β2m-peptide complexes [54].

In this study, we evaluated in pulse chase experiments if US2 and US11 are capable of targeting free HCs for degradation. For this purpose, we used a human melanoma cell line (FO-I), which does not express β2m [55]. β2m-reconstituted FO-I cells served as a control. Surprisingly, US2 as well as US11 could target free HCs for degradation. Moreover, this occurred with an efficiency that appeared to be similar to that observed in cells expressing β2m (Figure 2). This shows that US2 and US11 can both act at early stages of MHC class I assembly.

These data are in disagreement with a previous report, which suggested that US2-mediated dislocation of MHC class I HCs requires assembly with β2m [56]. This conclusion was based on experiments performed with a human astrocytoma cell line (U373-GM) in which RNA interference (RNAi) was used to knock down β2m-expression. US2-mediated dislocation of class I HCs was much less efficient in these β2m-knock out cells than in wild type cells, as indicated by a slower conversion of glycosylated to deglycosylated HCs in the presence of proteasome inhibitor. Our data suggest that another factor than the absence of β2m may be responsible for the slowed down US2-mediated retro-transport of HCs in these U373-GM β2m-knock out cells. In our experiments, we could see similar amounts of deglycosylated breakdown inter-mediates for both FO-I and β2m-reconstituted FO-I cells (expressing similar amounts of US2), when proteasome inhibitor was included (Figure 3). It may be that cell type specific factors render FO-I cells more suitable to facilitate US2-mediated degradation of free HCs than U373-MG cells. Alternatively, the RNAi construct used may, besides knocking down β2m-expression, also influence the expression of other factors important for the efficiency of the dislocation process.

We showed that HCs do not require assembly with β2m in order to become targets for US11 either. The efficiency of HC degradation in the presence of US11 is similar in $\beta2m^+$ and $\beta2m^-$ FO-I cells (Figure 2). Interestingly, the inclusion of proteasome inhibitor seriously obstructed the dislocation efficiency of HCs, but only for $US11^+$, $\beta2m^-$ cells (Figure 3). This was not observed in $US2^+$ $\beta2m^+$, $US2^+$, $\beta2m^-$, nor $US11^+$ $\beta2m^+$ cells. Why was this obstruction for dislocation seen only in the presence of proteasome inhibitor, and why only in cells lacking β2m-expression? And why is this observed in US11-positive cells, but not in cells expressing US2?

In the absence of US2 or US11, incompletely folded or assembled MHC class I molecules are also removed from the ER and transported to the cytosol where they are degraded by proteasomes [57]. We showed that the dislocation of unassembled HCs in FO-I cells occurred with lower efficiency in the presence of proteasome inhibitors, in the absence of US11 (Figure 4). A similar observation has been reported for β2m-negative Daudi cells [58]. Interestingly, treatment with chemicals that interfere with disulfide

bond formation (diamide, NEM), also abrogated dislocation of HCs in Daudi cells [59].

MHC class I HCs expressed in cell lines with or without β2m are known to differ for their interaction with ER chaperones. Analysis of human β2m-deficient cells has shown that the light chain is required for correct folding, binding to calreticulin and TAP, peptide loading, intracellular transport, and cell surface expression of HLA class I heavy chains [60,61]. In the absence of β2m, HCs do not enter the secretory pathway, but remain associated for a prolonged time with BiP and calnexin [62,63]. The exact mechanism by which misfolded and unassembled molecules are finally removed from the ER remains elusive, but there are indications that these ER chaperones may play a role in this process.

BiP is known to retain many misfolded proteins in the ER [64], including unassembled MHC class I HC's [65]. Studies with mutant Kar2p (the yeast homologue of BiP) and mutant glycoprotein (CPY*), have shown an association between the ATPase activity of Kar2p with release of malfolded proteins into the cytosol [66]. In another report, studying the release from BiP of a soluble nonglycosylated protein, unassembled Ig L chain, and its retro-translocation out of the ER, the dislocation seemed to be tightly coupled to proteasome activity [67].

Calnexin, a lectin chaperone, accompanies many glycoproteins during their folding [68,69]. It can also contribute to oxidative folding, as it acts in conjunction with the oxidoreductase ERp57 [70]. We showed that in the absence of β2m, the majority of HCs is fully oxidized shortly after synthesis. When these HCs are followed in time, a larger amount, relative to the total HC pool at that time, is found in a partially or completely reduced state (Figure 1). The total amount of HCs gradually becomes less, as unassembled HCs are targeted for degradation (Hughes, Hammond, and Cresswell 1997, and Figure 4). This conversion of HCs to a reduced state may be a prerequisite for efficient dislocation. The finding that diamide and NEM abrogated dislocation in the β2m-negative Daudi cell line supports an influence of protein redox status on dislocation [71]. There are indications that proteasome inhibitors may interfere with CNX/oxido-reductase interactions [72,73]. In cells treated with lactacystin, a redistribution of ER chaperones was observed: upon proteasome inhibition, CNX, CRT, and ER degradation substrates (but not BiP, PDI, glucosyltransferase, ERp57) accumulated in a pericentriolar quality control compartment derived from the ER [74,75].

The fact that we found an abrogation of US11-mediated dislocation, in the presence of proteasome inhibitor only and exclusively in cells lacking β2m-expression may imply that US11 uses partially similar mechanisms for discarding HCs as the endogenous pathway used by FO-I cells to dispose of unassembled class I molecules (Figure 3).

All in all, we conclude that US2 and US11 can act on MHC class I molecules at an early stage of folding and assembly. In addition, our data indicate a link between the endogenous pathway for disposal of terminally misfolded proteins and US11-mediated degradation of MHC class I HCs. More research will be required to unravel the exact partners that link up these processes.

Acknowledgments

We would like to thank Dr. Patrizio Giacomini (Rome, Italy) for providing us with the FO-I wild type and β2m-reconstituted cell lines and Dr. Hidde Ploegh for providing reagents.

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