

Impaired Intracellular Transport and Cell Surface Expression of Nonpolymorphic HLA-E: Evidence for Inefficient Peptide Binding

By Matthias Ulbrecht,* Josef Kellermann,† Judith P. Johnson,* and Elisabeth H. Weiss*

From the Institut für Immunologie, Ludwig-Maximilians-Universität München, W-8000 München 2; and the †Max-Planck-Institut für Biochemie, W-8033 Martinsried, Germany

Summary

The assembly of the classical, polymorphic major histocompatibility complex class I molecules in the endoplasmic reticulum requires the presence of peptide ligands and β_2 -microglobulin (β_2m). Formation of this trimolecular complex is a prerequisite for efficient transport to the cell surface, where presented peptides are scanned by T lymphocytes. The function of the other class I molecules is in dispute. The human, nonclassical class I gene, HLA-E, was found to be ubiquitously transcribed, whereas cell surface expression was difficult to detect upon transfection. Pulse chase experiments revealed that the HLA-E heavy chain in transfectants, obtained with the murine myeloma cell line P3X63-Ag8.653 (X63), displays a significant reduction in oligosaccharide maturation and intracellular transport compared with HLA-B27 in corresponding transfectants. The accordingly low HLA-E cell surface expression could be significantly enhanced by either reducing the culture temperature or by supplementing the medium with human β_2m , suggesting inefficient binding of endogenous peptides to HLA-E. To analyze whether HLA-E binds peptides and to identify the corresponding ligands, fractions of acid-extracted material from HLA-E/X63 transfectants were separated by reverse phase HPLC and were tested for their ability to enhance HLA-E cell surface expression. Two fractions specifically increased the HLA class I expression on the HLA-E transfectant clone.

A variety of the molecules involved in vertebrate immune recognition are encoded by the MHC. In humans these include three highly polymorphic class I transplantation antigens (HLA-A, -B, -C). These so-called classical class I MHC molecules are composed of a MHC-encoded transmembrane heavy chain ($M_r \sim 44,000$; 44K) noncovalently associated with β_2 -microglobulin (β_2m).¹ They present antigens to CTL, due to their capacity to bind peptides from intracellularly degraded proteins, and expose them on the cell surface (1, 2). Moreover, intracellular transport and cell surface expression of classical class I molecules are controlled by the efficient assembly of this trimolecular complex and depend on the availability of high affinity peptides within the endoplasmic reticulum (3–9). Whether or not the less polymorphic nonclassical class I loci (HLA-E [10, 11], -F [12], and -G [13, 14]) also function in antigen presentation in humans is unknown.

Like the classical HLA class I genes, HLA-E, localized be-

tween the HLA-C and the HLA-A loci, is highly transcribed in most tissues (10, 11, 15; M. Ulbrecht, manuscript submitted for publication). Conflicting results have been obtained concerning the cell surface expression of the HLA-E-encoded class I heavy chain. Upon transfection of a HLA-E 6.2-kb HindIII subclone of cosmid B1.1 (E*0102) into an HLA class I-negative mutant B-LCL, only an intracellular 41 K heavy chain could be coprecipitated with human β_2m (hu β_2m) (10, 16). In contrast, the transfer of a 16-kb EcoRI subclone derived from cosmid RS5 was reported to result in a detectable cell surface expression when transfected into mouse L cells, which also express hu β_2m (17). We recently found low cell surface expression of HLA-E antigen in the mouse myeloma cell line P3X63-Ag8.653 (X63) transfected with cosmid cd3.14 (E*0105) and hu β_2m . The discrepancy between the size of the metabolically labeled HLA-E heavy chain (M_r 41 K) and the size of the iodinated surface HLA-E chain (M_r 42 K) precipitated from these cells suggests a defect in N-linked glycan maturation. Here we present evidence that the lack of productive cell surface expression of HLA-E is the result of a low stability of the HLA-E molecule during transport and on the cell surface, which may be due to its defective binding of available endogenous peptides.

¹ Abbreviations used in this paper: BFA, brefeldin A; β_2m , β_2 -microglobulin; hu β_2m , human β_2 -microglobulin; endo H, endoglycosidase H.

Materials and Methods

Cell Culture. P3X63-Ag8.653 (X63) transfected with either human β_2m , human β_2m and HLA-E, or human β_2m and HLA-B27, as described (M. Ulbrecht, manuscript submitted for publication), was maintained in RPMI 1640 (10% FCS, 1% penicillin/streptomycin; all from Gibco Laboratories, Grand Island, NY) at 37°C in 6% CO₂.

10⁵ cells per microtiter plate well were kept for 12 h in 200 μ l of RPMI 1640 at either 37, 26, or 37°C in the presence of 12 μ g/ml purified human β_2m (Sigma Chemical Co., St. Louis, MO).

2 \times 10⁵ cells per microtiter plate well were preincubated for 30 min at 37°C in 200 μ l of RPMI 1640 containing 10 μ g/ml brefeldin A (Sigma Chemical Co.). Cells were then kept for 5 h either at 37°C with or without the addition of 12 μ g/ml of purified human β_2m , or at 26°C.

Fractions of reverse phase HPLC-separated acid-extracted material were dissolved in RPMI 1640. One-half of each fraction was added to 1.5 \times 10⁵ cells of either X63/HLA-E, hu β_2m , or X63/HLA-B27, hu β_2m transfectants. Incubation was performed for 7 h at 37°C in microtiter plate wells in a total volume of 200 μ l of RPMI 1640.

Antibodies. Supernatants of hybridomas producing mAbs specific for human class I, B9.12.1 (IgG2a [18]); human β_2m , BBM.1 (IgG2b; American Type Culture Collection, Rockville, MD); purified mAbs specific for human CD4, M-T151 (IgG2a), and M-T242 (IgG2b); and human class I heavy chain, A1.4 (IgG2b [19]; United Biomedical Inc., Hauppauge, NY) were used for immunofluorescence and immunoprecipitation.

Immunofluorescence Staining and FACS[®] Analysis. 1–2 \times 10⁵ cells per microtiter plate well were washed twice with ice-cold PBS, then incubated with 40 μ l of BBM.1 or B9.12.1 hybridoma supernatant or 40 μ l of A1.4 at a concentration of 20 μ g/ml for 30 min on ice. Cells were washed thrice with PBS and then incubated with 40 μ l dichlorotriazinyl aminofluorescein (DTAF)-conjugated goat anti-mouse Ig-specific antiserum (Dianova) at a concentration of 25 μ g/ml for 30 min on ice. After four washes with PBS, samples were fixed with 1% paraformaldehyde in PBS and analyzed on a FACScan[®] flow cytometer (Becton Dickinson & Co., Mountain View, CA). 5,000 viable cells were measured. Mean fluorescence was obtained with logarithmic amplification of fluorescence intensity expressed in channels, where 4 logs span 1,024 channels (256 channels per log). An increase in fluorescence intensity of 160 channels represents a 4.2-fold increase in expression, and 120 channels represent a threefold increase.

Preparation of Radiolabeled Proteins. For metabolic labeling, cells (1–3 \times 10⁶/ml) were preincubated for 1.5 h in methionine-free MEM supplemented with 10% dialyzed FCS (all from Gibco Laboratories) at 37°C. Cells were then pulse labeled with 20 μ Ci/ml [³⁵S]methionine (>1,000 Ci/mmol; Amersham Corp., Arlington Heights, IL) for 10 min at 37°C and chased in the presence of a 200–400-fold molar excess of cold methionine at 37°C, or where indicated at 26°C. At the time points indicated aliquots were removed and lysed.

Iodination of cell surface molecules was carried out using the lactoperoxidase-glucose oxidase method (20). In brief, 10⁷ cells cultured at either 37 or 26°C for 24 h were washed twice with PBS and labeled in 1 ml of PBS with 5 U lactoperoxidase (L-0515; Sigma Chemical Co.), 6 U glucose oxidase type V (Sigma Chemical Co.), and 1 mCi of carrier-free Na¹²⁵I (17.4 Ci/mg; DuPont Co., Wilmington, DE) in the presence of 0.01 mM KI and 5 mM D-glucose for 20 min at room temperature. Cells were washed five times in PBS/10% FCS and lysed.

Immunoprecipitation and SDS-PAGE. [³⁵S]-Methionine metabolically labeled cells were lysed at 4°C for 30 min in 0.6 ml lysis buffer (1% [wt/vol] NP-40, 2 mM benzamidinium HCl, 2 mM PMSF, 2% [vol/vol] aprotinin in PBS). After sedimentation of nuclei and debris at 15,000 g for 15 min at 4°C, lysates were supplemented with 50 μ l of FCS and precleared at 4°C one to three times for 12 h with 100 μ l horse serum-saturated Sepharose 6B (Pharmacia Fine Chemicals, Piscataway, NJ), one to two times for 2 h with 50 μ l of protein A-Sepharose CL-4B (Sigma Chemical Co.), and once for 1 h with 20 μ l of isotype control mAb(M-T151 or M-T242)-protein A-Sepharose. After a last preclearing step with 20 μ l of protein A-Sepharose for 1 h, lysates were incubated for 1 h at 4°C with 20 μ l of either A1.4- or B9.12.1-protein A-Sepharose. Immunoabsorbents were washed six times in PBS containing 0.2% (wt/vol) NP-40, resuspended in 50 μ l of SDS sample buffer (4.2% SDS, 0.1% 2-ME, 18% glycerol), boiled for 3 min, and analysed by SDS-PAGE on 9, 10, or 12% gels. The gels were treated with Amplify (Amersham Corp.), dried, and fluorographed at –85°C.

For ¹²⁵I-labeled proteins a modification of this protocol was adopted. After preclearing at 4°C for the first 15 h with 100 μ l of horse serum-saturated Sepharose and then for 2 h with 25 μ l of protein A-Sepharose, lysates were sequentially immunoprecipitated (1 h at 4°C) using the following reagents: 20 μ l isotype control M-T151-protein A-Sepharose, 20 μ l of B9.12.1-protein A-Sepharose, 20 μ l of isotype control M-T242-protein A-Sepharose, 30 μ l of A1.4-protein A-Sepharose, and finally 20 μ l of BBM.1-protein A-Sepharose. Between each round lysates were cleared with 20 μ l of protein A-Sepharose for 2 h at 4°C. Further treatment of immunoabsorbents was as described. For analysis, 10 or 12% SDS-PAGE was used.

Endo H Treatment of Radiolabeled Proteins. After washing with PBS containing 0.2% (wt/vol) NP-40, immunoabsorbents were split into two equal aliquots, resuspended in 26 μ l of sodium phosphate buffer (50 mM, pH 5.5) supplemented with 0.1 M 2-ME and 0.02% SDS, boiled for 1 min, and then cooled on ice. 1 μ l of 200 mM PMSF and 8 μ l of endo H (1 mU/ μ l; Boehringer Mannheim Biochemicals) were added (control samples receiving 8 μ l PBS), and digestion was performed at 37°C for 15 h under gentle agitation. Samples were analyzed as described by 10 or 12% SDS-PAGE.

Acid Extraction of Peptides and HPLC Separation. 3 \times 10⁶ human β_2m /HLA-E-transfected X63 cells kept at 37°C and washed twice with PBS were resuspended in 0.1% (vol/vol) TFA. Extraction was performed as described (21). Lyophilized material was resuspended in 0.1% TFA and subjected to a Sephadex G-25 gel filtration column (Pharmacia Fine Chemicals). Material of *M*, <10 K was collected and dried by vacuum centrifugation. The extract was dissolved in 0.1% TFA and separated by HPLC (LKB Instruments) on a reverse phase column, Hibar LiChrospher (Merck), using (a) 0.1% TFA/H₂O, and (b) 0.1% TFA/acetonitrile as a solvent system. A linear gradient from 0 to 70% (vol/vol) of *b* in *a* was applied over a period of 60 min at room temperature. The flow rate was adjusted to 1 ml/min. The effluent was monitored at 206 nm. Fractions of 1 min were collected and dried by vacuum centrifugation.

Results

Impaired Intracellular Transport of HLA-E Molecules. Post-translational modifications and intracellular transport of HLA-E and HLA-B27 heavy chains were investigated by pulse chase experiments (Fig. 1). Comparing X63 transfectants, expressing only hu β_2m , HLA-E and hu β_2m , or HLA-B27

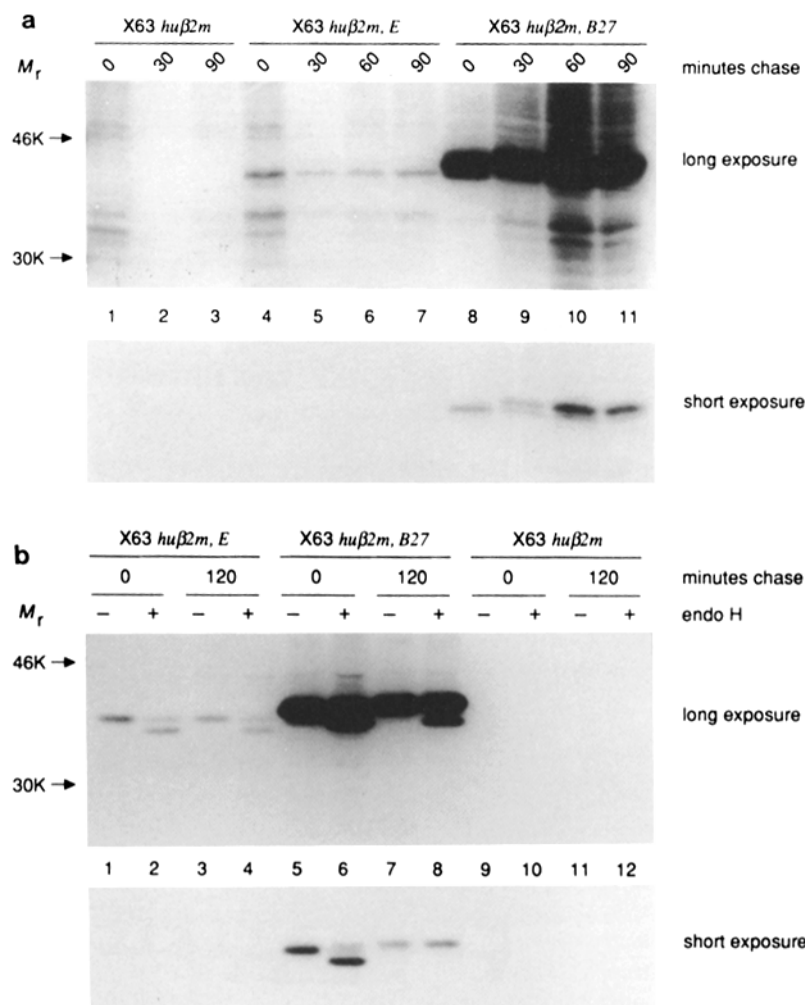


Figure 1. Inefficient oligosaccharide maturation of the HLA-E class I heavy chain. (a) X63 transfectant clones expressing either hu β_2m (lanes 1–3), HLA-E and hu β_2m (lanes 4–7), or HLA-B27 and hu β_2m (lanes 8–11) were metabolically labeled for 10 min and chased for the times indicated at 37°C. HLA class I molecules were immunoprecipitated with mAb A1.4 specific for HLA class I and separated by 10% SDS-PAGE. A short exposure is shown to visualize the process of glycan modification of the HLA-B27-encoded α chain. (b) X63 transfectant clones expressing either HLA-E and hu β_2m (lanes 1–4), HLA-B27 and hu β_2m (lanes 5–8), or hu β_2m (lanes 9–12) were metabolically labeled for 10 min and chased for the times indicated at 37°C. For each time point equal aliquots of anti-HLA class I immunoprecipitates with mAb B9.12.1 were either endo H (+) or mock (-) digested and analyzed by 10% SDS-PAGE.

and hu β_2m , revealed that the HLA-B27 molecules showed extensive glycan modification during the chase period of 90 min (Fig. 1 a) and expressed predominantly mature (endo H-resistant) glycans at 120 min (Fig. 1 b). In contrast, HLA-E molecules did not increase in size (Fig. 1 a) and remained endo H sensitive (Fig. 1 b). In biosynthetically labeled cells, both HLA-E and HLA-B27 heavy chains were precipitated at least partially complexed with β_2m (see Fig. 5), irrespective of the mAb used (B9.12.1 [18] or A1.4, which also recognizes denatured class I heavy chains [19]).

Enhancement of HLA-E Surface Expression at Low Temperature. A defect in carbohydrate side chain processing and intracellular trafficking of classical MHC class I molecules, which has been observed in the absence of peptide ligands, could be compensated for by a reduction in temperature (4, 22). Culturing the X63 transfectants at 26°C resulted in a three- to eightfold increase of human class I-specific cell surface immunofluorescence of the HLA-E transfectant, whereas no effect was observed on the HLA-B27 expression (Fig. 2). Maximum expression was reached after a 4-h culture period and persisted up to 48 h. Labeling of cell surface class I molecules and immunoprecipitation confirmed the increase of HLA-E molecules on the cell surface (Fig. 3). Whereas in metaboli-

cally labeled cells a M_r 41 K HLA-E heavy chain remained endo H sensitive, HLA-E antigen expressed on the cell surface at either 37 or 26°C was endo H resistant. A polypeptide of M_r 37 K coprecipitated with cell surface HLA-E polypeptides and with HLA-B27 heavy chains not complexed with β_2m . The origin of this protein is not clear. It might represent a proteolytically shortened class I heavy chain or another, possibly irrelevant molecule coprecipitated with human class I polypeptides.

Enhancement of HLA-E Surface Expression by Exogenous hu β_2m . Sequential immunoprecipitation using mAb B9.12.1 followed by A1.4 indicates that the majority of HLA-E and a substantial fraction of HLA-B27 are present on the cell surface of the X63 transfectants as free class I heavy chains, not complexed with β_2m (Fig. 3 b). Some HLA-E molecules are complexed with β_2m on the cell surface when immunoprecipitated with anti- β_2m (BBM.1; Fig. 3 b). Since immunoprecipitation of metabolically and cell surface-labeled cells was performed under the same conditions with concentrated cell lysates, the great reduction of β_2m -associated HLA-E polypeptide in cell surface-labeled lysates should not be due to a subsequent enhanced dissociation of the iodinated HLA-E molecule (23).

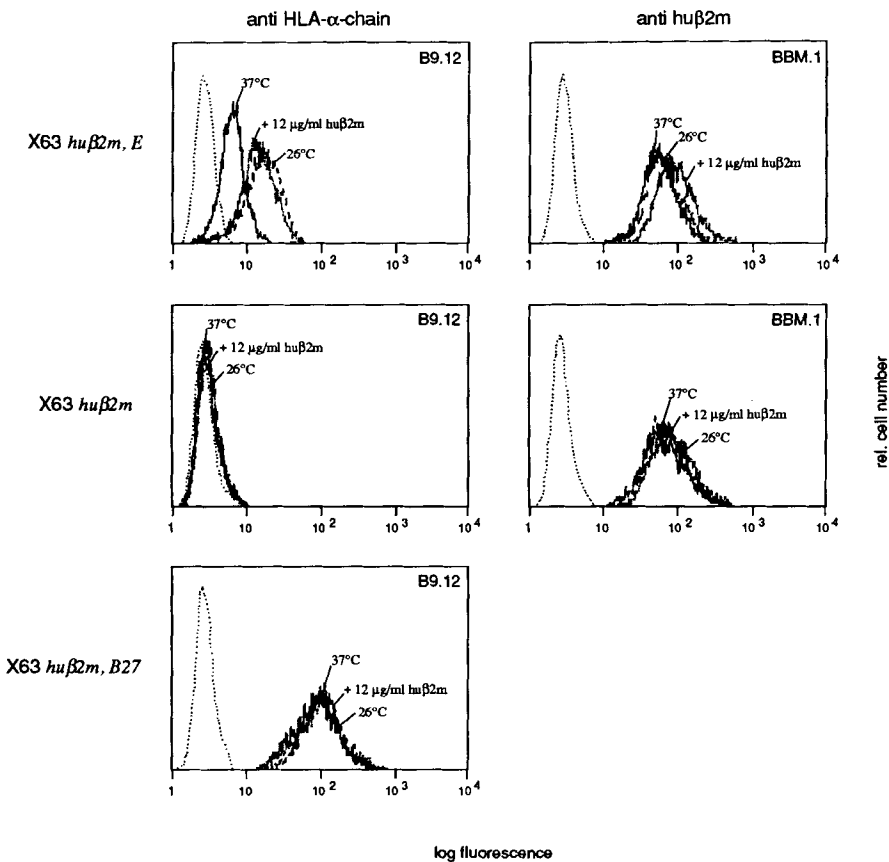


Figure 2. Enhancement of cell surface expression of the HLA-E class I molecules at low temperature or by excess hu β_2m in the medium. X63 transfectant clones expressing either hu β_2m alone, HLA-E and hu β_2m , or HLA-B27 and hu β_2m were cultured either at 37°C in absence or presence of hu β_2m , or at 26°C for 12 h and subsequently stained with mAbs against HLA class I (B9.12.1) and hu β_2m (BBM.1). Specific indirect fluorescence profiles were compared to second antibody alone (.....). The same staining profiles were obtained using the mAb A1.4.

Exogenous β_2m has been shown to stabilize free heavy chains on the cell surface (24). Incubation of the X63 transfectants for 12 h at 37°C in the presence of exogenous hu β_2m led to the same increase in HLA-E cell surface expression as observed after 12 h at 26°C (Fig. 2). No increase was detected after a 1-h incubation with hu β_2m . An increased level of hu β_2m at the cell surface of the HLA-E transfectant was only observed when hu β_2m was in the medium. Again, neither treatment affected HLA-B27 cell surface expression. The association of exogenous hu β_2m with mouse cells has been described to be rapid (24), reaching saturation after 15 min and reflecting the presence of a large pool of free class I heavy chains on the mouse cells tested. The prolonged time required for enhanced HLA-E expression suggests that free HLA-E is not present on the cell surface in a conformation undetectable by the reagents used, but that HLA-E polypeptides reaching the cell surface are continuously stabilized. This is confirmed by the lack of any increase of HLA-E cell surface expression in the presence of brefeldin A (BFA), which inhibits further intracellular transport (Fig. 4) (4, 25). In contrast, purified mouse β_2m did not enhance HLA-E cell surface expression (data not shown).

Low Temperature Stabilizes Mature HLA-E Molecules. To correlate the enhancement of HLA-E surface expression at low temperature with the underlying processes of intracellular transport, transfectants were pulse labeled at 37°C and equal aliquots chased at either 37 or 26°C. Low temperature reduces the turnover both of immature M_r 41 K HLA-E

heavy chains and HLA-E molecules of M_r 42 K with mature glycans, though the effect on the processed form predominates (Fig. 5). The mature M_r 42 K form of HLA-E is abundantly detectable when chased at 26°C, whereas only trace amounts precipitate after a 4-h chase at 37°C. This is not a result of an increase in processing rate, since equal amounts of immature HLA-E α chains are precipitated after a 4-h chase at both temperatures. In contrast, reduction of the chase temperature had little influence on the turnover and the oligosaccharide maturation of the HLA-B27 heavy chain. That the residual immature form can be detected after 4 h at 37°C, whereas only a mature product is seen in cells chased at 26°C, might indicate that HLA-B27-linked glycan modification was accelerated at 26°C. At either chase temperature the HLA-E molecules failed to show complete oligosaccharide maturation even after 8 h, implying a reduced rate of intracellular transport. The experiments suggest that after reaching the cell surface at 37°C, the mature form may dissociate from β_2m and be rapidly degraded. Since the mAb A1.4 also recognizes denatured HLA class I heavy chains, the turnover of the HLA-E polypeptide at 37°C should not be due to irreversible denaturation. Incubation at 26°C or the presence of exogenous hu β_2m stabilizes the conformation of HLA-E and thus inhibits its degradation.

Enhancement of HLA-E Cell Surface Expression by Eluted Peptide Material. The observation that cell surface expression of the HLA-E antigen was only detected upon transfection into X63 cells might indicate that these cells contain a low

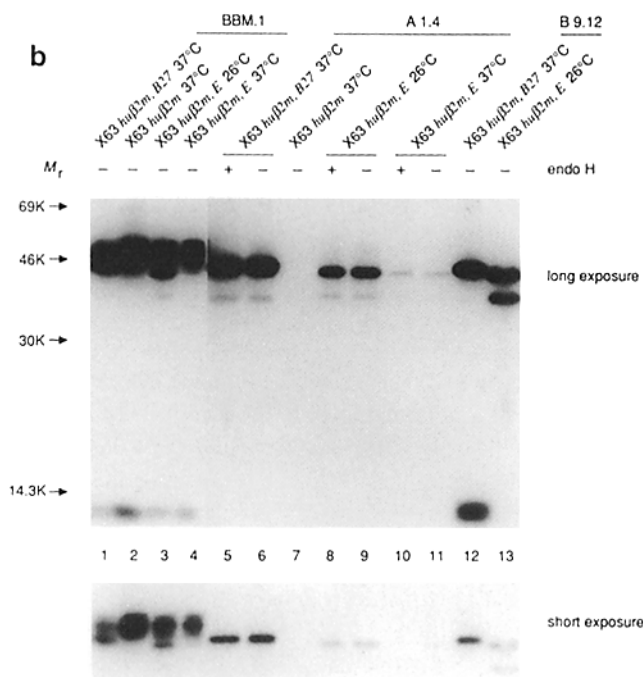
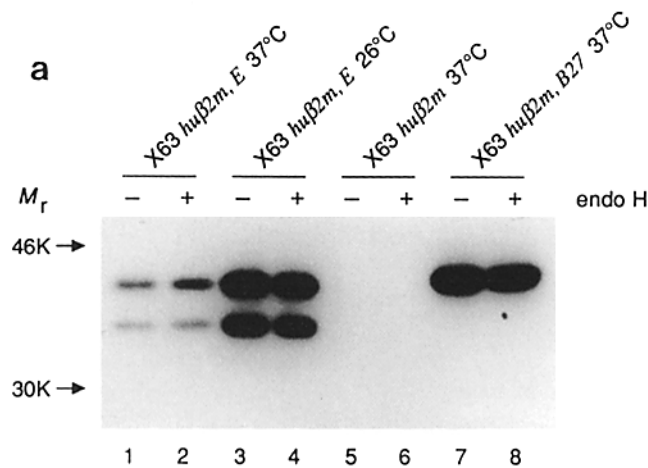


Figure 3. Increase of mature, endo H-resistant surface HLA-E molecules at low temperature. X63 cells expressing huβ_{2m} (a, lanes 5 and 6; b, lanes 2 and 7), HLA-B27/huβ_{2m} (a, lanes 7 and 8; b, lanes 1, 5, 6, and 12), or HLA-E/huβ_{2m} (a, lanes 1–4; b, lanes 3, 4, 8–11, and 13) were kept for 24 h at 26 or 37°C, as indicated, before surface-labeling by iodination and lysis. Lysates were sequentially immunoprecipitated with mAbs B9.12.1 (a, lanes 1–8; b, lanes 12 and 13), A1.4 (a, lanes 5–11), and BBM.1 (b, lanes 1–4). Equal aliquots of B9.12.1 (a) and A1.4 immune complexes (b) were either endo H (+) or mock (–) digested. Analysis was performed by SDS-PAGE on 10% (a) and 12% (b) gels.

amount of self-peptides capable of binding to this class I molecule, or that they are permissive to the cell surface transport of empty, otherwise retained, class I molecules. To analyze whether HLA-E has the ability to bind peptides and to identify the corresponding ligands, acid extracts of the X63/huβ_{2m}, HLA-E transfectant were prepared as described, and peptides of *M_r* <10 K were further fractionated by reverse phase HPLC (21, 26). We reasoned that the small amount

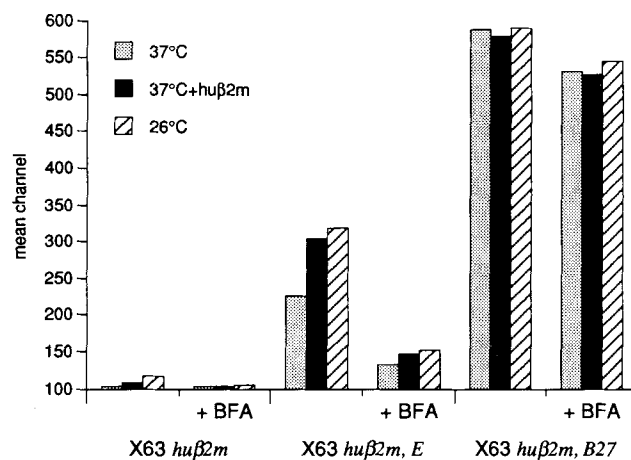


Figure 4. BFA inhibits enhancement of cell surface expression of the HLA-E class I molecules at low temperature or by excess huβ_{2m} in the medium. Aliquots of huβ_{2m}⁺, huβ_{2m}/HLA-E⁺, huβ_{2m}/HLA-B27⁺ X63 transfectant clones were preincubated for 30 min at 37°C in the presence of BFA. Cells were then kept for 5 h either at 37°C, with or without addition of 12 μg/ml of purified huβ_{2m}, or at 26°C. Cells were then stained with mAb A1.4 by indirect immunofluorescence. Fluorescence intensities are indicated as mean channels.

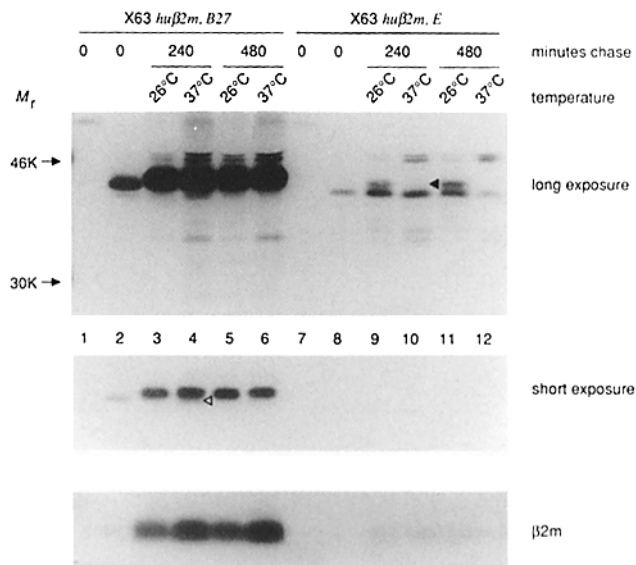


Figure 5. Posttranslational modifications of HLA-E α chains induced at low temperature. HLA-B27/huβ_{2m}⁺ (lanes 1–6) or HLA-E/huβ_{2m}⁺ (lanes 7–12) X63 transfectants were cultured and metabolically labeled for 10 min at 37°C. Equal aliquots were chased for the times indicated at either 37 or 26°C, and lysed. HLA class I molecules were immunoprecipitated with mAb B9.12.1 (lanes 2–6 and 8–12) and analyzed by 9% SDS-PAGE. Representative unspecific immunoprecipitates obtained with isotype control M-T151 during preclearing are shown for the 0-min time points (lanes 1 and 7). The upper bands in the HLA class I immunoprecipitates were endo H resistant (data not shown) and represent the mature form of the class I heavy chains. Trace amounts of mature HLA-E molecules after a 240-min chase at 37°C are indicated (◀). A short exposure visualizes oligosaccharide maturation of the HLA-B27 molecules. (◁) Small amounts of immature HLA-B27 molecules present after a 240-min chase at 37°C. To demonstrate coprecipitating β_{2m}, remaining immunoprecipitates were analyzed by 12% SDS-PAGE (bottom). Due to the pipetting scheme, the 0-min time points contained fewer cells.

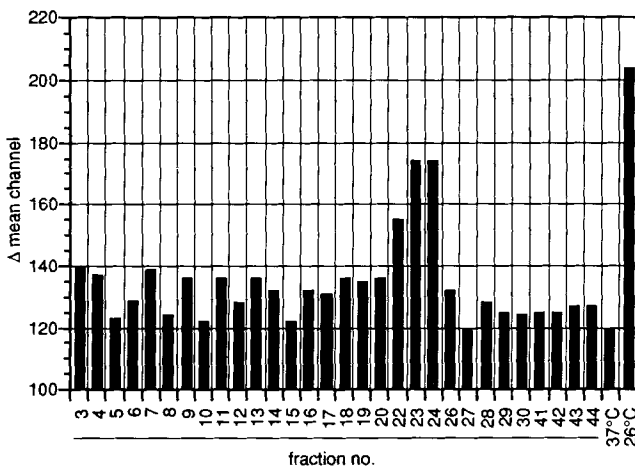


Figure 6. Enhancement of HLA-E cell surface expression by eluted peptide material. X63 transfectants expressing either hu β_2m and HLA-E, or hu β_2m and HLA-B27, were incubated for 7 h at 37°C with 50% of the reverse phase HPLC-separated fractions of acid extracts from X63/HLA-E, hu β_2m transfectants, and tested for the cell surface expression of HLA class I heavy chains by flow cytometry using mAb A1.4. (Δ mean channels) Specific fluorescence obtained by subtracting background staining (no first mAb) from A1.4 staining. The number of the HPLC fractions is indicated at the bottom. The two bars at the right show A1.4 binding to cells incubated with medium alone for 7 h at either 37 or 26°C.

of β_2m -associated mature HLA-E antigen on the cell surface might be peptide ligand associated. Individual peptide fractions were tested for the enhancement of HLA-E or HLA-B27 cell surface expression (Fig. 6). Two fractions (23 and 24) significantly enhanced the HLA class I expression on the HLA-E transfectant clone. None of the fractions had an effect on the HLA-B27 transfectant (data not shown). Adopting the same elution protocol, purified hu β_2m eluted from the reverse phase HPLC column in fraction 38.

Discussion

These findings suggest that the impaired expression of the HLA-E antigen may be the result of insufficient binding of peptide ligands (3, 7) or of complexing longer peptides, giving rise to unstable molecules (27). Further experiments are needed to determine whether the extracted ligands were originally complexed with HLA-E. Sequence analysis of the purified peptides and identification of the self-protein will reveal whether the aberrant expression of the HLA-E molecule results from an insufficient pool of self-peptides capable of binding to HLA-E, or whether the structure of its binding cleft only accommodates peptides with a rare composition. Protein sequence comparison does not indicate the presence of substitutions at positions known to be crucial for ligand binding (28).

In addition, HLA-E antigen expression may be affected by a reduced translation efficiency since the amount of HLA-E mRNA did not correlate with the HLA-E cell surface staining in transfectant clones. The clones used in the experiments

presented here contained similar amounts of steady-state hu β_2m and HLA class I mRNA, comparable with human tissues (M. Ulbrecht, manuscript submitted for publication). Regardless of the reagent used for immunoprecipitation of pulse-labeled class I, the amount of HLA-E polypeptide was drastically reduced compared with the HLA-B27 immunoprecipitates. The possibility of a rapid conversion of HLA-E into a non-hu β_2m -associated form that is not recognized by A1.4 cannot formally be excluded.

It is unlikely that the poor cell surface expression of the HLA-E molecule is only due to its expression in a mouse cell line. Class I antigen cell surface expression is easily detected upon transfection of HLA-A, -B, and -C genes (29, 30) and no species-specific restriction in peptide binding and class I assembly has been described so far, although variability in MHC-encoded transporter or proteasome genes might affect the endoplasmic peptide spectrum (7, 31). Indeed, defective cell surface expression of the HLA-E antigen was also observed upon transfection into human B-LCL 721.221 (16) cells. HLA-E might be representative for a class of relative invariant class I antigens found in all species studied so far that share weak cell surface representation (16, 32, 33).

Our results might suggest that the structure of HLA-E has evolved such that this class I antigen lost its function in presenting antigenic peptides to the immune system. This must not necessarily be true, since HLA-E, in its biochemical properties, behaves very similarly to H-2L^d, which serves as a restriction element for several virus-specific cytotoxic T cell responses in H-2^d mouse strains (34, 35). Moreover, the homologue of a nonpolymorphic HLA molecule, HLA-G, functions as major restriction element in a New World primate species (36).

A specific physiological role for nonclassical MHC class I molecules is only emerging now. In the mouse, such molecules can present synthetic peptides to T cells, and it has been postulated that they display a greater specialization of peptide binding than the classical MHC molecules (37–40). Several reports have suggested that TL region class I gene products might be involved in antigen presentation to γ/δ receptor-bearing T cells (38, 41). The influence of heat-shock proteins on Qa-1 cell surface expression (42), and the finding that several γ/δ T cell lines recognize heat-shock proteins (43), have led to the hypothesis that the TL molecules and other nonclassical MHC class I molecules might, as a primary line of defense, present peptides derived from an endogenous stress-response protein to γ/δ T cells (42). In addition, since they are not saturated with endogenous peptides, they might preferentially bind environmental antigens that can be recognized by the immune system. The observed stabilization of HLA-E class I antigens at reduced temperature might be of physiological significance in the skin, where the temperature is lower than elsewhere in the body.

It is also conceivable that HLA-E serves a more "ancient" function intracellularly as a "modulator" of endoplasmic peptide loading. This may be related to the function of heat-shock proteins (hsp70), in which the hypothetical structure of the peptide binding domain is similar to the MHC an-

tigen binding site (44, 45). Since HLA-E is retained and accumulates in the class I "peptide-loading" compartment, the endoplasmic reticulum, it might influence the spectrum of peptides presented by classical class I molecules through low affinity binding and easy release of peptides, thus modulating

the concentration of free peptides available. Finally, HLA-E might be involved in the postulated process of trimming preprocessed peptides to the biologically active length of eight to nine amino acids.

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Address correspondence to Elisabeth H. Weiss, Institut für Anthropologie und Humangenetik der Universität München, Richard-Wagner-Str. 10/1, D-8000 München 2, Germany.

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