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β2-Microglobulin required for cell surface expression of blastocyst MHC ☆,☆☆

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Abstract

Blastocyst MHC is a mouse MHC class Ib gene that is selectively expressed in blastocysts and placenta like human HLA-G, which protect fetal trophoblasts and some tumor cells from NK cell attack, and in TAP-dependent expression on the cell surface. We expressed blastocyst MHC cDNA in β2-deficient EL-4 S3 or β2m-transfected EL-4 S3 cells. In parental EL-4 S3 cells, only 47-kDa blastocyst MHC protein was expressed and retained in the cytoplasm. However, additional 51-kDa blastocyst MHC protein was expressed on the surface of β2m-transfected EL-4 S3 cells. The 51-kDa protein was resistant to Endo-H, whereas the 47-kDa protein was sensitive for Endo-H. The results suggested that β2m as well as TAP was necessary for the transportation of blastocyst MHC from endoplasmic reticulum to cell surfaces through the Golgi apparatus, similar to other MHC class I molecules.

Keywords: Blastocyst MHC; β2-Microglobulin; Surface expression; Glycosylation; Endoglycosidase H

During pregnancy in mammals, semiallogeneic fetal cells invade maternal uterine tissue but survive without rejection by the maternal immune system [1–3]. Human trophoblast cells lack classical major histocompatibility complex (MHC) class Ia molecules on their surface, and so can escape from recognition by maternal alloreactive T cells. Natural killer (NK) cells attack cells lacking MHC class Ia molecules after virus infection or malignant transformation. Recent studies have shown that NK cell effector function was controlled by several inhibitory and activating receptors on cell surfaces [4]. Inhibitory receptors on NK cell surfaces bind to MHC class I molecules and inactivate NK cell effector functions via an immunoreceptor tyrosine-based inhibitory motif in their cytoplasmic domain [4].

Human trophoblasts express a non-classical MHC class Ib molecule, HLA-G, which has been implicated in protection from NK cells in vitro and in vivo [5–11]. Two Ig-like receptors ILT2 (LIR-1) and p49 (KIR2DL4) directly bind to HLA-G molecules expressed on the surface of target cells and inhibit NK cell-mediated lysis [12–14]. In addition, it has been shown that the HLA-G leader fragment can efficiently stabilize and upregulate the expression of HLA-E, another class Ib molecule and a major ligand of the lectin-like CD94/NKG2A receptor [15–18]. However, despite this remarkable progress in our knowledge about the recognition of HLA-G by NK cells, the physiological functions of HLA-G remain

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☆☆ *Abbreviations:* MHC, major histocompatibility complex; NK, natural killer; bc1, blastocyst MHC isoform 1; bc2, blastocyst MHC isoform 2; TAP, transporter associated with antigen processing; β2m, β2-microglobulin; Endo-H, endoglycosidase-H.
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elusive [19], primarily due to the lack of an appropriate animal model.

It has recently been shown that a mouse MHC class Ib molecule, Qa-1^b, acts as a mouse homologue of HLA-E and is recognized by the mouse CD94/NKG2A receptor [20,21], and further that blastocyst MHC resembles HLA-G in its structure, selective mRNA expression, and in its inhibitory function of NK cells in vitro and in vivo [22,23]. Although its expression at the protein level and function on the cell surface are still unknown, β2-microglobulin (β2m), a polypeptide of relative molecular mass 12 kDa, is activated in mouse embryos by the 2-cell stage, and associates with the heavy chain of the classical MHC class I proteins H-2K, H-2D, and Qa. Surface expressions of the antigen peptides and these MHC complexes require β2m; numerous studies have indicated the importance of β2m for intracellular transportation, and for normal folding and surface stability of MHC class I molecules [24,25] as well as its important role in binding MHC complexes with antigen peptides [26–28].

In the present study, to investigate the molecular profiles of blastocyst MHC, we established blastocyst MHC in β2m-deficient EL-4 S3 cells, which lack classical class Ia molecules, by introducing full-length blastocyst MHC, which may be homologous to HLA-G1. We found that the surface expression of blastocyst MHC depended on β2m.

Materials and methods

Cell lines and culture. EL-4 S3 is a clone of murine thymoma EL-4 of C57BL/6 (B6) origin. The S3 clone was negative for surface expression of D^b and K^b, because of it was deficient in β2m gene expression. RMA is a B6 mouse-derived T lymphoma cell line; it was obtained from American Type Culture Collection (ATCC; Manassas, VA) and cultured in RPMI 1640 containing 10% FCS, 100 μg/ml penicillin G, 100 μg/ml streptomycin, and 2 mM L-glutamine.

RT-PCR and generation of transfectants. Total RNA was isolated from RMA cells using RNA STET-60 (TEL-TEST, Friendswood, TX). Single-stranded cDNA was synthesized with oligo(dT)$_{12–18}$ primer and Superscript reverse transcriptase (Life Technologies, Gaithersburg, MD) from 5 μg RNA and then used for PCR. PCR was performed at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, for 30 cycles. In accord with the published mouse β2m sequence, 5′-*Bam*HI β2m (5′-TGCGGATCCATGGCTCGCTCGGTGACC-3′) was used as the 5′ primer, and 3′-*Xho*I β2m (5′-TGCCTCGAGTCACATGTCTCGATCCCAGT-3′) was used as the 3′ primer.

PCR products were subcloned into the *Bam*HI and *Xho*I sites of the mammalian expression vector pcDNA3.1(+) (Invitrogen, Carlsbad, CA), and sequenced by a fluoresceinated dye terminator cycle sequencing method on an automated sequencer (Applied Biosystems, Foster City, CA).

EL-4 S3 cells were transfected with the pcDNA3.1(+) vector containing β2m cDNA by LipofectAMINE (Invitrogen, Carlsbad, CA), and selected with 1 mg/ml G418 (Sigma–Aldrich, St. Louis, MO). Transfectants were then sorted by the surface expression of β2m.

C-terminal Myc-His-tagged cDNA was subcloned into the MSCV-IRES-green-fluorescent protein (GFP) retroviral vector and infected into parental and β2m-transfected EL-4 S3 cells as described [23]. Stable GFP$^+$ cells were then isolated by FACS.

Blastocyst MHC cDNA was also subcloned into the pcDNA-CT-TOPO vector (Invitrogen, Carlsbad, CA) in accord with the manufacturer's instructions. Parental and β2m-transfected EL-4 S3 cells were transiently transfected with the pcDNA3.1 containing C-terminal cycle-3 GFP fused blastocyst MHC cDNA by LipofectAMINE for confocal microscopy analysis.

Immunofluorescence, flow cytometry, and confocal microscopy. To analyze the cell surface expression of H-2K^b, H-2D^b, and β2m, cells were stained with anti-H-2K^b mAb (AF6-88.5; BD Pharmingen), anti-H-2D^b mAb (KH95; BD Pharmingen), and anti-β2m mAb (Lym-11; BD Pharmingen), respectively, followed by PE-conjugated goat anti-mouse IgG Ab (Caltag, Burlingame, CA). Stained cells were analyzed on an FACSCalibur (BD Biosciences, San Jose, CA), and data were processed by the CellQuest program (BD Biosciences).

Western blotting. Cells were lysed with a lysis buffer containing 1% Nonidet P-40, 50 mM Hepes (pH 7.3), 250 mM NaCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM PMSF at a concentration of 1×10^7 cells/ml. Endoglycosidase-H and N-glycosidase treatments were performed according to the manufacturer's instructions (Calbiochem).

Total lysates were separated by 10% SDS–PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane (Immunobilon-P; Millipore, Bedford, MA). After blocking with Block Ace (Yukijirushi, Sapporo, Japan), the membrane was probed with anti-Myc mAb (9E10; ATCC). Ab binding was detected using HRP-conjugated rabbit anti-mouse IgG Ab (Jackson ImmunoResearch, West Grove, PA) and an chemiluminescence system (ECL Western blotting detection reagents; Amersham-Pharmacia Biotech, Little Chalfont, UK).

Cell surface biotinylation and immunoprecipitation. Cells (1×10^7/ml) were washed twice in PBS and then surface biotinylated with 100 μg/ml sulfo-*N*-hydroxysuccinimide-biotin (Sigma–Aldrich) at room temperature for 40 min. After washing three times with RPMI 1640, cells were lysed with lysis buffer for 30 min at 4 °C. Lysates were immunoprecipitated with anti-Myc mAb and protein G-conjugated beads (Amersham-Pharmacia). The immunoprecipitates were then subjected to 10% SDS–PAGE and detected using an HRP-containing ABC kit (Vector Laboratories, Burlingame, CA) and ECL.

Laser confocal microscopy. Cells were transiently transfected with Cycle 3 GFP-tagged bc1 and Cycle 3 GFP alone, mounted onto slides using Gelmount medium, and scanned with a Zeiss LSM 510 laser confocal microscope (Heidelberg, Germany). For detecting Cycle 3, GFP was excited using an argon laser at a wavelength of 488 nm, and the absorbed wavelength was detected at 510–520 nm for Cycle 3 GFP. The pinhole size was maintained at 1 Airy Unit for all images.

Results

Establishment of β2m- and/or bc1-transfected EL-4 S3 cell lines

To investigate that the surface expression of blastocyst MHC depended upon β2m, a murine thymoma cell line of B6 mouse origin, EL-4 S3 was used for transfection. The EL-4 S3 cell lines do not express functional MHC class I molecules on their surface due to their β2m deficiency and thus are susceptible to NK cell-mediated cytolysis in vitro [29]. EL-4 S3 was transfected with the gene for β2m by a lipofectamine method and stable neomycin-resistant transfectants were identified by the surface expression of β2m. To confirm the surface expression of classical MHC class I molecules on the β2m-transfected EL-4 S3, cells were stained with anti-H-2K^b mAb and anti-H-2D^b mAb, and analyzed by

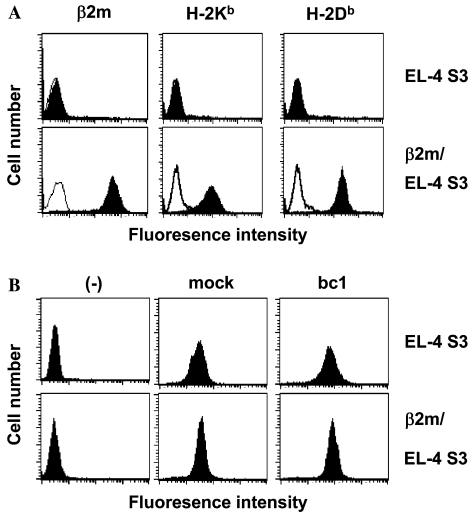

Fig. 1. Generation of β2m and/or bc1-transfected EL-4 S3 cell lines. (A) Surface expression of β2m, H-2K^b, and H-2D^b on parental and β2m-transfected EL-4 S3. Parental and β2m-transfected EL-4 S3 cells were stained with anti-β2m, H-2K^b, and H-2D^b mAb followed by PE-conjugated goat anti-mouse IgG antibody. The closed histograms represent staining with indicated mAb and the open histograms represent background staining with control mouse IgG. (B) GFP expression of bc1 infected parental and β2m-transfected EL-4 S3 cells. Parental and β2m-transfected EL-4 S3 cells were retrovirally infected using MSCV-IRES-GFP vector. The histograms represent the intensity of GFP.

flow cytometry. As previously reported, the β2m-transfected EL-4 S3 cells exhibited expressions of H-2K^b, H-2D^b, and β2m on their surfaces as compared with untransfected cells (Fig. 1A). The results suggested that transfected β2m functioned in transfectants and was possible for the association with bc1 protein.

There are no reports of the expression of blastocyst MHC at the protein level because of the lack of a specific Ab reagent. To overcome this problem, bc1 cDNAs were tagged with anti-Myc and anti-His epitope sequences at the C terminus. bc1-Myc-Hys cDNA was expressed in EL-4 S3 and β2m-transfected EL-4 S3 cells using the MSCV-IRES-GFP retroviral vector, and stable transfectants were then identified by the expression of GFP (Fig. 1B).

Protein expression of blastocyst MHC in EL-4 S3 and β2m-transfected EL-4 S3

To characterize the predicted bc1 protein product in parental and β2m-transfected EL-4 S3 cells, transfected cells were lysed and bc1-Myc-His protein was detected with anti-Myc mAb. As shown in Fig. 2A, the 51-kDa bc1-Myc-His protein could be recognized by anti-Myc mAb, and the 47-kDa protein could be recognized in bc1-Myc-His-transfected RMA cells, and β2m and bc1-Myc-His-transfected EL-4 S3 cells. In bc1-Myc-His-transfected EL-4 S3 cells, the 47-kDa, but not the 51-kDa, protein was detected.

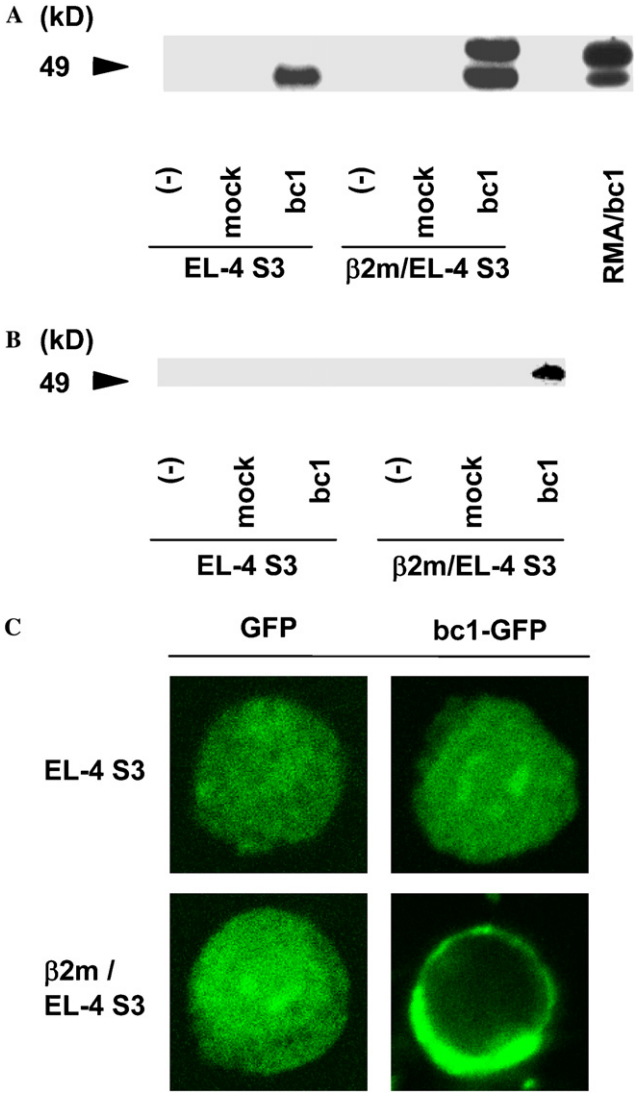

Fig. 2. Protein expression of bc1 in parental EL-4 S3 and transfectants. (A) Western blotting analysis of β2m and/or bc1-transfected EL-4 S3 cell lines The lysates from parental, mock-, and bc1, and/or β2m-transfected EL-4 S3 cells, and bc1-transfected RMA cells were subjected to SDS–PAGE and then blotted onto a PVDF membrane. The membrane was probed with anti-Myc mAb followed by HRP-conjugated rabbit anti-mouse IgG and then subjected to chemiluminescent detection. (B) Cell-surface biotinylation and immunoprecipitation. Parental, mock-, and bc1-transfected EL-4 S3 and/or β2m-transfected EL-4 S3 cells were biotinylated on the cell surface and the lysates were immunoprecipitated with anti-Myc mAb. The immunoprecipitates were subjected to SDS–PAGE and then blotted onto a PVDF membrane. The membrane was probed with HRP-containing ABC and then subjected to chemiluminescent detection. (C) Confocal microscopy analysis. C-terminal Cycle 3 GFP-tagged bc1 or Cycle 3 GFP with or without β2m-transfected EL-4 S3 cells adhered onto slides and were visualized using laser confocal microscopy according to the protocol detailed under Materials and methods. Each image is representative of all cells observed in three separate experiments.

As that predicted the bc1-Myc-His protein (the calculated molecular weight: 39,600) possesses a three potential N-glycosylation sites one in each of the α1, α2, and α3 domains at the Asn residues at positions 86, 176, and

256, that are present in the blastocyst MHC translated sequence [22]. The 47- and 51-kDa proteins were expected and confirmed to be different glycosylation forms of the blastocyst MHC protein (see below).

To determine whether these 47- and 51-kDa proteins express on cell surfaces, cells were biotinylated on the cell surface, lysed, and immunoprecipitated with anti-Myc mAb. As shown in Fig. 2B, a single protein was detected in bc1-Myc-His-transfected EL-4 S3/β2m cells. On the other hand, no protein was detected in bc1-Myc-His-transfected EL-4 S3 cells. To confirm whether this protein size was 47- or 51-kDa, the membrane was re-probed with anti-Myc mAb; the result being the same as in Fig. 2A, and that the protein was 51-kDa, not 47-kDa (data not shown). These results indicated that the 51-kDa protein of blastocyst MHC was present on cell surfaces, and that the 47-kDa protein was retained in the cytoplasm in β2m-transfected EL-4 S3 cells.

To visualize the subcellular distribution of blastocyst MHC, we performed confocal imaging analysis. First, we attempted to detect C-terminal Myc-His-tagged blastocyst MHC protein by staining with anti-His or anti-Myc antibody, followed by fluorescein-conjugated secondary antibody. However, the localization of bc1-Myc-His protein could not be clearly detected with anti-His and anti-Myc mAb because of background (data not shown). To overcome this problem, bc1 cDNA was directly fused with Cycle-3 GFP sequences at the C terminus. Cycle-3 GFP has its primary excitation peak at 395 nm and a secondary one at 478 nm. Excitation at either of these wavelengths yields a fluorescent emission peak with a maximum at 507 nm.

Parental and β2m-transfected EL-4 S3 were transiently transfected with the gene for bc1-Cycle3 GFP and Cycle 3 GFP alone by the lipofectamine method, fixed on slide glasses, and examined using confocal microscopy. As shown in Fig. 2C, confocal imaging analysis of these cells showed that only the GFP-tagged bc1 protein appeared to localize at the plasma membrane of EL-4 S3/β2m, and that it was mostly retained in the cytoplasm of EL-4 S3 cells. On the other hand, both parental and β2m-transfected EL-4 S3 retained Cycle 3 GFP in the cytoplasm.

These results showed that bc1 could be expressed on cell surfaces in β2m-transfected EL-4 S3 cells, and that bc1 was retained in the cytoplasm in β2m-deficient EL-4 S3 cells; suggesting that endogenous levels of β2m in EL-4 S3 cells are sufficient to mediate the regulation of bc1 subcellular localization.

Glycosylation form of blastocyst MHC protein in transfectants

To investigate whether surface blastocyst MHC was glycosylated in a manner similar to other class I molecules, cells were lysed and treated with endoglycosidase-H (Endo-H) and N-glycosidase. For MHC class I, Endo-H resistance is indicative of complex formation and transport through the Golgi to the cell surface [30]. This analysis did indeed show a correlation between surface expression and the presence of some Endo-H resistant material, consistent with that found for other MHC class II molecules.

As shown in Fig. 3, the 47-kDa bc1-Myc-His protein in RMA, EL-4 S3, and β2m-transfected EL-4 S3 cells was downsized by Endo-H treatment. On the other hand, the 51-kDa bc1-Myc-His protein in RMA and β2m-transfected EL-4 S3 cells was not downsized by Endo-H treatment. N-glycosidase treatment caused the downsizing of both 51-kDa and 47-kDa bc1-Myc-His proteins.

These results suggested that the 51 kDa bc1-Myc-His protein was associated with β2m, Endo-H resistance indicative of complex formation, and transport through the Golgi to cell surface-transfected EL-4 S3 cells, like other MHC molecules.

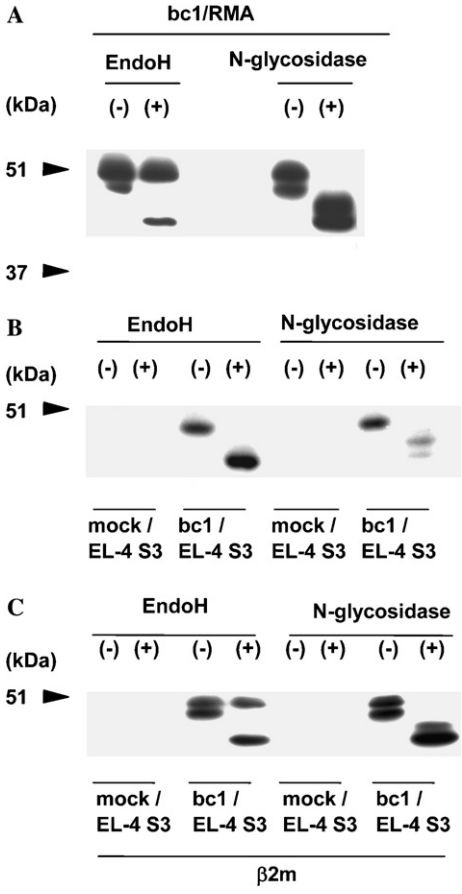

Fig. 3. Sensitivity of bc1 oligosaccharides to endoglycosidase-H (Endo-H) and N-glycosidase. Cell lysates from bc1/RMA (A), mock/EL-4 S3 and bc1/EL-4 S3 (B), and β2m/mock/EL-4 S3 and β2m/bc1/EL-4 S3 (C) cell lines were untreated or treated with Endo-H or N-glycosidase, subjected to SDS–PAGE, and then blotted onto a PVDF membrane. The membrane was probed with anti-Myc mAb followed by HRP-conjugated rabbit anti-mouse IgG and then subjected to chemiluminescent detection.

Discussion

In this study, we analyzed the expression profile of blastocyst MHC in β2m-deficient EL-4 S3 and β2m-transfected EL-4 S3 cells. We found that the two distinct forms of blastocyst MHC were present in the transfectant. One is a 47-kDa protein of high mannose hybrid-type form (Endo-H sensitive) retained in the cell and the other is a 51-kDa protein of form with complex-type N-glycosylation (Endo-H resistant) presented on the surface. Moreover, β2m was necessary for the surface expression of the 51-kDa protein.

In a previous study, the blastocyst MHC gene was transcripted encoding a full-length α-chain (bc1) and an alternatively spliced form lacking the α2 domain (bc2) [23]. When these cDNAs were expressed in TAP-deficient RMA-S or TAP-sufficient RMA cells, only bc1 protein was expressed on the surface of RMA cells, but both bc1 and bc2 proteins were retained in the cytoplasm of RMA-S cells. Moreover, RMA-S cells expressing either bc1 or bc2 were protected from lysis by NK cells in vitro and in vivo. This protection was at least partly mediated by up-regulation of Qa-1^b expression on the surface of RMA-S cells, which engaged the CD94/NKG2A inhibitory receptor on NK cells [23]. These results indicated that β2m as well as TAP was necessary for the transportation of blastocyst MHC from endoplasmic reticulum to cell surfaces through the Golgi apparatus, just like MHC class I molecules.

Since glycosylation presumably stabilizes the glycoprotein structure [31], a consequence may be a differential in the half-life of the two forms of blastocyst MHC. Post-translational glycosylation is also critical for the biological function of many proteins. For example, changes in the glycosylation of CD44 appear to have important regulatory effects on CD44 function [32], and the extent of glycosylation of the human prostacyclin receptor may be important for ligand binding and signal transduction [33]. On the other hand, high mannose structures on soluble CD154 compared with those on complex-type sCD154 glycoprotein showed no difference in complex formation, ligand binding, or function [34].

Glycosylation of MHC class I is not required for recognition by B or T cells; and at least for some of the inhibitory specificities examined, oligosaccharide is not necessary for activity of MHC class I and NK cell inhibitory receptors. However, intracellular trafficking appears to be significantly affected by changes in glycosylation or the lack thereof, as mutant class I heavy chains that lack glycosylation sites show inefficient transit to the cell surface [35]. High mannose-type glycans can interact with mannose-6-phosphate receptors to assist trafficking to the lysosomal compartment [36], speculatively in the example of blastocyst MHC, for peptide loading there.

Calnexin, a membrane protein of the endoplasmic reticulum (ER), transiently associates with MHC class I heavy chains [37,38]. Binding of calnexin to a glycoprotein depends on the presence of a monoglucosylated oligosaccharide and, consistent with its role as a lectin, calnexin selectively binds to oligosaccharides of this type. Studies of various cell lines that are defective in MHC class I synthesis show that calnexin retains class I heavy chains within the ER until they have folded sufficiently to bind β2m, and possibly peptide; for example, in cell lines that cannot make β2m, HLA class I heavy chains do not reach the cell surface because of intracellular retention by calnexin [38–40].

These findings suggested a scheme for blastocyst MHC biosynthesis. First, newly synthesized blastocyst MHC associated with calnexin. Second, binding of β2m to blastocyst MHC causes the release of calnexin and facilitates association with the peptide transporter proteins. After peptide loading, blastocyst MHC is released from the ER. Finally, blastocyst MHC is modified to the Endo-H resistant glycosylation form in the Golgi and transported to the cell surface.

HLA-G1, the human homologue of blastocyst MHC, binds to both antigen peptide and β2m [41,42]. HLA-G molecules present peptides essentially in the same way as classical HLA molecules do. The peptide motif is specifically recognized by HLA-G; its basic features are described by the sequence XI/LPXXXXXL. These studies have emphasized the importance of the peptide positions 1, 2, 3, and 9 for binding to HLA-G [42,43]. Surface expression of HLA-G on the HLA-G transfectant could be increased upon overnight incubation with synthetic binding peptide, RIIPRHLQL and KIPAQFYIL, but not weak binding peptide RLPKDFRIL; this result indicating that the stability of HLA-G on cell surface depends on the presenting peptide [42–44].

Whether blastocyst MHC interacts with antigen peptide and target receptors remains to be determined; however, given the cell line surface expression of blastocyst MHC, these questions might now be more directly addressed.

Acknowledgments

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References

[1] D.R. Bainbridge, Evolution of mammalian pregnancy in the presence of the maternal immune system, Rev. Reprod. 5 (2000) 67–74.

[2] A.M. Rodriguez, V. Mallet, F. Lenfant, J. Arnaud, M. Girr, S. Urlinger, A. Bensussan, P. Le Bouteiller, Interferon-gamma

rescues HLA class Ia cell surface expression in term villous trophoblast cells by inducing synthesis of TAP proteins, Eur. J. Immunol. 27 (1997) 45–54.
[3] W.P. Faulk, A. Temple, Distribution of beta2 microglobulin and HLA in chorionic villi of human placentae, Nature 262 (1976) 799–802.
[4] L.L. Lanier, NK cell receptors, Annu. Rev. Immunol. 16 (1998) 359–393.
[5] P. Paul, F.A. Cabestre, F.A. Le Gal, I. Khalil-Daher, C. Le Danff, M. Schmid, S. Mercier, M.F. Avril, J. Dausset, J.G. Guillet, E.D. Carosella, Heterogeneity of HLA-G gene transcription and protein expression in malignant melanoma biopsies, Cancer Res. 59 (1999) 1954–1960.
[6] P. Paul, N. Rouas-Freiss, I. Khalil-Daher, P. Moreau, B. Riteau, F.A. Le Gal, M.F. Avril, J. Dausset, J.G. Guillet, E.D. Carosella, HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance, Proc. Natl. Acad. Sci. USA 95 (1998) 4510–4515.
[7] L.L. Lanier, Natural killer cells fertile with receptors for HLA-G? Proc. Natl. Acad. Sci. USA 96 (1999) 5343–5345.
[8] N. Rouas-Freiss, R.E. Marchal, M. Kirszenbaum, J. Dausset, E.D. Carosella, The alpha1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc. Natl. Acad. Sci. USA 94 (1997) 5249–5254.
[9] L. Pazmany, O. Mandelboim, M. Vales-Gomez, D.M. Davis, H.T. Reyburn, J.L. Strominger, Protection from natural killer cell-mediated lysis by HLA-G expression on target cells, Science 274 (1996) 792–795.
[10] B. Seliger, M.J. Maeurer, S. Ferrone, TAP off-tumors on, Immunol. Today 18 (1997) 292–299.
[11] S. Kovats, E.K. Main, C. Librach, M. Stubblebine, S.J. Fisher, R. DeMars, A class I antigen, HLA-G, expressed in human trophoblasts, Science 248 (1990) 220–223.
[12] M. Ponte, C. Cantoni, R. Biassoni, A. Tradori-Cappai, G. Bentivoglio, C. Vitale, S. Bertone, A. Moretta, L. Moretta, M.C. Mingari, Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor, Proc. Natl. Acad. Sci. USA 96 (1999) 5674–5679.
[13] S. Rajagopalan, E.O. Long, A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells, J. Exp. Med. 189 (1999) 1093–1100.
[14] F. Navarro, M. Llano, T. Bellon, M. Colonna, D.E. Geraghty, M. Lopez-Botet, The ILT2 (LIR1) and CD94/NKG2A NK cell receptors respectively recognize HLA-G1 and HLA-E molecules co-expressed on target cells, Eur. J. Immunol. 29 (1999) 277–283.
[15] K. Soderstrom, B. Corliss, L.L. Lanier, J.H. Phillips, CD94/NKG2 is the predominant inhibitory receptor involved in recognition of HLA-G by decidual and peripheral blood NK cells, J. Immunol. 159 (1997) 1072–1075.
[16] M. Llano, N. Lee, F. Navarro, P. Garcia, J.P. Albar, D.E. Geraghty, M. Lopez-Botet, HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer, Eur. J. Immunol. 28 (1998) 2854–2863.
[17] F. Borrego, M. Ulbrecht, E.H. Weiss, J.E. Coligan, A.G. Brooks, Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis, J. Exp. Med. 187 (1998) 813–818.
[18] V.M. Braud, D.S. Allan, C.A. O'Callaghan, K. Soderstrom, A. D'Andrea, G.S. Ogg, S. Lazetic, N.T. Young, J.I. Bell, J.H. Phillips, L.L. Lanier, A.J. McMichael, HLA-E binds to natural killer cell receptors CD94/NKG2A B and C, Nature 391 (1998) 795–799.
[19] D. Bainbridge, S. Ellis, P. Le Bouteiller, I. Sargent, HLA-G remains a mystery, Trends Immunol. 22 (2001) 548–552.
[20] R.E. Vance, J.R. Kraft, J.D. Altman, P.E. Jensen, D.H. Raulet, Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1(b), J. Exp. Med. 188 (1998) 1841–1848.
[21] Z. Kurepa, C.A. Hasemann, J. Forman, Qa-1b binds conserved class I leader peptides derived from several mammalian species, J. Exp. Med. 188 (1998) 973–978.
[22] S.L. Sipes, M.V. Medaglia, D.L. Stabley, C.S. DeBruyn, M.S. Alden, V. Catenacci, C.P. Landel, A new major histocompatibility complex class I b gene expressed in the mouse blastocyst and placenta, Immunogenetics 45 (1996) 108–120.
[23] A. Tajima, T. Tanaka, T. Ebata, K. Takeda, A. Kawasaki, J.M. Kelly, P.K. Darcy, R.E. Vance, D.H. Raulet, K. Kinoshita, K. Okumura, M.J. Smyth, H. Yagita, Blastocyst MHC, a putative murine homologue of HLA-G, protects TAP-deficient tumor ells from natural killer cell-mediated rejection in vivo, J. Immunol. 171 (2003) 1715–1721.
[24] D.B. Williams, B.H. Barber, R.A. Flavell, H. Allen, Role of beta 2-microglobulin in the intracellular transport and surface expression of murine class I histocompatibility molecules, J. Immunol. 142 (1989) 2796–2806.
[25] A. Vitiello, T.A. Potter, L.A. Sherman, The role of beta 2-microglobulin in peptide binding by class I molecules, Science 250 (1990) 1423–1426.
[26] L.F. Boyd, S. Kozlowski, D.H. Margulies, Solution binding of an antigenic peptide to a major histocompatibility complex class I molecule and the role of beta 2-microglobulin, Proc. Natl. Acad. Sci. USA 89 (1992) 2242–2246.
[27] S. Kozlowski, T. Takeshita, W.H. Boehncke, H. Takahashi, L.F. Boyd, R.N. Germain, J.A. Berzofsky, D.H. Margulies, Excess beta 2 microglobulin promoting functional peptide association with purified soluble class I MHC molecules, Nature 349 (1991) 74–77.
[28] B. Arce-Gomez, E.A. Jones, C.J. Barnstable, E. Solomon, W.F. Bodmer, The genetic control of HLA-A and B antigens in somatic cell hybrids: requirement for beta2 microglobulin, Tissue Antigens 11 (1978) 96–112.
[29] K. Sturmhofel, G.J. Hammerling, Reconstitution of H-2 class I expression by gene transfection decreases susceptibility to natural killer cells of an EL4 class I loss variant, Eur. J. Immunol. 20 (1990) 171–177.
[30] H.G. Ljunggren, N.J. Stam, C. Ohlen, J.J. Neefjes, P. Hoglund, M.T. Heemels, J. Bastin, T.N. Schumacher, A. Townsend, K. Karre, Empty MHC class I molecules come out in the cold, Nature 346 (1990) 476–480.
[31] D.F. Wyss, G. Wagner, The structural role of sugars in glycoproteins, Curr. Opin. Biotechnol. 7 (1996) 409–416.
[32] A. Bartolazzi, A. Nocks, A. Aruffo, F. Spring, I. Stamenkovic, Glycosylation of CD44 is implicated in CD44-mediated cell adhesion to hyaluronan, J. Cell Biol. 132 (1996) 1199–1208.
[33] Z. Zhang, S.C. Austin, E.M. Smyth, Glycosylation of the human prostacyclin receptor: role in ligand binding and signal transduction, Mol. Pharmacol. 60 (2001) 480–487.
[34] S.S. Khandekar, C. Silverman, J. Wells-Marani, A.M. Bacon, H. Birrell, M. Brigham-Burke, D.J. DeMarini, Z.L. Jonak, P. Camilleri, J. Fishman-Lobell, Determination of carbohydrate structures N-linked to soluble CD154 and characterization of the interactions of CD40 with CD154 expressed in *Pichia pastoris* and Chinese hamster ovary cells, Protein Expr. Purif. 23 (2001) 301–310.
[35] P. Parham, Functions for MHC class I carbohydrates inside and outside the cell, Trends Biochem. Sci. 21 (1996) 427–433.
[36] S. Kornfeld, I. Mellman, The biogenesis of lysosomes, Annu. Rev. Cell Biol. 5 (1989) 483–525.
[37] E. Degen, D.B. Williams, Participation of a novel 88-kDa protein in the biogenesis of murine class I histocompatibility molecules, J. Cell Biol. 112 (1991) 1099–1115.

[38] E. Degen, M.F. Cohen-Doyle, D.B. Williams, Efficient dissociation of the p88 chaperone from major histocompatibility complex class I molecules requires both beta 2-microglobulin and peptide, J. Exp. Med. 175 (1992) 1653–1661.
[39] E. Nossner, P. Parham, Species-specific differences in chaperone interaction of human and mouse major histocompatibility complex class I molecules, J. Exp. Med. 181 (1995) 327–337.
[40] S. Rajagopalan, M.B. Brenner, Calnexin retains unassembled major histocompatibility complex class I free heavy chains in the endoplasmic reticulum, J. Exp. Med. 180 (1994) 407–412.
[41] S.A. Ellis, I.L. Sargent, C.W. Redman, A.J. McMichael, Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line, Immunology 59 (1986) 595–601.
[42] N. Lee, A.R. Malacko, A. Ishitani, M.C. Chen, J. Bajorath, H. Marquardt, D.E. Geraghty, The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association, Immunity 3 (1995) 591–600.
[43] M. Diehl, C. Munz, W. Keilholz, S. Stevanovic, N. Holmes, Y.W. Loke, H.G. Rammensee, Nonclassical HLA-G molecules are classical peptide presenters, Curr. Biol. 6 (1996) 305–314.
[44] C. Munz, S. Stevanovic, H.G. Rammensee, Peptide presentation and NK inhibition by HLA-G, J. Reprod. Immunol. 43 (1999) 139–155.