Takeuchi T, Miyasaka N, Zang C, Alvarez D, Fletcher T, Wajdula J, Yuasa H, and Vlahos B.	A phase 3 randomized, double-blind, multicenter comparative study evaluating the effect of etanercept versus methotrexate on radiographic outcomes, disease activity and safety in Japanese subjects with rheumatoid arthritis.	Mod Rheum.	23(4)	623-33	2013
Cho S, Sakai R, **Nanki T**, Koike R, Watanabe K, Yamazaki H, Amano K, Tanaka Y, Nakajima A, Atsumi T, Ihata A, Ezawa K, Sung Y, Bae S, Miyasaka N, Harigai M.	A comparison of incidence and factors influencing development of serious adverse events in patients with rheumatoid arthritis between biologic DMARDs registries in Korea and Japan.	Mod. Rheumatol.			2013 In Press
Komazaki Y, Miyazaki Y, Fujie T, Sakashita H, Tsuchiya K, Tamaoka M, Sumi T, Maruyama Y, **Nanki T**, Inase N.	Serodiagnosis of *Mycobacterium avium* complex pulmonary disease in rheumatoid arthritis.	Respiration.			2013 In Press
Umezawa N, Kohsaka H, **Nanki T**, Watanabe K, Tanaka M, Shane PY, Miyasaka N.	Successful treatment of eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg-Strauss syndrome) with rituximab in a case refractory to glucocorticoids, cyclophosphamide, and IVIG.	Mod. Rheumatol.			2013 In Press
Fukuda S, **Nanki T**, Morio T, Hasegawa H, Koike R, Miyasaka N.	Recurrent mitral valve regurgitation with neutrophil infiltration in a patient with multiple aseptic abscesses.	Mod. Rheumatol.			2013 In Press

Watanabe K, Sakai R, Koike R, Sakai F, Sugimaya H, Tanaka M, Komano Y, Akiyama Y, Mimura T, Kaneko M, Tokuda H, Iso T, Motegi M, Ikeda K, Nakajima Y, Taki H, Kubota T, Kodama H, Sugii S, Kuroiwa T, Nawata Y, Shiozawa K, Ogata A, Sawada S, Matsukawa Y, Okazaki T, Mukai M, Iwahashi M, Saito K, Tanaka Y, **Nanki T,** Miyasaka N, Harigai M.	Clinical characteristics and risk factors for *Pneumocystis jirovecii* pneumonia in patients with rheumatoid arthritis receiving adalimumab: a retrospective review and case-control study of 17 patients.	Mod. Rheumatol.	23(6)	1085-93	2013
Miyabe Y, Miyabe C, Iwai Y, Takayasu A, Fukuda S, Yokoyama W, Nagai J, Jona M, Tokuhara Y, Ohkawa R, Albers HM, Ovaa H, Aoki J, Chun J, Yatomi Y, Ueda H, Miyasaka M, Miyasaka N, **Nanki T.**	Necessity of Lysophosphatidic Acid Receptor 1 for Development of Arthritis.	Arthritis Rheum.	65(8)	2037-47	2013
Takayasu A, Miyabe Y, Yokoyama W, Kaneko K, Miyabe C, Fukuda S, Kubota T, Miyasaka N, **Nanki T.**	CCL18 activates fibroblast-like synoviocytes in patients with rheumatoid arthritis.	J. Rheumatol.	40(6)	1026-28	2013

Nanki T, Onoue I, Nagasaka K, Takayasu A, Ebisawa M, Hosoya T, Shirai T, Sugihara T, Hirata S, Kubota T, **Harigai M,** Miyasaka N	Suppression of elevations in serum C reactive protein levels by anti-IL-6 autoantibodies in two patients with severe bacterial infections.	Ann. Rheum. Dis.	72(6)	1100-02	2013
Uto-Konomi A, McKibben B, Wirtz J, Sato Y, Takano A, **Nanki T**, Suzuki S.	CXCR7 agonists inhibit the function of CXCL12 by down-regulation of CXCR4.	Biochem. Biophys. Res. Commun.	431(4)	772-76	2013
Shima Y, Hosen N, Hirano T, Arimitsu J, Nishida S, Hagihara K, Narazaki M, **Ogata A,** Tanaka T, Kishimoto T, Kumanogoh A.	Expansion of range of joint motion following treatment of systemic sclerosis with tocilizumab. Mod Rheumatol.	Mod Rheumatol.			2013 In Press
Ogata A, Tanimura K, Sugimoto T, Inoue H, Urata Y, Matsubara T, Kondo M, Ueki Y, Iwahashi M, Tohma S, Ohta S, Saeki Y, Tanaka T. and the MUSASHI Study Investigators.	A Phase 3 Noninferiority Study of the Efficacy and Safety of Subcutaneous Tocilizumab Monotherapy Versus Intravenous Tocilizumab Monotherapy in Patients With Rheumatoid Arthritis With an Inadequate Response to Synthetic and/or Biologic DMARDs in Japan (MUSASHI Study).	Arthritis Care Res (Hoboken).			2013 In Press

Shinzaki S, Kuroki E, Iijima H, Tatsunaka N, Ishii M, Fujii H, Kamada Y, Kobayashi T, Shibukawa N, Inoue T, Tsujii M, Takeishi S, Mizushima T, **Ogata A**, Naka T, Plevy SE, Takehara T, Miyoshi E.	Lectin-based Immunoassay for Aberrant IgG Glycosylation as the Biomarker for Crohn's Disease.	Inflamm Bowel Dis.	19(2)	321-31	2013
Watanabe K, Sakai R, Koike R, Sakai F, Sugiyama H, Tanaka M, Komano Y, Akiyama Y, Mimura T, Kaneko M, Tokuda H, Iso T, Motegi M, Ikeda K, Hiroshi Nakajima H, Taki H, Kubota T, Kodama H, Sugii S, Kuroiwa T, Nawata Y, Shiozawa K, **Ogata A**, Sawada S, Matsukawa Y, Okazaki T, Mukai M, Iwahashi M, Saito K, Tanaka Y, Nanki T, Miyasaka N, Harigai M.	Clinical characteristics and risk factors for Pneumocystis jirovecii 5 pneumonia in patients with rheumatoid arthritis receiving 6 adalimumab: a retrospective review and case; control study 7 of 17 patients.	Mod Rheumatol.	23(6)	1085-93	2013
Hishitani Y, **Ogata A**, Shima Y, Hirano T, Ebina K, Kunugiza Y, Shi K, Narazaki M, K Hagihara K, T omita T, H Yoshikawa H, T Tanaka T, Kumanogoh A.	Retention of tocilizumab and anti-tumour necrosis factor drugs in the treatment of rheumatoid arthritis.	Scand J Rheumatol.	42(4)	253-9	2013

Ogawa M, Matsuda T, **Ogata A**, Hamasaki T, Kumanogoh A, Toyofuku T, Tanaka T.	DNA Damage in Rheumatoid Arthritis: : An Age-Dependent Increase in the Lipid Peroxidation Derived DNA Adduct, Heptanone-Etheno-2'-Deoxycytidine.	Autoimmune Dis.	2013	183487	2013
Yoshikawa M, Mukai Y, Okada Y, Tsumori Y, **Tsunoda S**, Tsutsumi Y, Aird WC, Yoshioka Y, Okada N, Doi T, Nakagawa S.	Robo4 is an effective tumor endothelial marker for antibody-drug conjugates based on the rapid isolation of the anti-Robo4 cell-internalizing antibody.	Blood.	121	2804-13	2013
Tsunoda S.	Antibody Engineering for Next-Generation Biodrug Development.	Yakugaku Zasshi.	133(9)	923-24	2013
Morishige T, Yoshioka Y, Narimatsu S, Ikemizu S, **Tsunoda S**, Tsutsumi Y, Mukai Y, Okada N, Nakagawa S.	Mutants of lymphotoxin-α with augmented cytotoxic activity via TNFR1 for use in cancer therapy.	Cytokine.	61	578-84	2013
Mimoto F, Katada H, Kadono S, Igawa T, Kuramochi T, Muraoka M, Wada Y, Haraya K, Miyazaki T, **Hattori K**.	Engineered antibody Fc variant with selectively enhanced FcgRIIb binding over both FcgRIIaR131 and FcgRIIaH131.	Protein Eng Des Sel.	26	589-98	2013
Igawa T, Maeda A, Haraya K, Tachibana T, Iwayanagi Y, Mimoto F, Higuchi Y, Ishii S, Tamba S, Hironiwa N, Nagano K, Wakabayashi T, Tsunoda H, **Hattori K**.	Engineered monoclonal antibody with novel antigen-sweeping in vivo.	PLoS One.	8	e63236	2013

Sampei Z, Igawa T, Soeda T, Okuyama-Nishida Y, Moriyama C, Wakabayashi T, Tanaka E, Muto A, Kojima T, Kitazawa T, Yoshihashi K, Harada A, Funaki M, Haraya K, Tachibana T, Suzuki S, Esaki K, Nabuchi Y, **Hattori K**.	Identification and multidimensional optimization of an asymmetric bispecific IgG antibody mimicking the function of factor VIII cofactor activity.	PLoS One.	8	e57479	2013
Mimoto F, Igawa T, Kuramochi T, Katada H, Kadono S, Kamikawa T, Shida-Kawazoe M, **Hattori K**.	Novel asymmetrically engineered antibody Fc variant with superior FcγR binding affinity and specificity compared with afucosylated Fc variant.	MAbs.	13	229-36	2013
Ueda O, Tateishi H, Higuchi Y, Fujii E, Kato A, Kawase Y, Wada NA, Tachibe T, Kakefuda M, Goto C, Kawaharada M, Shimaoka S, **Hattori K**, Jishage K.	Novel genetically-humanized mouse model established to evaluate efficacy of therapeutic agents to human interleukin-6 receptor.	Scientific Reports.	3	1196	2013

MHC class I molecules are incorporated into human herpesvirus-6 viral particles and released into the extracellular environment

Running title: MHC class I expresses in HHV-6 virions

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Abstract

Human herpesvirus-6 (HHV-6) belongs to the betaherpesvirus subfamily and mainly replicates in T lymphocytes. Here, we show that major histocompatibility complex (MHC) class I molecules are incorporated into HHV-6 viral particles and released into the extracellular environment. In addition, HHV-6A/B-infected T cells showed reduced surface and intracellular expression of MHC class I molecules. The cellular machinery responsible for molecular transport appears to be modified upon HHV-6 infection, causing MHC class I molecules to be transported to virion assembly sites.

Key words

HHV-6A/B, MHC class I, viral particles

Introduction

Human herpesvirus 6 (HHV-6), which belongs to the betaherpesvirus subfamily (1) was first isolated from peripheral blood lymphocytes obtained from patients with lymphoproliferative disorders (2). HHV-6 isolates are classified as HHV-6A and HHV-6B (see the Virus Taxonomy List 2011), based on genetic and antigenic differences and their cell tropism (3, 4, 2, 5). Primary infection with HHV-6B causes exanthem subitum (6). The diseases caused by HHV-6A have been unknown. HHV-6B mostly infects infants and remains latent in more than 90% of the population (7).

In general, herpesviruses use several strategies to evade host immune responses. For example, viruses may inhibit major histocompatibility complex (MHC) class I-associated antigen presentation to escape detection by cytotoxic T lymphocytes (CTLs). Several proteins expressed by herpesviruses block the transport of antigenic peptides from the cytosol to the endoplasmic reticulum (ER) (8-11), whereas others retain (12-14) or destroy class I molecules, or deliver them to lysosomes for degradation (15-18). The result is reduced surface expression of MHC class I molecules, enabling the virus to evade host immune surveillance.

HHV-6A, but not HHV-6B, downregulates the expression of MHC class I in dendritic cells (19). HHV-6 U21 binds to and diverts MHC class I molecules to an endolysosomal compartment, effectively removing them from the cell surface and providing a possible means of immune escape (20).

Here, we show that the expression of MHC class I molecules by infected cells was downregulated with incorporation into HHV-6 viral particles, suggesting a possible mechanism by which the virus escapes host immune surveillance.

Materials and Methods

Cells and viruses

Umbilical cord blood mononuclear cells (CBMCs) were prepared as described previously (21). CBMCs were provided by K. Adachi (Minoh Hospital, Minoh, Japan) and H. Yamada (Kobe University Graduate School of Medicine, Kobe, Japan) and purchased from the Cell Bank of the RIKEN BioResource Center, Japan. Virus stocks were also prepared as described previously (22, 21). HSB-2 and MT-4 cell lines were used in this study (23). HHV-6A (GS strain) and HHV-6B (HST strain) were prepared as previously described (21).

Antibodies

Monoclonal antibody (Mab) OHV-1 (24) and a polyclonal antibody against gB (23, 25) have been described previously. Other Mabs were purchased: MHC class I (clone: W6/32; Bio Legend), CD63 (clone: CLB-gran/12, 435; Sanquin), and α-tubulin (clone: B-5-1-2; Sigma). The following secondary antibodies were used: Alexa Fluor 488- or 594-conjugated F(ab')2 fragment of goat anti-mouse or rabbit immunoglobulin G (IgG) (Invitrogen) and anti-mouse IgG, horseradish peroxidase-linked whole antibody (from sheep) (GE Healthcare).

Virion and exosome isolation

Virions and exosomes were purified as previously described (26, 23). The collected fractions were used for Western blotting, electron microscopy, or LC-MS/MS.

78

79 **Liquid chromatography-tandem mass spectrometry**

80 The fractions described above were analyzed by LC-MS/MS. Proteins were 10-times
81 diluted with 9.8M urea. The solutions were adjusted to pH 8.5, reduced with 13 mM
82 DTT at 37 °C for 1.5 h and alkylated with 27 mM iodoacetamide in the dark for 1 h.
83 The protein mixtures were further diluted with 100mM Triethylammonium bicarbonate
84 (TEAB) pH8.5 to reduce urea to 1 M, and digested with 4 μl of 1mg/ml trypsin-TPCK
85 solution. Samples were digested overnight at 37°C. Following digestion, lysates were
86 acidified by adding 10% TFA. The samples were desalted using peptide cleanup C18
87 spin tubes (Agilent Technologies) and vacuum-dried. NanoLC-MS/MS analyses were
88 performed on an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific,
89 Waltham, MA, USA) as described previously (27), while spray voltage was changed to
90 1800 V. Peptides and proteins were identified by automated database searches using
91 Proteome Discoverer v.1.1 (Thermo Fisher Scientific) against all entries of the Swiss
92 Prot protein database (version 3.26) with a precursor mass tolerance of 10 p.p.m., a
93 fragment ion mass tolerance of 0.8 Da, and strict trypsin specificity, allowing for up to
94 two missed cleavages. Cysteine carbamidomethylation was set as a fixed modification,
95 and methionine oxidation was allowed as a variable modification.

96

97 **Western blotting**

98 Western blotting was performed as described previously (28, 29).

99

100 **Electron microscopy**

101 Electron microscopy was performed as described previously (30).

Briefly, the virion-containing pellet was resuspended in 2% (wt/vol) paraformaldehyde solution buffered with 0.1 M phosphate (pH 7.2). Next, 5 μl of the resuspended pellet was loaded onto Formvar–carbon-coated grids to adsorb the virions. Immunostaining was then performed. The virions were incubated with mouse anti-gB, anti-MHC class I, or anti-CD63 antibody for 1 h at room temperature, followed by goat anti-mouse IgG conjugated to 10 nm colloidal gold particles (GE Healthcare) for a further 1 h at room temperature. After immunolabeling, the samples were washed in distilled water, stained for 5 min with uranyl oxalate, pH 7.0, washed again, embedded in a mixture of 1.8% methylcellulose and 0.4% uranyl acetate, pH 4.0, at 4°C, air-dried, and observed under a Hitachi H-7100 electron microscope. For the control experiments, samples were incubated with the secondary antibody alone.

Flow cytometry

MT-4 cells were infected with HHV-6B. At 72 h post-infection, the cells were fixed with 4% (wt/ vol) paraformaldehyde at room temperature for 15 min and incubated with the anti-MHC class I Mab at 37°C for 1 h. Cells were then stained with an appropriate secondary antibody at 37°C for 30 min. For the control experiments, samples were incubated with the secondary antibody alone. Stained cells were analyzed using a flow cytometer (ec800; SONY;).

Immunofluorescence assay

The immunofluorescence assay was performed as described previously (28). Briefly, MT-4 cells were infected with HHV-6B. At 72 h post-infection, the cells were fixed with cold acetone-methanol (7:3) and incubated at 37°C for 1 h with an anti-HHV-6 gB

126 rabbit antibody or an anti-MHC class I Mab. After washing for 10 min with PBS
127 containing 0.02% Tween-20, the cells were incubated with an appropriate secondary
128 antibody at 37°C for 30 min, followed by Hoechst33342 at 37°C for 40 min. After
129 washing as described above, signals were detected by a confocal laser-scanning
130 microscope (Olympus FluoView FV1000).
131
132
133
134 **Results**
135 **Virion and exosome isolation**
136 The extracellular viral particles containing exosomes were purified from the culture
137 supernatant of HHV-6A (strain GS)-infected HSB-2 or HHV-6B (strain HST)-infected
138 MT-4 cells. The particle-containing fractions were confirmed by Western blotting with
139 an anti-gB antibody (23, 25). Next, the particle-containing fractions were analyzed by
140 liquid chromatography-tandem mass spectrometry (LC-MS/MS) (27), which detected
141 many cellular proteins (unpublished data). Of the host proteins detected, we focused our
142 analyses on MHC class I molecules.
143
144 **Virion- or exosome-associated fractions contain MHC class I molecules**
145 To verify the expression of MHC class I within viral particles, the proteins in fractions
146 3–10 were separated by SDS-PAGE and analyzed by Western blotting with anti-gB
147 rabbit, anti-MHC class I, or anti-CD63 antibodies. As shown in Figure 1, gB protein
148 was detected in fractions 5–6 and MHC class I was detected primarily in fractions 6–8.
149 We previously reported that the multivesicular body (MVB) marker, CD63, was

150 incorporated into virions and exosomes (23); therefore, we also examined the
151 expression of CD63. As expected, CD63 was detected in fractions 5–10 (Fig. 1c). To
152 confirm the expression of MHC class I within both virions and exosomes, we performed
153 negative staining of fractions 6 and 7 followed by electron microscopy (30). Fraction 6
154 contained mainly viral particles of approximately 200 nm in diameter. Both MHC class
155 I (Fig. 1e) and gB protein (Fig. 1d) were present in these particles. Fraction 7 contained
156 mainly exosomes of approximately 50–100 nm in diameter (Fig. 1f). These exosomes
157 contained MHC class I, which conformed the results of the Western blotting
158 experiments. Taken together, these results indicate that MHC class I molecules are
159 present in exosomes and virions released from HHV-6B-infected cells.
160
161 **Downregulated expression of MHC class I molecules on the surface of**
162 **HHV-6B-infected cells**
163 Downregulation of MHC class I occurs in many different virus-infected cells (31-37).
164 Because MHC class I molecules were incorporated into virions, HHV-6-infected MT-4
165 cells might show an apparent downregulation in cell surface expression. To confirm this,
166 HHV-6B- or mock-infected cells, which were harvested at 72 h post-infection, were
167 fixed and then stained with an anti-MHC class I antibody. The surface expression of
168 MHC class I was then analyzed by flow cytometry. As expected, HHV-6B-infected cells
169 showed downregulated cell surface expression of MHC class I when compared with
170 mock-infected cells (Fig. 2a). This reduced expression was confirmed by Western blot
171 analysis (Fig. 2b), indicating that the expression of MHC class I molecules within
172 HHV-6-infected cells (not just expression on the cell surface) was also downregulated.
173 We next observed the localization of MHC class I molecules in these cells after they

were fixed and co-stained with anti-MHC class I and gB antibodies. MHC class I in infected cells was localized mainly within intracellular compartments, and colocalized with the envelope glycoprotein gB during the later stages of infection; however, MHC class I was mainly localized to the plasma membrane in mock-infected cells (Fig. 2c).

Discussion

Here, we used mass spectrometry-based proteomics analysis to show that MHC class I molecules are incorporated into HHV-6 viral particles. The downregulation of MHC class I molecules in virus-infected cells is an important mechanism by which viruses evade immune surveillance (31-37). We showed that downregulation of MHC class I molecules occurs in T cells infected by HHV-6. MHC class I molecules are incorporated into viral particles and exosomes and then released into the extracellular environment, suggesting a possible strategy for escaping host immune responses. In addition, MHC class I molecules incorporated into virions and exosomes may assist viral entry. Further studies are needed to address this question.

We previously reported that immature HHV-6 particles bud into TGN or TGN-derived vesicles (which are produced in HHV-6B-infected cells), that vesicles containing mature virions become MVBs, and that virions and exosomes are released into the extracellular environment via an exosomal secretary pathway (23). It is possible that MHC class I molecules are transported into the TGN-derived membranes from which the virions bud and then incorporated into virions within infected cells without being recycled (Fig. 3).

The MHC class I molecules within infected cells colocalized with the gB protein in the cytoplasm, indicating that, like viral glycoproteins, they are sorted into vesicles. The

reduction in the total (both cell surface and intracellular) expression of MHC class I in HHV-6-infected cells suggests that some of them may be transported to lysosomes and degraded, as this route is the same as that used to transport particles to MVBs.

Several host proteins are expressed in the same intracellular compartments incorporating viral particles, although they are usually expressed on the surface of uninfected cells. Newly formed compartments within HHV-6-infected cells may show the combined characteristics of early and late endosomes. Recycling to early endosomes in HHV-6-infected cells may be modified or defective; therefore, several cellular proteins that use the same recycling system may be incorporated into virions and exosomes.

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Competing interests

The authors have declared that no competing interests exist.

References

1 Roizmann, B., Desrosiers, R.C., Fleckenstein, B., Lopez, C., Minson, A.C. & Studdert, M.J. (1992) The family Herpesviridae: an update. The Herpesvirus Study Group of the International Committee on Taxonomy of Viruses. Arch Virol, **123**: 425-49.

2 Salahuddin, S.Z., Ablashi, D.V., Markham, P.D., Josephs, S.F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Staal, F., Kramarsky, B. & Gallo, R.C. (1986) Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science, **234**: 596-601.

3 Aubin, J.T., Collandre, H., Candotti, D., Ingrand, D., Rouzioux, C., Burgard, M., Richard, S., Huraux, J.M. & Agut, H. (1991) Several groups among human herpesvirus 6 strains can be distinguished by Southern blotting and polymerase chain reaction. J Clin Microbiol, **29**: 367-72.

4 Campadelli-Fiume, G., Guerrini, S., Liu, X. & Foa-Tomasi, L. (1993) Monoclonal antibodies to glycoprotein B differentiate human herpesvirus 6 into two clusters, variants A and B. J Gen Virol, **74 (Pt 10)**: 2257-62.

5 Wyatt, L.S., Balachandran, N. & Frenkel, N. (1990) Variations in the replication and antigenic properties of human herpesvirus 6 strains. J Infect Dis, **162**: 852-7.

6 Yamanishi, K., Okuno, T., Shiraki, K., Takahashi, M., Kondo, T., Asano, Y. & Kurata, T. (1988) Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet, **1**: 1065-7.

7 Okuno, T., Takahashi, K., Balachandra, K., Shiraki, K., Yamanishi, K., Takahashi, M. & Baba, K. (1989) Seroepidemiology of human herpesvirus 6 infection in normal children and adults. J Clin Microbiol, **27**: 651-3.

8 Ahn, K., Gruhler, A., Galocha, B., Jones, T.R., Wiertz, E.J., Ploegh, H.L., Peterson, P.A., Yang, Y. & Fruh, K. (1997) The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. Immunity, **6**: 613-21.

9 Hill, A., Jugovic, P., York, I., Russ, G., Bennink, J., Yewdell, J., Ploegh, H. & Johnson, D. (1995) Herpes simplex virus turns off the TAP to evade host immunity. Nature,

375: 411-5.

10 Tomazin, R., Van Schoot, N.E., Goldsmith, K., Jugovic, P., Sempe, P., Fruh, K. & Johnson, D.C. (1998) Herpes simplex virus type 2 ICP47 inhibits human TAP but not mouse TAP. J Virol, 72: 2560-3.

11 Wills, M.R., Ashiru, O., Reeves, M.B., Okecha, G., Trowsdale, J., Tomasec, P., Wilkinson, G.W., Sinclair, J. & Sissons, J.G. (2005) Human cytomegalovirus encodes an MHC class I-like molecule (UL142) that functions to inhibit NK cell lysis. J Immunol, 175: 7457-65.

12 Jones, T.R., Wiertz, E.J., Sun, L., Fish, K.N., Nelson, J.A. & Ploegh, H.L. (1996) Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. Proc Natl Acad Sci U S A, 93: 11327-33.

13 Wiertz, E.J., Jones, T.R., Sun, L., Bogyo, M., Geuze, H.J. & Ploegh, H.L. (1996) The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell, 84: 769-79.

14 Ziegler, H., Thale, R., Lucin, P., Muranyi, W., Flohr, T., Hengel, H., Farrell, H., Rawlinson, W. & Koszinowski, U.H. (1997) A mouse cytomegalovirus glycoprotein retains MHC class I complexes in the ERGIC/cis-Golgi compartments. Immunity, 6: 57-66.

15 Coscoy, L. & Ganem, D. (2000) Kaposi's sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC class I chains by enhancing their endocytosis. Proc Natl Acad Sci U S A, 97: 8051-6.

16 Hudson, A.W., Howley, P.M. & Ploegh, H.L. (2001) A human herpesvirus 7 glycoprotein, U21, diverts major histocompatibility complex class I molecules to lysosomes. J Virol, 75: 12347-58.

17 Ishido, S., Wang, C., Lee, B.S., Cohen, G.B. & Jung, J.U. (2000) Downregulation of major histocompatibility complex class I molecules by Kaposi's sarcoma-associated herpesvirus K3 and K5 proteins. J Virol, 74: 5300-9.

18 Reusch, U., Muranyi, W., Lucin, P., Burgert, H.G., Hengel, H. & Koszinowski, U.H. (1999) A cytomegalovirus glycoprotein re-routes MHC class I complexes to lysosomes for degradation. EMBO J, 18: 1081-91.

19 Hirata, Y., Kondo, K. & Yamanishi, K. (2001) Human herpesvirus 6 downregulates major histocompatibility complex class I in dendritic cells. J Med Virol, 65: 576-83.

20 Glosson, N.L. & Hudson, A.W. (2007) Human herpesvirus-6A and -6B encode viral immunoevasins that downregulate class I MHC molecules. Virology, 365: 125-35.

21 Mori, Y., Yagi, H., Shimamoto, T., Isegawa, Y., Sunagawa, T., Inagi, R., Kondo, K.,

287 Tano, Y. & Yamanishi, K. (1998) Analysis of human herpesvirus 6 U3 gene, which is
288 a positional homolog of human cytomegalovirus UL 24 gene. Virology, **249**: 129-39.
289 22 Mori, Y., Akkapaiboon, P., Yang, X. & Yamanishi, K. (2003) The human herpesvirus 6
290 U100 gene product is the third component of the gH-gL glycoprotein complex on the
291 viral envelope. J Virol, **77**: 2452-8.
292 23 Mori, Y., Koike, M., Moriishi, E., Kawabata, A., Tang, H., Oyaizu, H., Uchiyama, Y. &
293 Yamanishi, K. (2008) Human herpesvirus-6 induces MVB formation, and virus
294 egress occurs by an exosomal release pathway. Traffic, **9**: 1728-42.
295 24 Okuno, T., Shao, H., Asada, H., Shiraki, K., Takahashi, M. & Yamanishi, K. (1992)
296 Analysis of human herpesvirus 6 glycoproteins recognized by monoclonal antibody
297 OHV1. J Gen Virol, **73 (Pt 2)**: 443-7.
298 25 Tang, H., Kawabata, A., Takemoto, M., Yamanishi, K. & Mori, Y. (2008) Human
299 herpesvirus-6 infection induces the reorganization of membrane microdomains in
300 target cells, which are required for virus entry. Virology, **378**: 265-71.
301 26 Kawabata, A., Tang, H.M., Huang, H.L., Yamanishi, K. & Mori, Y. (2009) Human
302 herpesvirus 6 envelope components enriched in lipid rafts: evidence for
303 virion-associated lipid rafts. Virology Journal, **6**.
304 27 Yamada, M., Mugnai, G., Serada, S., Yagi, Y., Naka, T. & Sekiguchi, K. (2013)
305 Substrate-attached materials are enriched with tetraspanins and are analogous to
306 the structures associated with rear-end retraction in migrating cells. Cell Adh Migr,
307 **7**: 304-14.
308 28 Akkapaiboon, P., Mori, Y., Sadaoka, T., Yonemoto, S. & Yamanishi, K. (2004)
309 Intracellular processing of human herpesvirus 6 glycoproteins Q1 and Q2 into
310 tetrameric complexes expressed on the viral envelope. J Virol, **78**: 7969-83.
311 29 Mori, Y., Akkapaiboon, P., Yonemoto, S., Koike, M., Takemoto, M., Sadaoka, T.,
312 Sasamoto, Y., Konishi, S., Uchiyama, Y. & Yamanishi, K. (2004) Discovery of a
313 second form of tripartite complex containing gH-gL of human herpesvirus 6 and
314 observations on CD46. J Virol, **78**: 4609-16.
315 30 Raposo, G., Nijman, H.W., Stoorvogel, W., Liejendekker, R., Harding, C.V., Melief,
316 C.J. & Geuze, H.J. (1996) B lymphocytes secrete antigen-presenting vesicles. J Exp
317 Med, **183**: 1161-72.
318 31 Elboim, M., Grodzovski, I., Djian, E., Wolf, D.G. & Mandelboim, O. (2013) HSV-2
319 specifically down regulates HLA-C expression to render HSV-2-infected DCs
320 susceptible to NK cell killing. PLoS Pathog, **9**: e1003226.
321 32 Kubota, A., Kubota, S., Farrell, H.E., Davis-Poynter, N. & Takei, F. (1999) Inhibition
322 of NK cells by murine CMV-encoded class I MHC homologue m144. Cell Immunol,

191: 145-51.

33 Ma, G., Feineis, S., Osterrieder, N. & Van De Walle, G.R. (2012) Identification and characterization of equine herpesvirus type 1 pUL56 and its role in virus-induced downregulation of major histocompatibility complex class I. J Virol, 86: 3554-63.

34 Neumann, L., Kraas, W., Uebel, S., Jung, G. & Tampe, R. (1997) The active domain of the herpes simplex virus protein ICP47: a potent inhibitor of the transporter associated with antigen processing. J Mol Biol, 272: 484-92.

35 Raafat, N., Sadowski-Cron, C., Mengus, C., Heberer, M., Spagnoli, G.C. & Zajac, P. (2012) Preventing vaccinia virus class-I epitopes presentation by HSV-ICP47 enhances the immunogenicity of a TAP-independent cancer vaccine epitope. Int J Cancer, 131: E659-69.

36 Said, A., Azab, W., Damiani, A. & Osterrieder, N. (2012) Equine herpesvirus type 4 UL56 and UL49.5 proteins downregulate cell surface major histocompatibility complex class I expression independently of each other. J Virol, 86: 8059-71.

37 Vasireddi, M. & Hilliard, J. (2012) Herpes B virus, macacine herpesvirus 1, breaks simplex virus tradition via major histocompatibility complex class I expression in cells from human and macaque hosts. J Virol, 86: 12503-11.

Figure Legends

Figure 1. MHC class I molecules are incorporated into virions and exosomes and released from HHV-6B-infected cells. Virions and exosomes were collected from the culture medium of HHV-6B-infected cells by sucrose density gradient centrifugation and examined by Western blotting (a, b, and c) and electron microscopy (d, e, and f). Western blots with anti-gB rabbit (a), anti-MHC class I (W6/32) (b), or anti-CD63 (CLB-gran/12, 435) (c) antibodies are shown. The same amount of each protein fraction was added to each well of the gel. Immunogold labeling of gB (d) in Fraction 6 and of MHC class I in Fractions 6 and 7 (e and f). The Fraction number is shown at the top of each panel. The fractions were collected from the bottom of tube. Labeled virions (empty arrowheads) and exosomes (arrowheads). Scale bars: 200 nm (d–f).