Biochemical and Biophysical Research Communications 443 (2014) 422–427

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cell surface expression of MR1B, a splice variant of the MHC class I-related molecule MR1, revealed with antibodies

Hisateru Yamaguchi, Kentaro Tsukamoto, Keiichiro Hashimoto *

Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan

ARTICLE INFO

Article history:
Received 19 November 2013
Available online 2 December 2013

Keywords:
MR1
MHC
Splice variant
Cell surface expression

ABSTRACT

The major histocompatibility complex (MHC) class I-related molecule, MR1, is highly conserved in mammals and can present bacteria-derived vitamin B metabolites to mucosal-associated invariant T (MAIT) cells, possibly having important defense function in the microbial infection. MR1B is a splice variant of MR1 and possesses an intriguing domain structure with only two extracellular domains resembling some NKG2D ligand molecules. Thus far, cell surface expression of MR1B could not be analyzed with flow cytometry due to a lack of appropriate antibodies reactive with MR1B. Here we clarified the expression of MR1B recombinant protein on the cell surface of the transfected cells by flow cytometry analyses using the antiserum against MR1. Consistently, MR1B tagged with FLAG peptide at the N-terminus also could be detected with anti-FLAG monoclonal antibodies. Our result showed that MR1B can be recognized on the cell surface by macromolecules such as antibodies, indicating its potential of interaction with certain receptor(s). We discuss possibility of interaction of MR1B and/or the full-length MR1 with some receptor(s) other than αβ T cell receptor (TCR) of MAIT cells based on the highly conserved characteristic residues of the ligand-binding domains of MR1 and its MAIT cells αβTCR footprints.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

MR1 is a non-classical MHC class I molecule whose gene was originally found in the human chromosome 1, same as for CD1 genes, outside the MHC region where the genes for the classical MHC class I molecules reside [1]. Predicted MR1 protein, however, showed highest similarity to the classical MHC class I molecules among various MHC class I-related members [1]. Conservation of possible ligand-binding domains turned out to be surprisingly high between human and mouse reaching almost 90%, which suggested interaction of MR1 with some highly conserved molecule(s) and thereby exerting important biological function(s) [2]. Possible reactivity with some conserved TCR, like one with the invariant α chain for CD1, was also discussed [2]. MR1 was actually found to be critical for the development and expansion of a special T lymphocyte population called MAIT cells, whose αβTCR possesses an invariant α chain [3]. MAIT cells are abundant in human peripheral blood, up to 10% of T lymphocyte population, and also in mucosal tissues and liver [4]. It has been clarified that MAIT cells play an important role in microbial infections [5–10]. A recent intensive study finally revealed that MR1 ligands belong to microbial vitamin B metabolites [11]. The three-dimensional structure of the ligand-bound MR1 molecule was elucidated and its overall structure was very similar to that of the classical MHC class I molecule [11], also confirming the previous observation of its association with β_2-microglobulin (β_2-m) [12,13]. Very recently, the crystal structures of the complex of αβTCR of MAIT cells and MR1 molecule were revealed [14–16]. The results showed that ligand-bound MR1 molecule interacts with MAIT cells αβTCR like the conventional αβTCR/classical MHC class I molecule/peptide complex with respect to the binding orientation [14–16].

Studies on the transcripts of human MR1 gene showed the existence of several alternative spliced forms in addition to the full-length MR1 referred to MR1A [17]. One of those splice variants, called MR1B whose transcript was identified in the human fetal spleen cDNA library, possesses two extracellular domain architecture, namely, preserving its ligand-binding domains and lacking its membrane-proximal immunoglobulin-fold domain [17]. We became very interested in this form of MR1 as some NKG2D ligands such as ULBP3 and related molecules possess the same extracellular domain architecture [18]. MR1A is highly conserved in mammals including marsupials [19] and MR1B-like splice variants could also be found in the B-cell lines of other primates (chimpanzee and orangutan [20]), but not from mouse [17], ruminants (cow [21,22] and sheep [22]), and opossum [19].

Abbreviations: MHC, major histocompatibility complex; MAIT, mucosal-associated invariant T; TCR, T cell receptor; CDR, complementarity determining region; NK, natural killer; NKT, natural killer T; HLA, human leukocyte antigen; β_2-m, β_2-microglobulin.

* Corresponding author.
E-mail address: keihashi@fujita-hu.ac.jp (K. Hashimoto).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.096

Whether MR1B can be expressed on the cell surface and whether it retains the structure of the ligand-binding domains as observed in MR1A are intriguing important questions when one considers a possibility of interaction between MR1B and some certain molecules including cellular receptors. Very recently one group reported some characteristics of MR1B molecule tagged with GFP (green fluorescent protein) at the C-terminus [23]. The authors reported cell surface expression of GFP-tagged MR1B using small chemical reagents such as a biotinylating compound and a cross-linking compound, and also reported the activation of MAIT cells by MR1B. However, because of a lack of appropriate antibodies, flow cytometry analyses could not be conducted thus far. In the present study, taking advantage of the availability of the reactive antiserum, we studied cell surface expression of the natural form of MR1B using flow cytometry analyses, thereby showing that MR1B can react with macromolecules on the cell surface.

2. Materials and methods

2.1. Reverse transcription (RT)-polymerase chain reaction (PCR) of MR1A and MR1B

The MR1A and MR1B variants were amplified with a primer set using two multiple tissue cDNA panels, Human MTC Panel I and II normalized with four housekeeping genes (Clontech Laboratories Inc., Mountain View, CA, USA), as templates. The forward primer was 5′-AAAGAACCCGGAAAGAGAAGGA-3′ at 5′-untranslated region (UTR) and reverse primer was 5′-GAGGAAGGAGAACTGGAAAA GAGG-3′ at 3′-UTR. The GAPDH was used as control and amplified by 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ and 5′-CATGTGG GCCATGAGGTCCACCAC-3′ primers. The amplification was carried with PrimeSTAR HS DNA polymerase (Takara Bio Inc., Shiga, Japan). The PCR condition was as follows; 22 cycles for GAPDH or 30 cycles for MR1 of denaturation at 98 °C for 10 s, annealing at 55 °C for 15 s, and elongation at 72 °C for 1 min. The PCR products were analyzed in 2% agarose gel.

2.2. Production of rabbit anti-human MR1 antisera

The polyclonal antibodies against the α1 and α2 domains of human MR1 (1–179 residues of mature peptide) recombinant protein (anti-MR1-α1α2) and against the synthetic peptide (CRRPREQNGAIYLPTPDR) matching the C-terminal residues of human MR1 protein (anti-MR1-C-term) were raised in rabbits.

2.3. Cell culture and stable transfectants

293F cells (Invitrogen, Carlsbad, CA, USA) were cultured in FreeStyle 293 (Gibco, Carlsbad, CA, USA) at 8% CO_2 or in Dulbecco's modified Eagle's medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) supplemented with 5% fetal bovine serum (FBS) (BioWhittaker, Ontario, Canada) at 5% CO_2.

Stable transfectants expressing MR1A, MR1B, FLAG-tagged MR1A, FLAG-tagged MR1B, and FLAG-tagged HLA-A2 were established and were designated as 293-MR1A, 293-MR1B, 293-FLAG-MR1A, 293-FLAG-MR1B, and 293-FLAG-HLA, respectively.

2.4. Immunoblotting

The procedure for the preparation of cell lysates and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analyses of the lysates (Fig. 2A) were described previously [12]. In the other figures, the lysates were subjected to SDS–PAGE based on bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane-HCl with 3-(N-morpholino) propane sulfonic acid running buffer [24]. The MR1, β_2-m, and FLAG-tagged proteins were detected by immunoblotting conducted as described previously [12] using anti-MR1-α1α2 (this study), rabbit polyclonal anti-human β_2-m antibody (Dako, Glostrup, Denmark), and anti-FLAG M2 antibody (Sigma–Aldrich, St. Louis, MO, USA) as primary antibody, respectively. Anti-Rabbit IgG, HRP-Linked Whole Ab Donkey (GE Healthcare, Uppsala, Sweden) and goat anti-mouse HPR conjugated (Thermo Fisher Sci., Waltham, MA, USA) were used as secondary antibody. Detection was performed with Pierce Western Blotting Substrate Plus (Thermo Fisher Sci.) and imaging was done by ImageQuant LAS 4000mini (GE Healthcare).

2.5. Endoglycosidase H and Glycopeptidase F digestion

Digestions with Endoglycosidase H (New England Biolabs, Ipswich, MA, USA) and Glycopeptidase F (Takara Bio Inc.) were performed according to manufacturer's instruction.

2.6. Immunoprecipitation

The MR1A and MR1B proteins were immunoprecipitated from total cell lysates of 293-MR1A and 293-MR1B cells, respectively, using anti-MR1-C-term cross-linked to Protein G Sepharose (GE Healthcare). The elution was performed by 0.1% trifluoroacetic acid.

2.7. Flow cytometry

The cells were harvested and incubated with primary antibodies in FC buffer (phosphate-buffered saline containing 2% FBS and 0.1% sodium azide) for 1 h on ice. The cells were washed with ice-cold FC buffer prior to incubation with secondary antibody in FC buffer for 1 h on ice. The cells were washed with ice-cold PBS and were analyzed using a Gallios cytometer (Beckman Coulter Inc., Brea, CA, USA). Dead cells were excluded using propidium iodide (Invitrogen) staining. The collected data were analyzed using Kaluza v1.2 (Beckman Coulter). Anti-MR1-α1α2 (this study) and mouse monoclonal anti-FLAG M2 antibody (Sigma–Aldrich) were used as the primary antibody. The rabbit and mouse antibodies were detected using CELL LAB Goat $F(ab')_2$ Anti-Rabbit IgG Phycoerythrin Conjugate and CELL LAB Goat $F(ab')_2$ Anti-Mouse IgG Phycoerythrin conjugate (Beckman Coulter), respectively.

3. Results

3.1. Expression of MR1B in various human tissues

At first, we examined the expression of MR1B in various human tissue samples using RT-PCR (Fig. 1). With two primers designed at the 5′- and 3′-UTRs of MR1 gene, two major bands were detected which correspond to the full-length MR1 (MR1A; Fig. 1, upper band,) and MR1B (Fig. 1, lower band), respectively. As shown in Fig. 1, MR1B could be detected in various tissues, and its level of expression appears generally comparable to that of MR1A.

3.2. Characteristics of MR1B-transfected cells

We next asked whether MR1B transcript could give rise to a stable protein product and, if so, whether it could reach the cell surface for possible interaction with certain molecule(s). As the detection of the endogenous MR1B protein was difficult, we established stable MR1B-transfected cell lines and prepared rabbit anti-MR1-α1α2 antiserum using α1 and α2 domains of human MR1 as an immunogen. Fig. 2A shows that MR1B protein

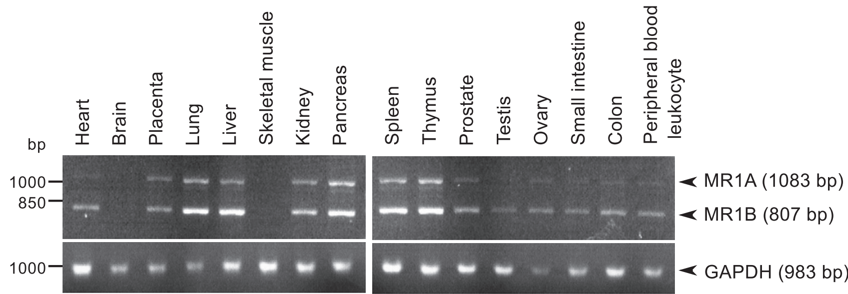

Fig. 1. Expression of splicing variants of MR1, MR1A and MR1B, in human tissues. These two transcripts were amplified by RT-PCR using two sets of human MTC panels as template. GAPDH was used as control.

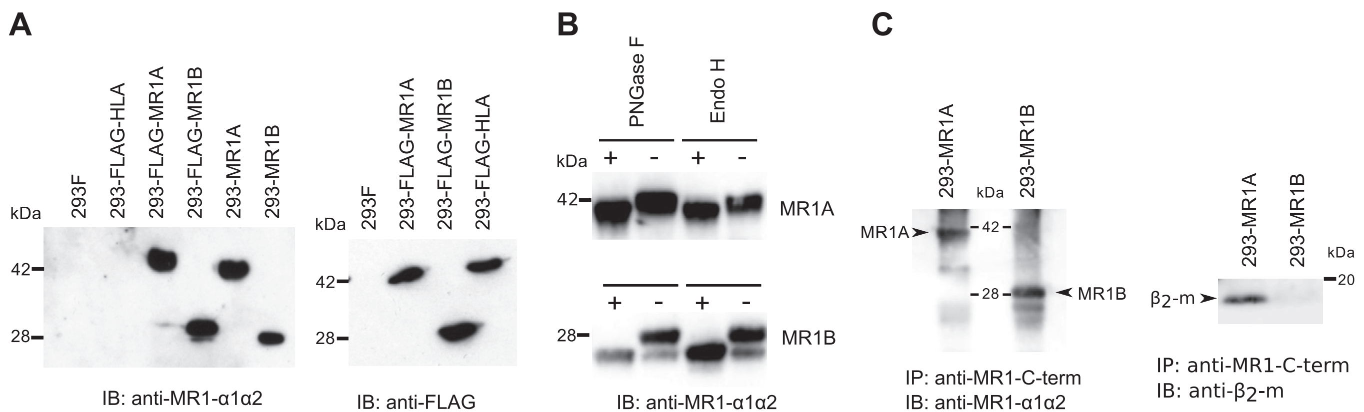

Fig. 2. Characteristics of MR1B-transfected cells. (A) Immunoblotting of transfected cell lines. Proteins in total cell lysates were separated by SDS–PAGE (10% gel), and MR1 and FLAG-tagged protein were detected using anti-MR1-α1α2 and anti-FLAG antibody, respectively. (B) Total cell lysates of 293-MR1A and 293-MR1B cells were treated with (+) or without (−) PNGase F or Endo H. The proteins were separated by SDS–PAGE (10% gel). The MR1A and MR1B proteins were detected using anti-MR1-α1α2 antiserum. (C) Proteins in total cell lysates were immunoprecipitated using anti-MR1-C-term. The immunoprecipitated proteins were separated using SDS–PAGE (10% and 12% gels for MR1 and β_2-m, respectively). The MR1 and β_2-m were detected using anti-MR1-α1α2 and rabbit polyclonal anti-human β_2-m antibody, respectively. Each result is a representative of three independent experiments.

product could be detected in the cell lysate of a stably transfected 293F cell line (293-MR1B) by immunoblotting with the anti-MR1-α1α2 antiserum. We also prepared stable cell lines transfected with MR1B added with FLAG tag-coding sequence at the 5′ side (293-FLAG-MR1B) along with 293-FLAG-MR1A and 293-FLAG-HLA. The anti-MR1-α1α2 antiserum also detected FLAG-MR1B as well as MR1A and FLAG-MR1A, but not FLAG-HLA (Fig. 2A). The anti-FLAG monoclonal antibody specifically detected all the FLAG-tagged molecules including FLAG-MR1B, FLAG-MR1A and FLAG-HLA (Fig. 2A).

Fig. 2B shows that MR1B is glycosylated and could be digested with Glycopeptidase F (PNGase F). The glycosylation of MR1B is immature as it was digested by Endoglycosidase H (Endo H). This is essentially similar to the situation of MR1A (Fig. 2B). As the α3 domain is important for the interaction with β_2-m, we also checked association between MR1B and β_2-m using the immunoprecipitation methods. The results show that β_2-m was coprecipitated with MR1A but not with MR1B (Fig. 2C).

3.3. Antibodies can recognize MR1B on the cell surface of MR1B-transfected cells

Next, we checked if MR1B is expressed on the cell surface of the transfected cells using flow cytometry analyses. Fortunately our anti-MR1-α1α2 antiserum exhibits reactivity toward MR1A and MR1B not only in the immunoblotting analyses but also in the flow cytometry analyses. The third panel of Fig. 3A shows that the anti-MR1-α1α2 antiserum could detect MR1B on the cell surface of the stably transfected cells. The same antiserum also could detect MR1A although the reactivity was somewhat lower compared to that toward MR1B, and reactivity was undetectable for HLA (Fig. 3A). We further conducted flow cytometry analyses using the cell line transfected by MR1B with FLAG tag at its N-terminus. The anti-FLAG monoclonal antibody could detect N-terminally FLAG-tagged MR1B (the fifth panel of Fig. 3B). The reactivity of the anti-FLAG monoclonal antibody was specific for FLAG as it did not detect untagged MR1B and MR1A (Fig. 3B). The anti-MR1-α1α2 antiserum could detect the FLAG-tagged MR1A and MR1B (Fig. 3A). The reactivity of anti-MR1-α1α2 antiserum toward MR1A or MR1B was not weakened even if FLAG tag was introduced to those molecules based on the comparison between reactivity toward MR1A and toward FLAG-MR1A or between MR1B and FLAG-MR1B. Using the same anti-FLAG monoclonal antibody, we could compare the amount of FLAG epitope accessible to this antibody in three kinds of transfected cell lines, FLAG-MR1A, FLAG-MR1B and FLAG-HLA. FLAG-MR1B showed intermediate amount of epitopes between FLAG-MR1A and FLAG-HLA (Fig. 3B), indicating that the lack of α3 domain itself did not result in significant decrease in the expression on the cell surface.

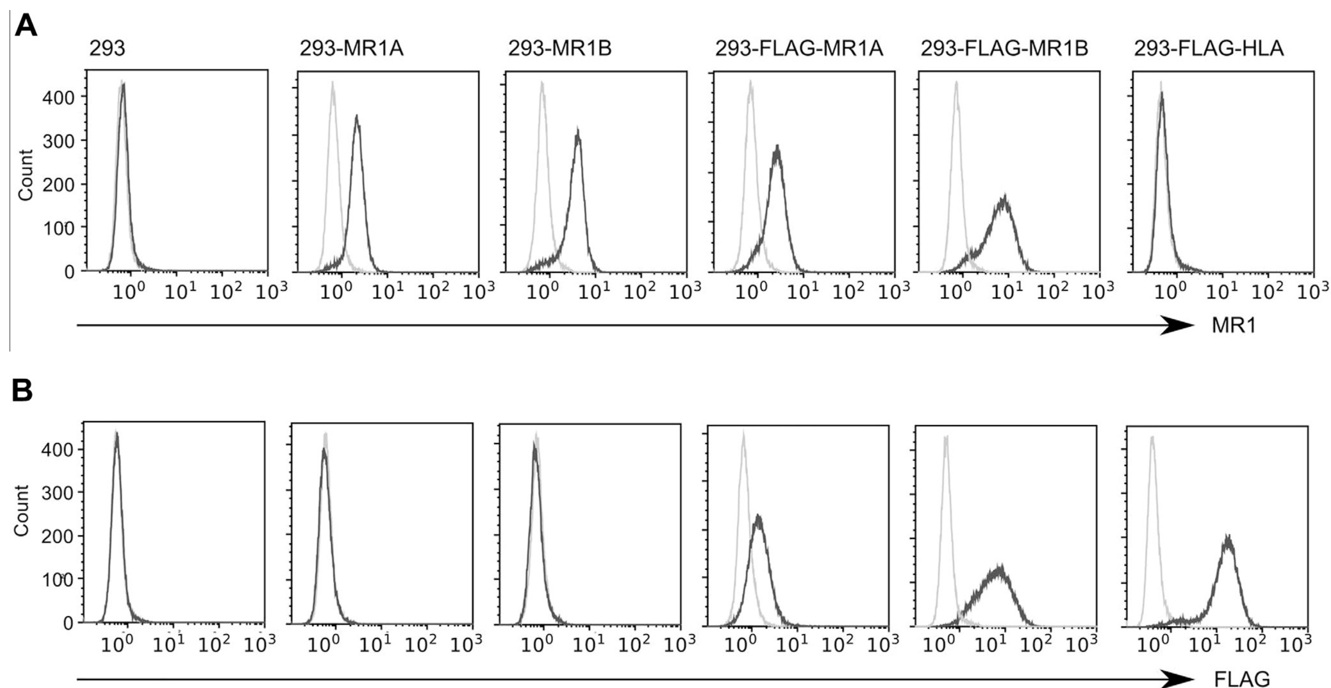

Fig. 3. Antibodies can recognize MR1B on the cell surface. Flow cytometry analyses of cell surface expression of MR1A and MR1B. (A) Cell surface expression was assessed using anti-MR1-α1α2 (black line) compared with negative control by rabbit pre-immune serum (gray line). (B) Cell surface expression was assessed using anti-FLAG antibody (black line) compared with negative control using secondary antibody only (gray line). Each histogram is a representative of three independent experiments.

4. Discussion

This study demonstrates that MR1B, a splice variant of MR1, can be detected on the transfected cell surface by flow cytometry analyses. The transcript for MR1B could be found in various human tissues at a comparable level to the full-length MR1 (MR1A). Thus, if once MR1B transcript is translated in the natural situation, it may reach the cell surface and could be accessible by various receptor(s).

In the present study, the anti-MR1-α1α2 antiserum was raised with the α1 and α2 domains of MR1 as an immunogen. Therefore, the results of flow cytometry analyses show that some portion of the α1 and α2 domains of MR1B is accessible by the rabbit antibodies on the MR1B-transfected cell surface. For the tagged MR1B, we added the FLAG tag peptide (eight amino acids) to the amino terminus of MR1B molecule (Fig. 4). The result of flow cytometry with this FLAG-tagged MR1B and the anti-FLAG monoclonal antibody (Fig. 3B) indicated that antibodies can have access to the amino terminal region of MR1B. Introduction of the small FLAG tag into the amino-terminus of MR1B did not significantly change the reactivity of the anti-MR1-α1α2 antiserum toward MR1B (Fig. 3A). Therefore, the results suggested that the addition of the FLAG tag did not affect the gross conformation of MR1B and MR1B seems to possess some structural integrity on the cell surface and not to have grossly misfolded. The anti-MR1-α1α2 antiserum did not stain a stable cell line producing the secreted form of MR1B lacking both the transmembrane and cytoplasmic regions, and the antiserum raised against the C-terminal peptide of MR1 did not stain the MR1B-transfected cells (data not depicted). Collectively the results suggest that MR1B exists at the cell surface with its transmembrane region being embedded in the cell membrane and with its α1 and α2 domains pointing to the extracellular space.

Lion et al. recently reported the characteristics of MR1B tagged with GFP at its C-terminus [23]. They could not detect MR1B-GFP on the cell surface when they used anti-MR1 monoclonal antibody in the flow cytometry analyses. Because of unavailability of antibodies reactive with MR1B, they used the cell surface biotinylation labeling method to study cell surface

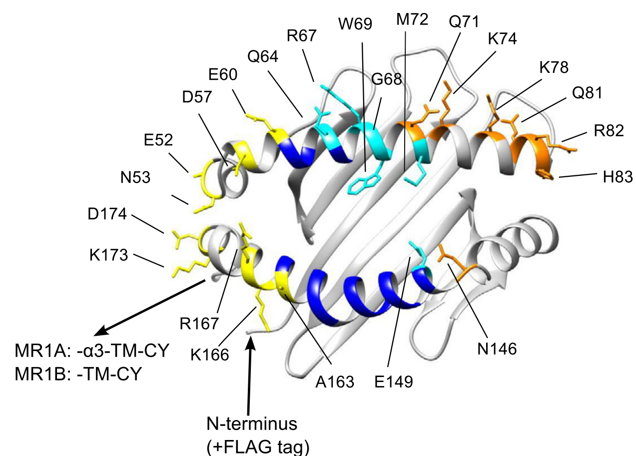

Fig. 4. Positions of some conserved residues of MR1 and MAIT αβTCR footprints. The ribbon structure of the ligand-binding α1 and α2 domains of human MR1A (PDB: 4L4V) is shown using the UCSF Chimera program (http://www.cgl.ucsf.edu/chimera/). MR1B possesses these domains although its structure remains undetermined. In the MR1A, the α2 domain is linked to the α3 domain, then to TM (transmembrane) and CY (cytoplasmic) regions, while in the MR1B, directly linked to TM and CY regions as indicated in the figure. In some of our experiments, the FLAG tag (composed of eight amino acids) was linked to the amino (N−) terminus of the molecule. The footprints of αβTCR of human MAIT cells on human MR1 are shown [15] with the following coloring: blue, the amino acid residues with which CDR(s) of Vα or CDRs of both Vα and Vβ interact; cyan, the residues with which only CDR(s) of Vβ interact. Except for these footprints, some of the amino acid residues whose side-chains are pointing outward and conserved among placental or placental plus marsupial mammals [19] are shown as a left-side (yellow) or a right-side (orange) group for convenience' sake. Various receptors can interact with some of those residues. The side-chains of cyan-, orange-, and yellow-colored residues are depicted. See main text and Supplementary Fig. S2 for further details.

expression of MR1B-GFP. Although they showed biotinylation and also crosslinking of MR1B-GFP on the cell surface using small reactive molecules, it was not clear whether larger molecules actually can interact with MR1B on the cell surface or not. Fortunately, our antiserum exhibited reactivity toward MR1B, thus we could demonstrate that MR1B can interact with macromolecules on the cell surface in the present study. Lion et al. reported some other features of MR1B-GFP, namely, its immature glycosylation state and non-association with β_2-m, and our results of untagged MR1B are largely consistent with their results. They also reported that MR1B-GFP mainly accumulated in the endoplasmic reticulum and exists as a homo-dimer both in the cell lysate and on the cell surface. In the immunoblotting analyses under the non-reducing condition, we could observe molecular species of MR1B in cell lysate whose superficial sizes are quite similar to those observed under the reducing condition, which suggested the existence of monomeric forms of MR1B as for MR1A (Supplementary Fig. S1). The molecular nature of untagged MR1B expressed on the cell surface needs further investigation. We actually observed several bands of higher molecular weights for both MR1B and MR1A under the non-reducing condition (Supplementary Fig. S1), however, the molecular nature of these fractions remains to be analyzed.

Importantly, Lion et al. reported that the cell line transfected with MR1B-GFP could activate MAIT cells if the preincubation of the transfected cells with bacteria had been conducted [23]. It is very interesting to know whether MR1B actually can bind some bacterial ligand(s) and interact with αβTCR of MAIT cells like MR1A. As MR1B lacks the α3 domain which may interact with CD8αα, the reactivity of MR1B with αβTCR of MAIT cells may become different compared with MR1A, even if MR1B retains the conformation of the ligand-binding domains.

Very recently, based on the crystal structures, the footprints of αβTCR of MAIT cells on MR1 molecule were reported [14–16]. The binding orientation of MAIT cells αβTCR over MR1 molecule looks like those observed for the conventional αβTCR/classical MHC class I/peptide and not like that of type I NKT αβTCR/CD1d/α-GalCer complex [14–16] (Fig. 4). The high conservation of some residues in the α1 and α2 helix regions of MR1 [19] could be explained by the interaction with the invariant α chain of MAIT cells αβTCR (Fig. 4, Supplementary Fig. S2). However, there are other highly conserved residues whose side-chains are pointing outward and whose conservation currently cannot be explained (e.g., orange-colored residues in Fig. 4, Supplementary Fig. S2). The right reason for the high conservation of those residues in MR1 may not be found in the interaction with the αβTCR of MAIT cells, when one considers the absence of mutational effects of some conserved MR1 residues [25,26] and also of many Vβ residues [25,26] on MAIT cells activation, and high variability of Vβ repertoire of MAIT cells TCR [27,28] (Supplementary Fig. S2).

In this respect, it is noteworthy that amino acid positions supposedly corresponding to the highly conserved positions of MR1 are involved in the interactions between MHC class I related members and various receptors including not only αβTCR but also NKG2D, CD94/NKG2A and KIRs (e.g., orange-colored residues in Fig. 4, Supplementary Fig. S2). Further, there are examples of extreme cases in which the footprints of αβTCR on a single CD1d ortholog differ enormously, with distinct bound-ligands and different groups of αβTCR (Supplementary Fig. S2). Based on the existence of some highly conserved residues facing outward in the ligand-binding domains of MR1 and the currently available MAIT αβTCR footprints, we speculate that some receptor(s) other than αβTCR of MAIT cells and/or some ligands other than the currently known ones interact with MR1 molecule with receptor footprints distinct from the reported ones.

The present study showed that a splice variant of human MR1B could be detected on the cell surface of the transfected cells by flow cytometry analyses. The conformation of MR1B on the cell surface remains to be clarified. The molecules which possess similar domain architecture are well known to have important biological functions (e.g., ULBP3 [18] and endothelial protein C receptor [29,30]). We already have an example in which the same receptor (e.g., NKG2D) can interact with two kinds of domain architecture, namely, MR1B-type lacking the immunoglobulin-fold α3 domain (e.g., ULBP3) and MR1A-type retaining it (e.g., MICA) (Supplementary Fig. S2). Thus, if the ligand-binding domains of MR1B on the cell surface possess a structure similar to that of MR1A, MR1B may become another target of various receptors. Even if MR1B exhibits a structure distinct from MR1A, it may create novel biological system(s) in primates.

Acknowledgment

This work was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2008–2012.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.096.

References

[1] K. Hashimoto, M. Hirai, Y. Kurosawa, A gene outside the human MHC related to classical HLA class I genes, Science 269 (1995) 693–695.
[2] H. Yamaguchi, M. Hirai, Y. Kurosawa, K. Hashimoto, A highly conserved major histocompatibility complex class I-related gene in mammals, Biochem. Biophys. Res. Commun. 238 (1997) 697–702.
[3] E. Treiner, L. Duban, S. Bahram, et al., Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1, Nature 422 (2003) 164–169.
[4] L. Le Bourhis, Y.K. Mburu, O. Lantz, MAIT cells, surveyors of a new class of antigen: development and functions, Curr. Opin. Immunol. 25 (2013) 174–180.
[5] L. Le Bourhis, E. Martin, I. Péguillet, et al., Antimicrobial activity of mucosal-associated invariant T cells, Nat. Immunol. 11 (2010) 701–708.
[6] M.C. Gold, S. Cerri, S. Smyk-Pearson, et al., Human mucosal associated invariant T cells detect bacterially infected cells, PLoS Biol. 8 (2010) e1000407.
[7] P. Georgel, M. Radosavljevic, C. Macquin, S. Bahram, The non-conventional MHC class I MR1 molecule controls infection by Klebsiella pneumoniae in mice, Mol. Immunol. 48 (2011) 769–775.
[8] W.J. Chua, S.M. Truscott, C.S. Eickhoff, et al., Polyclonal mucosa-associated invariant T cells have unique innate functions in bacterial infection, Infect. Immun. 80 (2012) 3256–3267.
[9] A. Meierovics, W.J. Yankelevich, S.C. Cowley, et al., MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection, Proc. Natl. Acad. Sci. USA 110 (2013). E3119–3128.
[10] L. Le Bourhis, M. Dusseaux, A. Bohineust, et al., MAIT cells detect and efficiently lyse bacterially-infected epithelial cells, PLoS Pathog. 9 (2013) e1003681.
[11] L. Kjer-Nielsen, O. Patel, A.J. Corbett, et al., MR1 presents microbial vitamin B metabolites to MAIT cells, Nature 491 (2012) 717–723.
[12] H. Yamaguchi, K. Hashimoto, Association of MR1 protein, an MHC class I-related molecule, with β_2-microglobulin, Biochem. Biophys. Res. Commun. 290 (2002) 722–729.
[13] M.J. Miley, S.M. Truscott, Y.Y.L. Yu, Biochemical features of the MHC-related protein 1 consistent with an immunological function, J. Immunol. 170 (2003) 6090–6098.
[14] J. Lopez-Sagaseta, C.L. Dulberger, J.E. Crooks, et al., The molecular basis for mucosal-associated invariant T cell recognition of MR1 proteins, Proc. Natl. Acad. Sci. USA 110 (2013) E1771–1778.
[15] O. Patel, L. Kjer-Nielsen, J. Le Nours, et al., Recognition of vitamin B metabolites by mucosal-associated invariant T cells, Nat. Commun. 4 (2013) 2142, http://dx.doi.org/10.1038/ncomms3142.
[16] J. Lopez-Sagaseta, C.L. Dulberger, A. McFedries, et al., MAIT recognition of a stimulatory bacterial antigen bound to MR1, J. Immunol. 191 (2013) 5268–5277.
[17] P. Riegert, V. Wanner, S. Bahram, Genomics, isoforms, expression, and phylogeny of the MHC class I-related MR1 gene, J. Immunol. 161 (1998) 4066–4077.
[18] S. Radaev, B. Rostro, A.G. Brooks, M. Colonna, P.D. Sun, Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3, Immunity 15 (2001) 1039–1049.

[19] K. Tsukamoto, J.E. Deakin, J.A. Graves, K. Hashimoto, Exceptionally high conservation of the MHC class I-related gene, MR1, among mammals, Immunogenetics 65 (2013) 115–124.

[20] J.F. Parra-Cuadrado, M.G. del Moral, P. García-Pavía, F. Setién, E. Martínez-Naves, Characterization of the MHC class I-related MR1 locus in nonhuman primates, Immunogenetics 53 (2001) 643–648.

[21] S. Huang, E. Martin, S. Kim, et al., MR1 antigen presentation to mucosal-associated invariant T cells was highly conserved in evolution, Proc. Natl. Acad. Sci. USA 106 (2009) 8290–8295.

[22] N. Goldfinch, P. Reinink, T. Connelley, et al., Conservation of mucosal associated invariant T (MAIT) cells and the MR1 restriction element in ruminants, and abundance of MAIT cells in spleen, Vet. Res. 41 (2010) 62.

[23] J. Lion, V. Debuysscher, A. Wlodarczyk, et al., MR1B, a natural spliced isoform of the MHC-related 1 protein, is expressed as homodimers at the cell surface and activates MAIT cells, Eur. J. Immunol. 43 (2013) 1363–1373.

[24] D.R. Graham, C.P. Garnham, Q. Fu, J. Robbins, J.E. Van Eyk, Improvements in two-dimensional gel electrophoresis by utilizing a low cost "in-house" neutral pH sodium dodecyl sulfate-polyacrylamide gel electrophoresis system, Proteomics 5 (2005) 2309–2314.

[25] R. Reantragoon, L. Kjer-Nielsen, O. Patel, et al., Structural insight into MR1-mediated recognition of the mucosal associated invariant T cell receptor, J. Exp. Med. 209 (2012) 761–774.

[26] M.H. Young, L. U'Ren, S. Huang, et al., MAIT cell recognition of MR1 on bacterially infected and uninfected cells, PLoS One 8 (2013) e53789.

[27] F. Tilloy, E. Treiner, S.H. Park, et al., An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted α/β T cell subpopulation in mammals, J. Exp. Med. 189 (1999) 1907–1921.

[28] R. Reantragoon, A.J. Corbett, I.G. Sakala, et al., Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells, J. Exp. Med. 210 (2013) 2305–2320.

[29] V. Oganesyan, N. Oganesyan, S. Terzyan, et al., The crystal structure of the endothelial protein C receptor and a bound phospholipid, J. Biol. Chem. 277 (2002) 24851–24854.

[30] C.R. Willcox, V. Pitard, S. Netzer, et al., Cytomegalovirus and tumor stress surveillance by binding of a human $\gamma\delta$ T cell antigen receptor to endothelial protein C receptor, Nat. Immunol. 13 (2012) 872–879.